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Review Article

LECITHIN- CHOLESTEROL BIO- MEMBRANES AND THEIR CORRELATION WITH LIQUID MEMBRANES

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ABSTRACT

Permeation through bio-membranes is a scenario which may give a clue to the mechanism of action of a variety of moieties as also drugs. Phospholipid bilayers form the cell membranes of animal tissues. Lecithin is a similar substance. Plasma membranes permit the entry of a variely of media through them by diverse mechanisms. Drugs, which are surfactant in nature have been shown to accumulate about the biomembranes and might modify the entry of neurotransmitters and drugs through them. These liquid membranes of drugs are somewhat similar to Lecithin and Lecithin- Cholesterol membranes with respect to their hydraulic permeability, electro-osmotic velocity streaming potential and electric current as has been demonstrated. This similarity with regard to permeation might open new vistas in the mode of action of a variety of drugs.

Keywords: Solute permeability, Liquid membranes, Lecithin, Cholesterol, Hydraulic permeability, Critical micelle concentration, Ionic fluxes

INTRODUCTION

Cholesterol, an important constituent of bio-membranes, though very slightly soluble, lowers the surface tension of water, considerably (1-4). The maximum solubility [5, 6] is 4.7µM in water and the measured surface tension is 33 dyne/cm at 25°C. Critical micelle concentration (CMC) is in the range [7,8] of 25-40 µM. Thus Cholesterol is a very effective surfactant capable of generating a liquid membrane at the interface in accordance with Kesting's liquid membrane hypothesis, and this has been established by the data on hydraulic permeability, electro-osmotic velocity and streaming current [9] The existence of liquid membrane has also been demonstrated (1-9) experimentally .

In all these experiments [1-9], the aqueous solutions of Cholesterol were obtained by dissolving the required amount of ethanol and adding this to water with constant stirring [10]. The stirring was continued, always for more than 120 hours. The CMC of aqueous cholesterol, was determined from the variation of surface tension with concentration, and in all such cases the concentration of ethanol was never allowed to exceed 0.1% by volume because it does not affect the surface tension of water to any measurable extent [10].

STUDIES ON LIQUID MEMBRANES

In all these [1-9] studies the all-glass cell as shown in fig.1 was used .A Sartorius cellulose acetate micro filtration membrane (average pore size, 0.2 µM of thickness, $1x10^{-4}$ m and area, $2.55x10^{-5}m^2$) in fact acted as a support for the liquid membrane dividing the transport cell into two compartments C and D. The two compartments of the transport cell were filled with the solutions of desired concentrations (1-6).

The concentration range from 0 to 56.4nM was chosen for the measurement of hydraulic permeability, electro-osmotic velocity, streaming potential and current in order to obtain data on both the lower and the higher side of the CMC of cholesterol (11). For measurements of hydraulic permeability the electrodes E_1 and E_2 were short-circuited and the volume flow consequent to the various pressure difference (Δp) applied across the membrane, was noted in the capillary L_1 L_2. For electro osmotic velocity measurements, the condition of no net volume flux, ΔP =0 was imposed on the system and the volume flow, induced by the electrical potential differences across the membrane, was noted. For measurements of streaming potentials/current, known pressure differences were applied across the membrane, when flow in the capillary, L_1L_2 became steady. The electrodes E_1 and E_2 were joined with measuring devices (12-15).

For transport data [table-1], for various concentrations of cholesterol reported [3], the straight line plots (Fig-2) signifies the linear equations for hydraulic permeability, electro-osmotic velocity, streaming potential and current. The values of the various phenomenological coefficients, viz., L_{11}, L_{12}, L_{21} and L_{22} at various concentrations of cholesterol, estimated from the slopes of the straight lines in Fig.2 validate, of Onsager's equality, viz. $L_{12}=L_{21}$, for all concentrations of cholesterol.

From observation of hydraulic permeability data and by the variations of the coefficient L_{11} with cholesterol concentration, plotted in Fig.2, it is obvious that as concentration of cholesterol increased, the resistance to volume flow also increased in a progressive manner and it was maximum when the concentration of cholesterol equaled its CMC beyond which it became more or less constant. [11-16]

As concentration of the surfactant increased, the supporting membrane- the cellulose acetate micro filtration membrane in this case, got progressively covered with the surfactant layer liquid membrane; at the CMC, was completely covered and when the concentration of the surfactant increased beyond the CMC almost all of the added surfactant went into the bulk of the solution in the form of micelles, and did not go to the interface. This was why the resistance to flow did not increase beyond the CMC of the surfactant. Analysis of the transport data in light of the mosaic membranes [12-14] furnishes further evidence in favor of the liquid membranes formation. The value of the coefficient L_{11} for half the CMC of cholesterol has been computed on the basis of the mosaic model, and comes out to be $(3.14\pm0.03)x10^{-8}$ m^3 N^{-1} s^{-1} , which compares favorably with the experimentally determined value (table-3). Similar considerations apply to other phenomenological coefficients as well.

CHOLESTEROL MEMBRANES

The transport data obtained in the case when both compartments C and D of the transport cell were filled with the cholesterol solution of concentration equal to its CMC, were utilized [3] to demonstrate the formation of bilayers of the cholesterol liquid membrane. Since at the CMC, the supporting membrane in this case got completely covered with the liquid membrane, the supporting membrane in this case would be sandwiched between the two layers of the liquid membrane generated, one on either side of it. In dealing with a situation like this it is more convenient to express it in the form of equations [1] between thermodynamic forces, X and fluxes, J, i.e.

$$X_i = \Sigma R_{jk} . J_k \quad (1)$$

Where the resistance coefficients, R_{ik} are related to the coefficients, L_{ik} by

$$R_{11} = (L_{22}/|L|),\ R_{12} = (-L_{12}/|L|),\ R_{21} = (-L_{21}/|L|),\ R_{22} = (L_{11}/|L|) \quad (2)$$

In Eq. (2),

$$|L| = L_{11}L_{22} - L_{12}L_{21} \quad (3)$$

Utilising Kedem and Katchalsky's theory [16,17,18] for permeability of composite membranes, one can write the following relationship among the resistance coefficients, R_{ik} for the series composite membrane of the supporting membrane and the corresponding resistance coefficients for the constituent membrane elements,

$$\mathbf{R^*_{ik} = R^s_{ik} + 2R^l_{ik}} \quad (4)$$

The superscripts s and 1 stand for the supporting membrane and the liquid membrane, respectively. Similarly, for the situation when one of the compartments of the transport cell , the compartment C, was filled with cholesterol solution of concentration equal to its CMC, and the other compartment, the compartment D, was filled with water, one can write,

$$\mathbf{R^t_{ik} = R^s_{ik} + R^l_{ik}} \quad (5)$$

Where the superscript t stands for the series composite membrane consisting of the supporting membrane and the cholesterol liquid membrane in series array. Using Eq. (5), Eq. (4) can be rewritten as.

$$\mathbf{R^*_{ik} = 2\,R^t_{ik} - R^*_{ik}} \quad (6)$$

The values of R^t_{ik} and R^s_{ik} can be computed from experimentally determined value of coefficients L_{ik} (table 1). Values of the various resistance coefficients R_{ik}^* match with the experimental values (table 3), lending support to the existence of the liquid membrane bilayers- one layer of the liquid membrane on either side of the supporting membrane (14-16).

LECITHIN- CHOLESTEROL BIOMEMBRANES

Similar experiments have been conducted [4-8] on lecithin and lecithin-cholesterol mixtures to demonstrate the formation of liquid membranes and bilayers of liquid membranes by them. In all these studies the same transport cell, has been used and a Sartorius cellulose acetate micro filtration membrane/aqueous interface has been used as site for the formation of liquid membrane. The data on hydraulic permeability have been exploited to demonstrate the formation of liquid membranes and bilayers of liquid membranes. The CMC value for aqueous lecithin was found to be 12.951 ppm. For measurement of hydraulic permeabilities, the two compartments of the transport cell (Fig.1) were filled with the aqueous solutions of lecithin or cholesterol and lecithin cholesterol mixtures of desired composition. The aqueous solution of lecithin, cholesterol and their mixtures were prepared using the method described by Gershfeld and Pagano [10]. The concentration ranges chosen for hydraulic permeability data in the case of lecithin, were such that the data are obtained on both the lower and the higher side of the CMC of lecithin. During the hydraulic permeability measurements of the solution in the compartment C, it was kept well stirred, and the electrodes E_1 and E_2 were short-circuited [11-16].

The hydraulic permeability data [4] for various concentrations of lecithin and for lecithin-cholesterol mixtures of various compositions is reproduced in table -2 and Fig- 2. In all the cases, the proportional relationship, $J_v = L_{11}\Delta P$, where L_{11} is the hydraulic conductivity coefficient, is obeyed. The values of the coefficients L_{11} for various concentrations of lecithin and for lecithin-cholesterol mixtures of various composition estimated from the slopes of the curves in Fig.2 are recorded in Table 4 and Table 2. The data in Table 2 are for the solutions of various concentrations of cholesterol, prepared in 15.542 ppm solution of lecithin. The trend in the values of L_{11} at various concentrations in the case of lecithin is similar to that observed in the case of cholesterol, i.e. in accordance with the liquid membrane hypothesis [20-23] and indicates complete formations of the liquid membrane in series with the supporting membrane when concentration of lecithin equals its CMC.

The values of L_{11} at several concentrations of lecithin below its CMC, were computed using mosaic membrane model [20-23] which is in agreement with the corresponding experimentally determined values (table-4). This furnishes additional support in favor of liquid membrane formation.

The values of L_{11} when both the compartments of the transport cell (fig-1) were filled with the lecithin solutions of concentration equal to its CMC, were utilized to demonstrate the formation of bilayers of lecithin liquid membranes. Utilizing Kedem and Katchalsky's theory [16,17,18] for the permeability of composite membranes and following the arguments given in the case of cholesterol, Eqs. (4) to (6), one can write for such case,

$$\mathbf{(I/L^*_{11}=(2/L^t_{11})-(1/L^s_{11})} \quad (7)$$

The value of L_{11} decreased regularly with increase in concentration of lecithin, and became constant when the concentration of lecithin equaled or exceeded its CMC, [table 4] An examination of the values recorded in table 2 reveals that when cholesterol was added to a solution of lecithin of concentration equal to or greater than its CMC 15.542 ppm, in the present case, the value of L_{11} decreased further and went on decreasing with the increasing concentration of cholesterol holding the concentration of lecithin constant at 15.542 ppm. The decreasing trend in the values of L_{11} continued up to when the cholesterol. concentration equalled to 1.175×10^{-6} M and, thereafter, it again became constant [11-15]. An obvious implication of this observation is that the increase in the resistance to water flow was due to incorporation of added cholesterol in the lecithin liquid membrane which already existed at the interface. At cholesterol concentration equal to 1.175×10^{-6} M, the lecithin liquid membrane was saturated with cholesterol. The decreasing trend in the values of L_{11} with increasing concentration of cholesterol was consistent with the results obtained on phospholipid-cholesterol bimolecular lipid membrane (BLM) [20,21]. There also water permeability has been found to decrease with the increase in concentration of cholesterol, and has been attributed to the fact that cholesterol strengthens the hydrophobic core and increases its viscosity. In order to assess whether the added cholesterol reached straight up to the interface or not, surface tensions of solutions of various concentration of cholesterol prepared in 15.152 ppm aqueous solution of lecithin were measured. Surface tensions of all such solutions were found to be equal to the surface tension of 12.951 ppm solution of lecithin. This was in keeping with literature reports that in mixed phospholipid-cholesterol films, cholesterol molecules occupy the cavities in lecithin monolayer caused by the thermal motions [20,21].

The value of L_{11} for the case when both the compartments, C and D of the transport cell, were filled with a solution of lecithin-cholesterol mixture, was 15.152 ppm with respect to lecithin and 1.175×10^{-6} M with respect to cholesterol and was utilized to demonstrate the existence of bilayers of the liquid membrane generated by the lecithin-cholesterol mixture. By the same arguments as given in the case of lecithin, the supporting membrane in this case also was sandwiched between the two liquid membranes generated on either side of it by the lecithin-cholesterol mixtures. The values of hydraulic permeability coefficient L^*_{11} in this case also were computed. However, in this case L_{11} represented the value of L_{11} for the case when compartment C of the transport cell was filled with the solution which was 15.542 ppm with respect to lecithin and 1.175×10^{-6} M with respect to cholesterol, i.e., the concentration at which the supporting membrane was completely covered with the mixed liquid membrane and the compartment D was filled with water. The value of L_{11} thus computed, comes out to be equal to $(0.871+0.09) \times 10^{-8}$ m^3 N^{-1} s^{-1} which agrees with the experimentally determined value lending support to the formation of bilayers of liquid membranes generated by the lecithin-cholesterol mixture.

Because of the surface active nature of lecithin and cholesterol, it is natural to expect that in the liquid membrane bilayers generated by lecithin, cholesterol and the lecithin-cholesterol mixture, the hydrophobic supporting membrane, i.e., the cellulose acetate micro filtration membrane in these experiment [2,4] and their hydrophilic ends will be drawn outwards away from the supporting membrane. The values of electrical resistance of freely formed [20,21] BLMs, in general, are very high [22,23], much higher than those reported for biomembranes [24]. Also the rate of ionic diffusion through BLMs was much slower [25,26] than through biomembrane [27,28]. This was ascribed [29] to a tight molecular arrangement in BLMs in contrast to biomembrane where the lipid bilayers generated in these experiments are expected to have more fluidity and, hence are expected to be closer to the state of lipid bilayers in biomembranes.

For solute permeability (ω) measurements of sodium, potassium and calcium ions, the compartment C of the transport cell was filled with a solution of known concentration of sodium. potassium or calcium chlorides prepared in aqueous solutions of known concentrations of the liquid membrane generating substances, viz., lecithin, cholesterol or the lecithin-cholesterol mixture and the compartment D was filled only with the solutions of the liquid membrane generating substances. The concentrations of liquid membrane generating solutions were such at which a complete liquid membrane was expected to be formed in series with the supporting membrane [30-33]. The concentrations of lecithin and cholesterol mixture was 15.542 ppm i.e. 1.175×10^{-6} M with respect to cholesterol. In control experiments no liquid membrane generating substances were used. To estimate the value of the solute flux J_s the condition, J=0, i.e. no net volume flux was imposed on the system and the contents of the compartments C and D were analysed for the cation concentration after a known period of time which was of the order of 6 to 8 hours. The amount of the permeant gained by the compartment D divided by the time and the area of the membrane, gave the value of the solute flux (J_s). Knowing the values of J_s , the value of ω were estimated. The value of osmotic pressure difference (Δл) was the average of the values of (Δл) at the beginning of the experiment (t=0) and at the end of the experiment. During the measurements of ω, the solutions in the compartment C were kept well stirred.

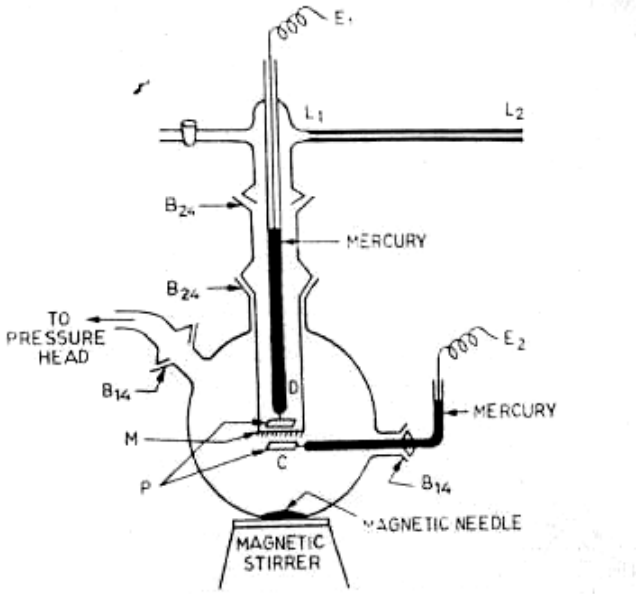

Fig. 1: The transport cell: M-supporting membrane. P-bright platinum electrodes, E_1 E_2-electrode terminals, L_1L_2-capability.

Table 1: Values of the phenomenological coefficients L_{ik} at various cholesterol concentrations

Cholesterol conc. (nM)	L_{11}x 10^8(m^3 s-1 N^{-1})	L_{12} x 10^6(mAJ^1)	L_{21}x10^6(mAJ^1)	L_{22}x10^6(ohm^{-1} m^{-2})
0.00	4.17±0.08	5.36±0.03	5.36±0.12	2.62±0.17
9.40	3.43±0.03	4.65±0.05	4.67±0.03	2.31±0.13
15.04	3.14±0.02	3.99±0.03	3.99±0.06	2.07±0.11
28.20	2.22±0.03	2.83±0.02	2.85±0.04	1.78±0.08
30.08*	2.11±0.04	2.62±0.04	2.60±0.04	1.63±0.07
37.60	2.13±0.03	2.63±0.04	2.62±0.04	1.57±0.06
56.40	2.i4±0.01	2.61±0.02	2.59±0.01	1.51±0.06
C **	1 .34±0.04	1.70±0.03	1.67±0.04	1 .26±0.04

CMC ** Values of L_{ik} for the system both the compartments, C and D were filled with cholesterol solution of concentration equal to its CMC

Table 2: Values of L_{11} at various concentrations of cholesterol, lecithin-cholesterol mixtures; lecithin concentration kept constant at 15.542 ppm (>CMC).

$C_c^*X10^6M$	0.0	0.235	0.470	0.705	1.175	1.645	2.350	C[a]
$L_{11}X\ 10^8$	2.304	2.152	2.033	1.885	1.741	1.755	1.723	0.925
($m^3s^{-1}N^{-1}$)	±0.077	±0.032	±0.044	±0.068	±0.092	±0.087	±0.057	±0.101

[a]Values for the systems when both the compartments C and D were filled with the lecithin-cholesterol mixture of the composition, 15.542 ppm with respect to lecithin and 1.750×10^{-6} M with respect to cholesterol. *[e]C cholesterol concentration.

Table 3: Values of the resistance coefficients for the case when both the compartments of the transport cell were filled with cholesterol solution of concentration equal to its CMC.

	Computed values using Eq. (6)	Experimental Values
R^*_n x $10^{-7}/m^{-3}$ N s	7.10 ±0.16	7.47±0.23
- R^*_{12} x 10^{-2} m^{-1} A^{-1} J	10.33±0.31	9.79±0.42
- R^*_{12} x 10^{-2} m^{-1} A^{-1} J	10.23±0.40	9.62±0.36
R^*22 / ohm m^{-2}	8.41±0.26	7.92±0.25

Table 4: Value of $L_{11}/m^3s^{-1}N^{-1}$, at various concentrations of lecithin .

C_L/ppm	0.0	1.2951	3.238	6.475	9.714	12.951[a]	32.381	38.853	C[b]
	3.721	3.457	3.222	2.958	2.631	2.304	2.281	2.327	1.681
$L^c_{11}x10^8$	±0.077	±0.094	±0.089	±0.061	±0.072	±0.077	±0.040	±0.018	±0.036
	-	3.580	3.367	3.012	2.65	-	-	-	1.669
$L^c_{11}x10^8$	-	±0.076	±0.077	±0.077	±0.078	-	-	-	±0.077c

[a]CMC, [b]Values for the system when both the compartments, C and D were filled with lecithin solution of concentration equal to its CMC. [c]Experimental Values. [d]Calculated values using the mosaic model. [c] Calculated value. *[CL] Lecithin

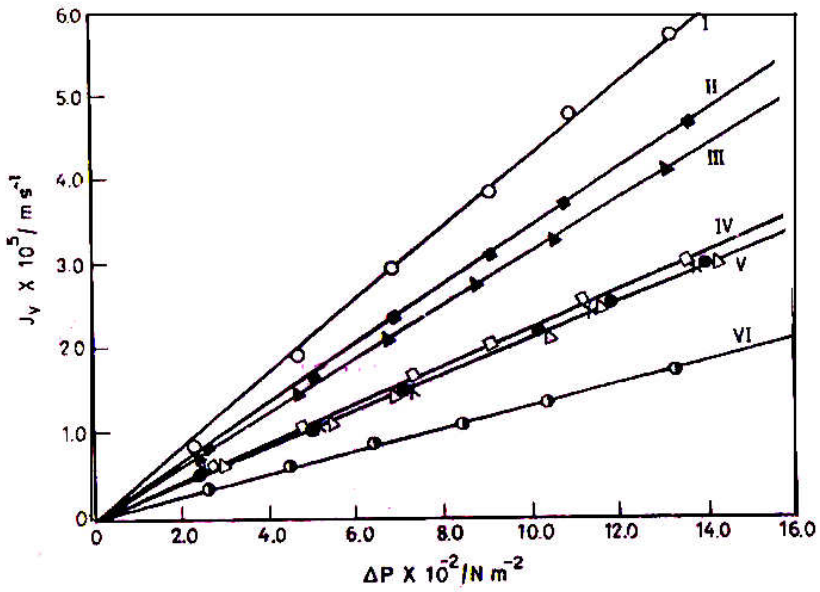

Fig. 2: Hydraulic-permeability data. Curves 1-V are for the case when compartment C was filled with cholesterol solutions and compartment D with water. Cholesterol concentration: (O)0, (■9.4. (▲)15.04, (□)28.2, (Δ)30.08. (●)37.6 and (x)56.4 nM. Curve VI is for the case when both compartments were filed with cholesterol solution of concentration equal to its CMC.

SUMMARY & CONCLUSION

Experimentations so far conducted, as also in this communiqué, demonstrate that lecithin, cholesterol, and lecithin-cholesterol liquid membranes formed by drugs which are surfactant in nature share a common platform in the biological systems, as also in in-vitro experimentation. Moreover the permeability and transport of ions, molecules, neurotransmitters through these in biological systems is worth studying and might unravel, mechanisms behind the action of such drugs.

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