Role of planar lipid structure in transmembrane charge conduction

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Biological membranes provide individuality and specificity to cells and intercellular organelles and is the site of action for many life sustaining biological processes. Transmembrane charge conduction plays an important role since it regulates some of the vital membrane-bound processes necessary for survival. It is known that in the biological membrane ions are the charge carriers and the ion transport is facilitated by the membrane embedded proteins. However, in spite of the increasingly detailed study of the transmembrane charge transport and the structure of membranes, the role of membrane lipids in this charge conduction mechanism is not yet thoroughly understood and the exact nature of charge carriers, whether it is ion or electron, is still a controversial one.

The simplest way to study transmembrane charge conduction is to use model membranes which mimic the rather complex biological membranes. Out of the several models available, the most widely used model is the artificially constituted bilayer lipid membrane (BLM) of planar configuration, because of its resemblance in many respect with the natural biological membrane.

Using planar membrane of oxidized cholesterol as the model for biological membranes, we have reviewed here our studies on the role of lipid medium in the mechanism of transmembrane charge transport. (Oxidized cholesterol has been chosen as the membrane forming material mainly due to the fact that it forms highly stable membrane and comparison of data using oxidized cholesterol to that obtained with for example, egg lecithin, shows that both yield similar results). An attempt has been made here to explain these observations in the light of semiconducting nature of lipid molecules, double electrode behaviour of the membrane and the theory of charge conduction in binary, inhomogeneous, disordered systems.

Formation of bilayer lipid membrane (BLM)

BLM is formed by brushing a saturated solution of oxidized cholesterol in n-decane on a 1.5 mm diameter hole in the teflon cup separating two bathing solutions (Fig. 1a). To monitor the formation of BLM, a beam of white light, reflected by the membrane is viewed by a microscope of power 10X. Immediately after brushing the solution over the orifice, a rainbow-like pattern is observed through the microscope, which ensures that the lipid structure is much thicker and the planar bilayer structure has not been attained. The electrical resistance at this stage is higher than that when the membrane thins down. The transition from the bulk solution phase to the bilayer structure occurs within a short time (1 - 2 min) and the membrane appears black as viewed by the reflected light as the beams reflected from the two surfaces of the BLM has a phase difference of 180° and hence destructive interference occurs at the eye piece of the microscope. At this stage the excess lipid molecules accumulated in a torus around the opening and this region, termed as Plateau-Gibbs region, supported the membrane. BLM (bilayer lipid membrane/black lipid membrane) thus formed is stable for several hours. Proper cleaning of the BLM chamber is a necessary condition for stable BLM formation.
The BLM thus formed is about 100 Å thick, the dielectric constant of the lipid milieu is approximately 2-3. When the physical properties of BLM are compared with that of an average natural membrane, it shows that except the electrical conductance and water permeability, the properties of the two membrane systems compare quite well. This is expected, as in the real membrane the presence of proteins facilitates the transmembrane water and ion transport.

**Planar lipid membrane**

Even when prepared and handled according to the best practice in the art of BLM research, these membranes being very delicate, are unstable. In order to overcome this difficulty, planar lipid membrane is reconstituted on a millipore filter. These become quite stable to bear with the asymmetry in the bathing solution and the high voltage gradient across the membrane. The cells consist of two L-shaped glass tubes (Fig. 2) on one of which a micropore glass filter (porosity - 10 μm) is mounted and this tube is inserted into the other by means of standard joints in order to prevent leakage. Saturated solution of oxidized cholesterol in n-decane is brushed on the glass filter. The increase in electrical resistance ensures the formation of planar lipid membrane on the sintered disc.

**Electrical measurements**

Two platinum electrodes, 2 cms apart from each other are placed symmetrically on either side of the BLM (Fig. 1b). For DC measurements, a constant voltage applied by means of a potentiostatic arrangement and for AC measurements a variable AC voltage source are used. The current is measured by a Keithley Electrometer. The whole experimental set up is enclosed in a Faraday cage in order to minimise the external electrical disturbances.

The membrane specific conductivity is calculated from the values of voltage across the BLM, the cur-
The corresponding voltage fluctuations measured during studies. The noise level of the electronic device used is passed through the planar lipid membrane. Data points are averaged over and used in fast Fourier transforms. The membrane bias, an average noise at steps are repeated for different currents for a particular sample. To examine it as a function of applied external agent present in the bathing solution.

**Noise measurements**

A constant current from a programmable current source is passed through the planar lipid membrane. The corresponding voltage fluctuations measured using a digital multimeter are stored in a computer at an interval of 250 ms and the data taken in sets of 1024 data points, are used in fast Fourier transforms. The resulting power spectra (512 points) are typically averaged over 10-20 sets at each current level. These steps are repeated for different currents for a particular sample. To examine it as a function of applied bias, an average noise at 0.5 Hz has been used in our studies. The noise level of the electronic devices used in our experiments has been found to be lower by at least five orders of magnitude when compared to the experimental noise spectrum.

In presence of an aqueous bathing solution, the electrical conductivity ($\sigma$) of BLM is about $5 \times 10^{-3}$ ohm cm$^{-1}$. The value of $\sigma$ depends upon the nature of external agent present in the bathing solution. It is also a function of the applied electric field, its frequency and the temperature of the bathing solution.

**Effect of different electrolytes on $\sigma$**

When an electrolyte is present in the bathing solution, $\sigma$ increases with the increase in electrolyte concentration and attains a saturation value just above 1 mM in all cases with the electrolytes having common cations ($\text{Na}_2\text{SO}_4, \text{NaCl, NaBr and NaI}$) or common anions ($\text{HCl, ZnCl}_2, \text{MgCl}_2, \text{NaCl, CaCl}_2, \text{KCl}$ and $\text{LiCl}$). This $\sigma$ value increases by three orders of magnitude when the bathing solution contains iodine or electrolytes saturated with iodine of 1 mM concentration. The charge transfer or electron donor-acceptor interaction of lipids (phospholipids and cholesterol) as electron donors with iodine as electron acceptor leads to an increase in the effective dielectric constant value which in turn is responsible for the increase in the $\sigma$. Table I shows the values of $\sigma$ at 1 mM concentration of the salts, both in the presence and in the absence of iodine.

In order to interpret the results so far obtained, we see that if the charge carriers were ions, we should have got higher $\sigma$ value in $\text{Na}_2\text{SO}_4$ solution (among the solutions with common cation) or in HCl solution (among the solutions with common anions). This is contrary to our experimental observations. Again the ratio of specific conductance of BLM in HCl and in KCl at 1 mM concentration is 0.75, whereas the standard value for the same ratio in an electrolytic solution where ions are the charge carriers, is 2.85. Thus the ionic conduction across BLM cannot be justified from our experimental results.

Table 1 also reveals that the $\sigma$ values at the same electrolyte concentration bear a good correlation with the standard electrode potential of the ions both in the presence and in the absence of iodine. This indicates generation of electrons and holes from the electrolytes at the interface as shown in Fig. 3. Under an applied electric field, the electrolyte MX dissociates to $M^+ + X^-$. When concentration of the oxidants and reductants are small and the electric field is low, the charges are deposited at the electrodes and at the membrane/water interface giving rise to the reductant (M) and oxidant (X). The regeneration of $M^+X^-$ is possible by either of the following reactions

\[ M + X \rightarrow M^+ X^- \]

or,

\[ M + H^+ + 1/2 \text{H}_2 \text{ and } 2X + 2 \text{OH}^- \rightarrow 2X^- + \text{H}_2\text{O} + 1/2 \text{O}_2 \]

only when they are very active, otherwise deposition

<table>
<thead>
<tr>
<th>Electrolytes in aqueous solution</th>
<th>Standard oxidation potential at 25°C (V)</th>
<th>Specific conductivity (ohm-cm)$^{-1}$</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Anions</td>
<td>Cations</td>
</tr>
<tr>
<td>$\text{Na}_2\text{SO}_4$</td>
<td>-2.010</td>
<td>3.85</td>
</tr>
<tr>
<td>$\text{NaCl}$</td>
<td>-1.360</td>
<td>4.31</td>
</tr>
<tr>
<td>$\text{NaBr}$</td>
<td>-1.065</td>
<td>4.75</td>
</tr>
<tr>
<td>$\text{NaI}$</td>
<td>-0.536</td>
<td>5.95</td>
</tr>
<tr>
<td>$\text{HCl}$</td>
<td>0.0</td>
<td>3.48</td>
</tr>
<tr>
<td>$\text{ZnCl}_2$</td>
<td>0.763</td>
<td>3.60</td>
</tr>
<tr>
<td>$\text{MgCl}_2$</td>
<td>2.370</td>
<td>4.25</td>
</tr>
<tr>
<td>$\text{NaCl}$</td>
<td>2.714</td>
<td>4.31</td>
</tr>
<tr>
<td>$\text{CaCl}_2$</td>
<td>2.870</td>
<td>4.43</td>
</tr>
<tr>
<td>$\text{KCl}$</td>
<td>2.925</td>
<td>4.60</td>
</tr>
<tr>
<td>$\text{LiCl}$</td>
<td>3.045</td>
<td>4.77</td>
</tr>
</tbody>
</table>
of metal on BLM and liberation of free oxidant in solution are expected.

If we consider the coupled redox reactions of BLM/electrolyte interfaces, then in that case $\sigma$ will become a function of the standard electrode potential of the ions. The semiconducting nature of BLM allows the flow of conduction electrons/holes across it and conductivity is controlled by the number of processes such as generation, movement across BLM and recombination of these carriers. So it is expected that $\sigma$ will bear a relation as follows

$$\sigma = g + k E_1^0 (E_1^0 + E_2^0) E_2^0$$  \hspace{1cm} (1)$$

where $E_1^0$ and $E_2^0$ are the standard oxidation potentials of the anions and cations respectively and $g$ and $k$ are empirical constants. When $\sigma$ values are plotted as a function of $E_1^0 (E_1^0 + E_2^0) E_2^0$, a straight line is obtained (Fig. 4), both in the presence and in the absence of iodine in the bathing solution. The values of the slope ($k$) and the intercept ($g$) are respectively $0.35 \times 10^{11}$ (ohm cm V$^{-1}$) and $4.2 \times 10^{11}$ (ohm cm)$^{-1}$ with iodine and $0.16 \times 10^{-14}$ (ohm cm V$^{-1}$) and $3.7 \times 10^{-14}$ (ohm cm)$^{-1}$ without iodine.

Thus, the correlation between the conductivity and the standard electrode potential can be explained on the basis of double electrode behaviour of BLM for generating charge carriers that are electrons and holes which then move across the semiconducting lipid milieu of BLM. The positive intercept in Fig. 4 indicates that besides the electronic conduction, there is some other mode of charge conduction present which may arise due to the presence of ions outside the BLM. The variation of $\sigma$ with respect to the electrolyte concentration before attaining the saturation value at 1 mM also supports this hypothesis. Thus the total conductivity across BLM may be considered to be a sum of the ionic conductivity due to the presence of the electrolyte outside the BLM and electronic conductivity outside the BLM and electronic conductivity due to the semiconducting nature of BLM and its double electrode behaviour. In Eqn. 1 the first term $g$ represents the contribution from the ionic conduction and the second term represents the electronic contribution to the value of $\sigma$.

**Semiconductivity of BLM**

Lipids are semiconductors in solid state. The semiconducting property of oxidized cholesterol in solid state as well as in the BLM form is known. The electrical conductivity $\sigma(T)$ of BLM increases exponentially with temperature according to the following relation

$$\sigma(T) = \sigma_0 \exp(-E_a/2kT)$$

where $E_a$ is the activation energy for the process, $k$ is Boltzmann’s constant and $\sigma_0$ is the extrapolated conductivity at infinite temperature. When the logarithm of the specific conductivity of oxidized cholesterol BLM is plotted against $T^{-1}$, straight lines are obtained when the bathing solutions are (i) aqueous medium (ii) aqueous medium containing electrolytes like KCl (iii) aqueous medium containing electron donor (acridine) (iv) electron acceptor (iodine) and (v) aqueous medium containing electron donor/acceptor and an electrolyte. From the slope of these straight lines, the values of $E_a$ have been found to be 2.02 eV in aqueous medium and 0.78 eV and 1.16 eV in presence of...
iodine and acridine respectively. The presence of an electrolyte does not change the value either in the aqueous solution or in the aqueous bathing solution saturated with iodine or acridine.

We interpret the result as follows. Because of its intrinsic semiconducting nature, the conduction through BLM is generally due to the mobility of holes and electrons\(^ {25}\). The intrinsic carrier concentration \((n_i)\) can be calculated using standard formula\(^ {26}\) from the measured value of \(E_g\) and assuming equal masses \((m)\) for electrons and holes. For aqueous bathing solution the calculated value of \(n_i\) is 115/cc. In the limiting approximation of equal mobility for electrons and holes, the calculated value of mobility is \(1.4 \times 10^{-2} \text{ cm/volt-sec}\). Addition of iodine, an electron acceptor, in the bathing solution is equivalent to introducing hole donating impurities into BLM\(^ {26}\), causing manifold rise in the conductivity. Similarly addition of an electron donor, like acridine, is equivalent to introducing electron donating impurities into the BLM system, which in turn increases the conductivity. Comparing the conductivity values in presence of these agents with that in the aqueous bathing solution, we have calculated the density of charge carriers in all these systems (Table 2).

When an electrolyte is added in the bathing solution containing an electron acceptor like iodine or an electron donor like acridine, a peculiar behaviour is observed in the variation of \(\sigma\) with the concentration of the electrolyte.

If \(R_1\) and \(R_2\) denote the electrical resistances across the platinum electrodes in presence and in absence of BLM respectively (Fig. 1c), then in the absence of any electron acceptor/donor in the bathing solution \(R_1 >> R_2\) and the resistance of the BLM can be approximated to be equal to \(R_1\). But when an electron donor/acceptor is present in the bathing solution, BLM resistance decreases by several orders of magnitude and in that case \(R_1\) is of the same order of magnitude as \(R_2\) and then BLM resistance \(R\) is equal to \((R_1 - R_2)\). From the value of \(R\) and from the dimension of BLM, \(\sigma\) the specific conductivity of BLM, can be calculated.

As mentioned earlier the initial value of \(\sigma\) in aqueous medium increases by several orders of magnitude in presence of iodine\(^ {6}\). When KCl is added in this

<table>
<thead>
<tr>
<th>Bathing solution</th>
<th>(E_g) (eV)</th>
<th>(\sigma) (ohm-cm(^{-1}))</th>
<th>Semiconducting characteristics</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>(H_2O)</td>
<td>2.02</td>
<td>4.5 \times 10^{15}</td>
<td>Intrinsic Conduction by electrons and holes: (n_i = 115/\text{cc})</td>
<td>Conduction by electrons and holes: (n_i = 115/\text{cc})</td>
</tr>
<tr>
<td>KCl (1 mM)</td>
<td>2.02</td>
<td>4.6 \times 10^{14}</td>
<td>Intrinsic Charge injection at BLM/electrolyte interface</td>
<td>Charge injection at BLM/electrolyte interface</td>
</tr>
<tr>
<td>I(_2) (1 mM)</td>
<td>0.98</td>
<td>7.0 \times 10^{11}</td>
<td>p-type Holes are majority charge carriers, (n_p = 3.6 \times 10^{6}/\text{cc})</td>
<td>Holes are majority charge carriers, (n_p = 3.6 \times 10^{6}/\text{cc})</td>
</tr>
<tr>
<td>I(_2) (1 mM) + KCl (0.2 mM)</td>
<td>0.98</td>
<td>3.7 \times 10^{11}</td>
<td>p-type Hole electron recombination reduces charge carriers</td>
<td>Hole electron recombination reduces charge carriers</td>
</tr>
<tr>
<td>I(_2) (1 mM) + KCl (1 mM)</td>
<td>0.98</td>
<td>6.8 \times 10^{11}</td>
<td>p-type Equilibrium attained</td>
<td>Equilibrium attained</td>
</tr>
<tr>
<td>Acridine (1 mM)</td>
<td>1.16</td>
<td>8.0 \times 10^{13}</td>
<td>n-type Electrons are majority charge carriers, (n_e = 4.1 \times 10^{7}/\text{cc})</td>
<td>Electrons are majority charge carriers, (n_e = 4.1 \times 10^{7}/\text{cc})</td>
</tr>
<tr>
<td>Acridine (1 mM) + KCl (0.2 mM)</td>
<td>1.16</td>
<td>1.2 \times 10^{12}</td>
<td>n-type More electrons are injected at BLM/electrolyte interface</td>
<td>More electrons are injected at BLM/electrolyte interface</td>
</tr>
<tr>
<td>Acridine (1 mM) + KCl (1 mM)</td>
<td>1.16</td>
<td>2.75 \times 10^{12}</td>
<td>n-type Equilibrium attained</td>
<td>Equilibrium attained</td>
</tr>
</tbody>
</table>
solution, then with the rise in electrolytic concentration $\sigma$ value lowers initially, attains a minimum at around 0.2 mM concentration. The $\sigma$ value rises again and reaches a saturation value for electrolyte concentration 1 mM. The same qualitative pattern is followed by all the electrolytes we have used, with slight variation in the magnitude of their values. Curves of similar nature are also obtained with other electron acceptors like picric acid and 2,4-dinitrophenol. The representative curves for variation of $\sigma$ with electrolyte concentration in presence of iodine are given for KCl, CaCl$_2$ and Na$_2$SO$_4$ (Fig. 5A).

This result can be interpreted from the semiconductivity of BLM. When an electrolyte is added in the aqueous bathing solution, the electron injection at the interface of the BLM/electrolyte will depend on (a) the standard electrode potential of the cations and anions at the interface and (b) the activation energy. For higher activation energy, electron injection will be less and vice versa. At low concentration of the electrolyte and in presence of an electron acceptor when the BLM behaves as a p-type semiconductor these injected electrons will partially combine with the holes in BLM, reducing the number of charge carriers and consequently the conductivity will decrease.

As the electrolyte concentration increases, more and more electrons pass through the BLM and the conductivity increases till the saturation value is reached. The situation is different when an electron donor (acridine, bipyridyl) is present in the bathing solution. Here the intrinsic semiconducting property of the BLM changes to an n-type one and consequently with increase in the electrolyte concentration more and more electrons are available and the conductivity increases until it reaches a saturation value (Fig. 5 B). Hence the electronic conduction through BLM, which behaves as a double electrode, explains the qualitative nature of the conductivity-concentration curve in presence of an electron donor/acceptor in the bathing solution.

A closer look at Table 2 reveals that the addition of electron donor or acceptor in the bathing solution changes the activation energy whereas the addition of electrolytes do not. That would not have been the case if ions were the majority charge carriers through BLM.

**Nonlinear dc electrical response in BLM**

An interesting aspect of the electrical behaviour of a BLM in an aqueous bathing solution is the transi-
tion from a linear to a nonlinear domain above a certain critical field value. It was suggested by earlier workers that the nonlinearity is caused by the transport of charges through pores, which are formed above a certain critical field strength as a result of inversion of the spontaneously arising hydrophobic pores in the lipid moiety. The critical field at which the structural change occurs is a function of the pH and ionic concentrations of the bathing solution and hence the major characteristics of the transition from linear to nonlinear electrical responses of the BLM are probably monitored by the outer surface of the membrane.

In order to verify whether nonlinearity arises solely due to ion transport through hydrophobic pores, we have studied the I-V characteristics in thin films of lipids. In spite of the absence of any ion source in this case, the I-V curve shows a similar deviation from linearity above a certain field. The ohmic nature, however, is recovered with the increase in frequency of an applied AC field. This study indicated that besides pore formation, some other factors were also responsible for the observed nonlinearity.

The two most important factors that might contribute to the nonlinearity in the I-V characteristics are: the intrinsic semiconducting property of BLM and the nature of bathing solution. These two are interrelated as we have noted earlier that the intrinsic semiconducting nature of lipid molecules changes to a p- or n-type semiconductor in presence of an electron acceptor or donor in the bathing solution. Let us now investigate the nature of the I-V curves in presence of an electron donor and electron acceptor.

In Fig. 6 we have plotted the current I across BLM against the applied voltage V in presence of iodine, an electron acceptor (Fig. 6A) and acridine, an electron donor (Fig. 6B) in the bathing solution. The I-V characteristic curves show deviation from linearity above a certain field in both the cases. However, there is a distinct difference between the two in the region where the transition from linear to nonlinear electrical response takes place. It seems that this distinction arises due to the fact that in presence of iodine or acridine, the intrinsic semiconducting nature of BLM changes to an extrinsic one. At low voltage, the current increases with increase in voltage due to the semiconducting property of BLM. When voltage reaches a critical value, ions from the bathing solution move towards BLM and due to the double electrode behaviour of BLM, electron injection at the BLM/water interface takes place. These electrons tend to recombine with the holes as BLM becomes p-type semiconductor in presence of iodine in the bathing solution. When the effect of recombination becomes significant, the current reaches a low value and with further increase in voltage the current increases further. The situation is different when acridine, an electron donor, is present in the bathing solution. Here the majority charge carrier is electron and so when more electrons are injected at the interface, the current keeps on increasing with the increase in voltage. The I-V characteristics of BLM in aqueous bathing solution is of the same nature as that in presence of acridine and similar argument follows here too.

Now besides electrons, ions can also transport across the BLM through pores that are formed due to thermal fluctuations in the bilayer structure producing transient defects. These hydrophilic pores along with the nonconducting lipid molecules form a binary mixture. To explain the charge transport phenomena
through a binary mixture, Gefen et al.\textsuperscript{33,34} have put forward a model, called the dynamic random resistor network (DRRN) model. Our preliminary investigation shows that this model can be applied to describe the trans-BLM charge transport. The thermally created water filled pores are the hydrophilic pathways which in the otherwise nonconducting lipid moiety, are randomly oriented. The onset of nonlinearity is triggered at higher field values when these microscopic components aggregate to form pores. The size, frequency and conductivity of these pores are nonlinear functions of voltage and open up the ionic pathways.

Thus from our experimental observations with BLM systems formed in aqueous bathing solutions as well as in the presence of electron donors and acceptors, we may conclude that the possible factors which contribute to the nonlinear electrical response in these systems are: (i) pore formation in the hydrated state of lipid aggregates. The number, size and conductivity of these pores are nonlinear functions of applied voltage\textsuperscript{27,30,31}. The repulsive action among the ions moving through the pores attenuate the ion current. The attenuation factor, which is a measure of the internistic interaction is a function of ion conduction, dielectric constant and pore geometry\textsuperscript{5}. (ii) the characteristic semiconducting property of the lipid molecules. This property monitors the finer details of the concentration of the bathing solution, as the critical field value is a function of these parameters\textsuperscript{28}.

**Flicker noise study in planar lipid membrane**

As nonlinear I-V characteristics were obtained in thin lipid films\textsuperscript{5} as well as in planar bilayer membrane\textsuperscript{10}, we came to the conclusion that nonlinearity is not the only criterion to infer the formation of pores and thus the exact mechanism of charge conduction in lipid membrane remained a debatable one.

Lipid membrane is an inhomogeneous, disordered system and nonlinear charge conduction is a common feature of these systems\textsuperscript{57,58}. Since the noise power depends on the spatial current distribution throughout the system, a comprehensive view of the microstructure and hence current distribution in the inhomogeneous system can be obtained from the noise spectrum\textsuperscript{30}. With this idea we have carried out noise spectrum measurements in planar lipid membrane and compared that with the noise spectra obtained in different composite/one component systems\textsuperscript{38,11} where the mechanism of charge conduction is well established.

A modified Hooge's empirical formula for noise spectral density (\(S_f\)) as a function of frequency (f), voltage (V) and chordal resistance (R= V/I) is given by\textsuperscript{38}:

\[ S_f = R(V) V f^{\lambda} \]  

The functional dependence of \(R\) is determined by the microstructure of the system and \(\lambda\) depends upon the conduction mechanism.

The noise spectrum in BLM shows a basic 1/f feature (\(\lambda = 1\); flicker noise). In Fig. 7 the noise power at 0.5 Hz (solid symbol) and chordal resistance (R= V/I, open symbol) have been plotted as a function of voltage. The slope of the noise data changes from 1.97 to 1.48 at the onset of nonlinearity where \(R\) also deviates rapidly from its linear value, suggesting a strong correlation between the noise power and the nonlinear conductance and it is actually the conduction mechanism that determines the nature of these curves. Another interesting observation is that while the resistance decreases in the nonlinear regime by a factor of 2, the noise increases by three orders of magnitude, making noise measurement a more sensitive tool for detection of any change in the conduction mechanism.

Working with thin solid films of lipid, we obtained similar results, viz., noise power had an 1/f dependence and the slope of the \(S_f-V\) plot changed at the onset of nonlinearity\textsuperscript{11}. However, there the slope was more in the nonlinear region compared to that in the linear regime (1.78 and 1.07 respectively) which is just the opposite of the result obtained with the planar membranes indicating that there is a difference in the charge conduction mechanism in these two systems.

Nonlinear conduction at high field may arise either due to a change in the existing charge conduction mechanism or due to some other new charge conduction channels being created at high field allowing more charge carriers to pass through the system. We see that nonlinearity in conduction in the case of lipid membrane is accompanied by a decrease in resistance as well as in relative noise. Using a simple resistive circuit, Nandi et al.\textsuperscript{38} have shown that this occurs when parallel resistances are added to the circuit. Once again this result can be explained from Gefen's DRRN model\textsuperscript{33,34}. Here the thermally created water filled pores are the randomly placed conducting ele-
NANDY: ROLE OF PLANAR LIPID STRUCTURE IN TRANSMEMBRANE CHARGE CONDUCTION

...ments. The onset of nonlinearity is triggered when at high field the lipid molecules start orienting to provide more ionic pathways parallel to the existing ones. This reduces the resistance and decreases the relative noise.

When we compare our noise spectral measurement with those obtained by Nandi et al. with a composite system like carbon-wax and disordered but one component systems like conducting polymer and V$_2$O$_4$, we notice that the characteristic nature of the noise spectrum for planar membrane is similar to that obtained in the case of carbon-wax mixture, where charge conduction mechanism has been satisfactorily explained by applying Gefen's DRRN model.

**Nonlinear AC response in BLM**

Upon application of an AC field across the BLM, the conductivity increases, while the nonlinear nature of the curve is retained at all frequencies upto 5000 Hz (unpublished data, Fig. 8). This behavior is similar to that observed in case of thin lipid films and in carbon-wax mixture. This result can be interpreted by treating the BLM system as a random binary mixture of pure resistors and capacitors, forming an "impedance surface" where the impedance is a function of frequency and amplitude. At low frequency the current path is purely resistive, which manifests nonlinearity. As the frequency increases, the conduction through the capacitor becomes comparable to that through the resistive backbone and the onset of nonlinearity steadily shifts to higher voltages. On further increase of frequency it is expected that the conductance will become purely capacitative and the nonlinear nature of the I-V characteristics will disappear. The rupture of BLM at higher frequency and higher voltages prevents us from continuing this experiment further.

**Effect of temperature on the onset of nonlinearity in BLM**

The nonlinearity in the I-V characteristics in BLM has been explained by considering formation of pores in the BLM and the charge conduction mechanism has been interpreted by means of Gefen's DRRN model.

A more definite understanding of charge conduction can be had by studying the system at different conducting states. In composite systems different conductivity states can be attained either by changing...
the fraction of conducting component or by changing the temperature. There are technical limitations to alter the fraction of conducting components in the BLM system, but the temperature can be changed.

The I-V characteristics for BLM of oxidized cholesterol are in general nonlinear for all values of T with the degree of nonlinearity increasing as T decreases (unpublished data, Fig. 9). When I and V are normalised by their respective values at the onset of nonlinearity, \( I_c \) and \( V_c \), the \( \frac{I}{I_c} \) vs \( \frac{V}{V_c} \) curves at different temperatures collapse into a single curve, emphasizing the universality in their nature. A log-log plot of \( I_c \) against \( \sigma_0 \) (linear conductance) is a straight line (unpublished data, Fig. 10) which shows that \( I_c \) scales with \( \sigma_0 \) as,

\[ I_c \propto \sigma_0^x, \quad x = 1.56 \]

This equation gives a very basic description of linear to nonlinear cross-over phenomena in all disordered systems\(^4\). Comparison of our result with those obtained in other disordered systems shows that \( x \) has different value in different systems, e.g. \( x \approx 1.47 \) in 2 dimensional gold films\(^{33} \), 1.41 in the three dimensional composites of carbon-wax\(^{40} \) and 0.67 in conducting polymer system\(^{41} \).

Judging from our experimental observations we suggest that the charge conduction mechanism in planar lipid membrane can be explained from the DRRN model of Gefen and propose the following: (i) due to thermal fluctuations, lipid molecules orient themselves to form hydrophilic pathways, which are oriented at random; (ii) with increasing field intensity, these pores get oriented in the direction of the electric field and provide pathways for ion transport; number and size of the pores increase with the field strength, leading to nonlinear conductance; (iii) with increase in temperature, the increased thermal fluctuation helps to orient the lipid molecules. Hence conductivity increases with increase in temperature; (iv) \( V_c \) shifts to higher field values with increase in temperature. This is a characteristic feature of the disordered systems for which no suitable explanation is available in the literature\(^{41} \).

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**Fig. 9**—Nonlinear I-V characteristics of oxidized cholesterol in the bathing solution of saturated iodine with temperature. (With increase in temperature, nonlinearity decreases and \( V_c \), the value of voltage at the onset of nonlinearity shifts to higher value. (Unpublished data.))

**Fig. 10**—Log-log plot of \( I_c \) (value of current at the onset of nonlinearity) against \( \sigma_0 \) (linear conductance). [The straight line indicates a power law fit with exponent \( x = 1.56 \). (Unpublished data.)]
Transmembrane charge conduction and role of lipid molecules in it is a subject of great research interest. It is known that there is a large internal dipole potential associated with membrane, but this cannot be measured by placing electrodes across the membrane, because of the symmetry of the dipole layers. This implies that the field induced transmembrane charge transport may not get affected by this dipole potential. However, it has been observed that for the potential energy barrier associated with membrane, but this cannot be explained by an integrated metric distribution of charges across it. This has been explained by an integrated Nernst-Planck equation with trapezoidal energy barrier. The experimental steady state current-voltage relation for the transmembrane fluxes across a membrane have nonlinear dependence on the membrane potential (ψm), created by an asymmetric distribution of charges across it. The consideration of double electrode behaviour of BLM explains the source of charge carriers at the membrane - water interface. Another approach to explain the nonlinearity in the I-V characteristics of lipids, both in the form of film as well as BLM is to consider their semiconducting properties. The fact that lipid membrane (film or BLM) is an inhomogeneous, disordered system allows one to look at this problem from another viewpoint.

Our results show that whatever be the exact mechanism, the planar lipid bilayer skeleton of the biological membrane does take active part in the charge transport process. The study of the functional dependence of the electrical conductivity of these membranes on external agents present in the aqueous bathing solution throws light on the behaviour of the biological membrane in varied environment. The nonlinear electrical response in this model system has parallel in the case of charge conduction in synaptic membranes. Our results also indicate that both electrons and ions participate in the charge conduction across membranes and by approximating the hydrated lipid membrane as a binary mixture of lipids and hydrophilic pores, we have satisfactorily explained the results of flicker noise measurements in this system. The conclusion drawn has been further verified by studying this system at varying conductivity states at different temperatures. Our findings can be extrapolated with proper understanding of the biological systems to realise the charge conduction mechanism in the wider and more complex field of natural membranes.

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