Academic Sciences International Journal of Pharmacy and Pharmaceutical Sciences

ISSN- 0975-1491 Vol 5, Suppl 1, 2013

Research Article

LIQUID MEMBRANE PHENOMENON IN THE BIOLOGICAL ACTION OF VENLAFAXINE

P.K. MISHRA RAHUL[1*] AND S.S. PANCHOLI[2]

[1]Professor and Head, Department of Phamacology, Rajiv Academy for Pharmacy, P.O. Chhatikara, Delhi-Mathura National Highway # 2, Mathura, UP, India. [2]Principal, Babaria Institute of Pharmacy, Vadodara-Mumbai NH#8, Varnama, Vadodara, Gujarat, India.
*** Email: clares73@gmail.com**

Received: 31 Dec 2012, Revised and Accepted: 11 Feb 2013

ABSTRACT

The role of surface activity has been studied in the mechanism of action of venlafaxine. Venlafaxine (vnf) has been shown to generate liquid membranes in series with a supporting membrane by virtue of it's amphiphilicity. Transport of important biogenic amines (adrenaline, nor-adrenaline, dopamine and 5-hydroxytryptamine) and ions (potassium, sodium and calcium) have been studied in the presence of liquid membranes generated by surface-active venlafaxine. The data on the modifications in the permeability of important biogenic amines and ions indicates that the liquid membranes generated by vnf may play a significant role in the mechanism of it's anti depressant action. The surface-active nature of the drug has been discussed with relevance to it's pharmacological effects. The present study includes work on venlafaxine.

Keywords: Venlafaxine, Liquid membranes, Surface tension, Solute permeability, Hydraulic permeability, Volume flux.

INTRODUCTION

Many pharmacologically active compounds are amiphiphillic or hydrophobic molecules, which may undergo different types of association, and whose site of action is frequently, the plasma membrane. In many cases, excellent correlation between surface activity of drugs and their biological actions has been demonstrated[1-4] . Amphiphillic compounds contain an ionic (zwitterionic, anionic or cationic) or non-ionic polar head group and a hydrophobic portion. In aqueous medium, they are able to organize themselves as micelles, bilayers, monolayer, and hexagonal or cubic phases.

Investigations on a wide variety of surface active drugs belonging to different pharmacological categories have revealed that liquid membranes likely to be generated by the drugs either by themselves or in association with membrane lipids at their respective sites of action may modify access of relevant permeants to these sites[1-3]. This modification in the access of relevant permeants to the respective sites of action due to the presence of the liquid membrane barrier, though passive in nature, is likely to make a significant contribution to biological actions of such drugs. A consolidated account of this point of view and its implications substantiated in a good number of cases,—some of the recent examples being the studies on prostaglandins, vitamins, anticancer drugs and ant-antiarrhythmic drugs has been presented[4-9].

In an earlier study, the role of liquid membrane phenomenon in the anti-anxiety action of diazepam had been investigated[7]. Since benzodiazepines in addition to anxiolytic action are also known to exert myorelaxant and anticonvulsant actions, involving multiplicity of neurotransmitter systems including caiecholamines, serotonin, gamma-aminobutyric acid (GABA) and glycine, a more detailed study with the novel anti-depressent agent by the name of venlafaxine (vnf) is described in this communication. The present study has been conducted on venlafaxine. Data on hydraulic permeability have been obtained to demonstrate the formation of liquid membranes by vnf in series with a supporting membrane and also the incorporation of this drug into the liquid membranes generated by the lecithin-cholesterol mixtures. Transport of the relevant permeants, viz. noradrcnaline, dopamine and serotonin, through the liquid membranes generated by the lecithin-cholesterol-vnf mixtures has been studied and the data obtained have been utilized to throw light on the role of liquid membrane phenomenon in the biological actions of vnf. In the present studies, a non-living supporting membrane has been deliberately chosen so that the possibility of active interaction with the constituents of biomembranes is totally ruled out and the role of passive transport through the liquid membranes in the biological actions of vnf is highlighted.

MATERIALS AND METHODS

Venlafaxine (Sigma Aldrich) Lecithin (Patel Chest Institute, CSIR Centre for Biochemicals, Delhi), Cholesterol (Centron Research Laboratories, Bombay), L-noradrenaline (Sigma Aldrich), serotonin creatinine sulphate (Sigma Aldrich), dopamine chlorhydrate (Sigma-Aldrich) and water twice distilled in an all pyrex glass still have been used in the present experiments.

The critical micelle concentrations (CMCs) of aqueous solutions of vnf determined from the variation of surface tensions with concentrations was found to be $4x10^{-6}$M The surface tensions were measured using a Fischer Tensiomat Model 21. The aqueous solutions of vnf which are sparingly soluble in water, were prepared by adding the requisite volume of ethanolic stock solution of known concentration of the drug to the aqueous phase with constant stirring. In the aqueous solutions of the drugs, thus prepared, the final concentration of ethanol was never allowed to exceed 0.1% by volume because it was shown by control experiments that a 0.1% solution of ethanol in water does not affect the surface tension of water to any measurable extent[3]

The all glass transport cell used in the earlier studies[7] was also used in the present studies (For description of the transport cell see refs 2 or 3). In the all glass cell, a sartorius cellulose acetate microfiltration membrane (Cat. no. 11307, average pore size 0.2 μ,m) of thickness 1 x 10^{-4} m and area 2.55 x 10^{-5} m^2 which also acted as a supporting membrane for the liquid membrane divided the cell into two compartments C and D (see Fig. 1).

The hydraulic permeability data in the presence of various concentrations of the drug in the lower compartment C of the transport cell were obtained to demonstrate the formation of liquid membranes by the drugs in series with the supporting membrane. The information about the incorporation of the vnf in the liquid membranes generated by the lecithin-cholesterol mixture was obtained from the data on hydraulic permeability in the presence of varying concentrations of the drug in the aqueous solution of the lecithin-cholesterol mixture of fixed composition ie,15.542 ppm with respect to lecithin and 1.175 x 10^{-6} M with respect to cholesterol—taken in the lower compartment, C, of the transport cell. This particular composition of the lecithin-cholesterol mixture is derived from an earlier study[9]wherein it has been demonstrated that at this composition the liquid membrane generated by lecithin completely covers the interface and is saturated with cholesterol. The method used for obtaining the hydraulic permeability data was the same as used in an earlier study[10].

The values of solute permeability (ω) were estimated using the equation[11,12]

$(J_s/\Delta\pi) = \omega$ --------- 1

$J_v = 0$

Where, J_S and J_v are the solute flux and the volume flux per unit area of the membrane and $\Delta\Pi$ is the osmotic pressure difference across the membrane. In experiments for determining ω, a solution of desired concentration of the permeant prepared in the aqueous solution of the lecithin-choleslerol-vnf mixture of known composition was filled in compartment C and water in compartment D of the transport cell. The details of the method were the same as described [10-11] earlier. The composition of the lecithin-cholesterol-vnf mixture used in the experiments for measurements was derived from the hydraulic permeability data in the presence of varying concentrations of vnf in the aqueous solution of the fixed composition of the lecithin-cholesterol mixture. The composition of the lecithin-cholcsterol- vnf mixture used in the solute permeability (ω) measurements were those at which the liquid membrane generated by lecithin completely covers the interface and is saturated with both cholesterol and the drug under study.

All measuements were made at constant temperature using a thermostat set at 37° ± 0.1°C,[12-13]

RESULTS AND DISCUSSION

The hydraulic permeability data in all cases were found to be in accordance with the equation $J_v = L_p . \Delta_p$ ---------- 2

where J_v is the volume flow per unit area of the membrane, ΔP is the applied pressure difference and Lp is the hydraulic conductivity coefficient. The values of Lp, as estimated from the J_v versus ΔP plots, in the case of both drugs (Table 1) show trends which are indicative of the formation of the drug liquid membrane in series with the supporting membrane. According to Kesting's hypothesis[12-14] when a surfactant is added to an aqueous phase the surfactant layer which forms spontaneously at the interface acts as a liquid membrane and modifies transport across the interface. As the concentration of the surfactant is increased, the interface gets progressively covered with the surfactant layer liquid membrane and at the CMC it is completely covered, The values of Lp decrease progressively with the increase in the concentrations of the drugs up to their CMCs, beyond which they become more or less constant (Table 1). Analysis of the data on L_p values in the light of mosaic model[14]-[17] further confirms the formation of the drug liquid membrane in series with the supporting membrane. Utilizing the concept of progressive coverage of the supporting membrane by the liquid membrane, it was shown earlier[14] that when the concentration of the surfactant is n times its CMC, n < 1, the values of L_p can be computed using the expression $[(1—n).L^{\circ}_p$-t-n L^c_p *]*where L^0_p and L^c_p are the values of L_P, when the concentration of the surfactant is equal to zero and the CMC respectively. The values of *Lp* thus computed at different concentrations below the CMC of the drug compare, favorably with the corresponding experimentally determined values[18-20].(Table-1).

The hydraulic permeability data at varying concentrations of the vnf in aqueous solutions of the lecithin-cholesterol mixture of the fixed composition also obeyed Eq. 2. The values of L_P (Table 1) decrease with the increase in concentration of the drug up to a particular concentration where after they become more or less constant. The decreasing trend in the values of L_P indicates the incorporation of the vnf in the liquid membrane generated at the interface by the lecithin-cholesterol mixture and the concentration where after the values of L_p become constant are the concentrations at which the lecithin-cholesterol liquid membrane at the interface is saturated with the vnf. It is these particular compositions of the lecithin-cholesterol-vnf mixtures that were used in the experiments for solute permeability (ω) determinations1[17-19].

Solute permeability data and biological actions— Solute permeability data for the various permeates as recorded in table 2 appear relevant to the reported biological actions of vnf.

Solute permeabilities (ω) of the relevant permeants in presence of the liquid membranes generated by lecithin- cholesterol and vnf were measured (table2). Measurements of various ion and permeants evaluated are calcium chloride (for calcium), potassium chloride (for potassium transport), sodium chloride (for sodium transport). The endogenous amines like dopamine HCl, Scrotonin, nor-adrenaline and adrenaline were also evaluated so as to study the effect of the liquid membranes generated by drug on the transport of these endogenous amines.

The procedure adopted for the measurement is as earlier described[13-15]. Compartment C of the transport cell was filled with the solution of known concentration of permeant (ions and endogenous amines) in the aqueous solution of vnf of concentration $8x10^{-6}$(2CMC). The compartment D was filled with distilled water. In control experiments, however no drug was used in compartment C. Vnf has both hydrophobic and hydrophilic parts in it's structure ,and it is supposed that they are involved in the formation of liquid membrane in these experiments. The hydrophobic tails of vnf will preferentially be oriented towards the hydrophobic supporting membrane and the hydrophilic moieties would be drawn outwards away from it. So in the present experiments, the permeants would face the hydrophilic surface of the liquid membrane generated by vnf[18-20].

The values of solute permeability (ω) were measured using the definition.

$\omega = (J_s/\Delta\pi)\ J_v = o$ --- (1)

Where, Jv and Js are the volume flux and solute flux per unit area of the membrane, respectively and Δπ is the osmotic pressure difference.

All measurements were carried out at constant temperature using a thermostat set at 37±0.1 ^{o}C After a time period of 2h, the concentration of the permeant in the compartment D was measured. Ten repeals were taken for each value of ω.

For solute permeability (ω) measurements, the concentrations of vnf taken was always higher than it's CMCs. This was done to ensure that the supporting membrane was completely covered with liquid membrane generated by the drugs.

Estimation of various ions and endogenous amines

The amounts of various permeants transported across compartment D were estimated as follows.

Cations

The amount of sodium, potassium and calcium ions were determined using a flame photometer (model-CL-22A, Elico-India).

Endogenous amines

The amount of adrenaline, nor adrenaline, dopamine and 5-HT (serotonin) were estimated by using UV-visible spectrophotometer (SL 159, Elico-India) The absorption maxima for the biogenic amines (adrenaline, nor adrenaline, dopamine and serotonin) were determined and calibration curves were constructed by plotting concentrations versus absorbance of standard solutions of respective biogenic amines. The calibration curves thus constructed were used to determine the concentrations of unknown solutions of biogenic amines.

Inference

Amphiphillic drugs interact with membranes and biological systems, causing a variety of effects. A theoretical analysis of the effects of drugs on lipid bilayer has been discussed by Mouritsen *et al* [19-20].

Assuming that, vnf gets inserted into membranes as interstitial component, it was shown that it affects the organization and thermo-tropic properties of the lipids. Computer simulations indicate that the partitioning drugs, accumulate heterogeneously in the membrane, higher concentration being attained at the interface between gel phase and liquid crystal domains of membranes[21-24]

The values of solute permeability for biogenic amines (adrenaline, non adrenaline, dopamine and 5-HT) and cations (potassium, sodium and calcium) are recorded in Table-2. In order to ensure that the supporting membrane of the transport cell is completely covered with

the liquid membrane, the concentration of vnf chosen for the present experiment was $8x10^{-6}$, which was much higher than it's CMC.

When lecithin-cholesterol aqueous mixture was employed along with vnf, the values of the solute permeability still decreased as compared with the solute permeability studies when carried out using only vnf. This was because of the formation of liquid membrane by drug along with the lecithin-chloesterol aqueous mixture. This provided an additional parameter for determining the permeability characteristics of the drug[25-26].

The studies on cations were done, as cations play an important role in the electrophysiological properties of the membrane .Vnf is found to cause postural hypotension. Calcium ion when enters inside the vascular smooth muscles cells, causes it's excitation and contraction and there by increasing the total peripheral resistance and this causes increased blood pressure. In the present study, it was found that the liquid membrane formed by vnf decreased the permeability of calcium, sodium and potassium ions. This aspect of liquid membrane phenomenon if viewed in the light of permeation of ions might contribute to the blood pressure lowering effect of vnf. The liquid membranes formed around the vascular smooth muscle cells may decrease the permeation of extra cellular calcium and sodium into the cell. Decrease in intracellular sodium concentration in the vascular smooth muscle may decrease stiffiness of vessel wall, increase It's compliance and dampen responsiveness to constrictor stimuli (by nor adrenaline, and Angiotensin II) [26,27]

vnf is shown to inhibit the active reuptake of biogenic amines i.e. nor adrenaline, 5-HT, dopamine into their respective neurons. Vnf inhibits 5-HT as well as nor-adrenaline reuptake, but the effect on former is greater. By the observation of solute permeability data, (Table-2) it was found that the drug under study decreased the permeation of 5-HT, nor-adrenaline, dopamine and nor-adrenaline; as well as decreased the permeation of cations (potassium, sodium and calcium). Depression is assumed to occur due to the deficiency of biogenic amines, i.e. nor-adrenaline, 5-HT, dopamine in their respective neurons in the central nervous system. So, by impeding the transport of monoamines (nor-adrenaline, 5-HT, Dopamine) to its storage vesicles in the neurons leads to enhancement of monoamines in the synaptic cleft as a result of its antidepressant activity. Apart from the above mechanism of action, governing the role of vnf; on it's permeability characteristics with biogenic amines might also play a role in it's mechanism of action[28].

According to liquid membrane phenomenon, the surface-active drugs forms liquid membranes in series with the supporting membrane, and these liquid membranes might affect the permeation of several molecules and ions to their respective sites of action and this may contribute to their mechanism of action. Above the CMC several multi layers of the liquid membranes are formed, thus the permeation of relevant molecules might be altered.

If this facet of the mode of action of the surface-active drugs is considered, then a more rational approach to their mechanism of action can be achieved. In the present context, the aspect of surface-activity of the drug might be assumed to play a vital role.

Vnf above it's CMC, when accumulates in the brain in high concentrations, will form a liquid membrane that will envelop the neurons in the brain, thus affecting the permeation of biogenic amines, ions and other molecules in the neurons. As it has been established by the present study that the drug, forms liquid membranes in series with the supporting membrane, thereby decreasing the permeation of nor adrenaline, 5-HT, dopamine, adrenaline and cations (potassium, sodium and calcium). Thus, by the formation of liquid membranes in vivo, vnf might be assumed to act by decreasing the permeation of biogenic amines, i.e. 5-HT, nor-adrenaline, dopamine and adrenaline in the neurons of CNS, there by inhibiting the active reuptake of these biogenic amines. Thus, present study in no way refutes the already established mechanism of action of Vnf, but provides a more rational and dynamic approach to it's mechanism by highlighting the role played by the liquid membranes generated by vnf.

Further, in-vivo studies are required to be designed and done for further confirmation of the hypothesis.

Table 1: Values of L_p at different concentrations of Vnf.

S. No.	Concentration of the drug ($x10^{-6}M$)	$L_p x10^6$ ($m^3S^{-1}N^{-1}$) (Experimental)	$L_p x 10^6$ ($M^3s^{-1}N^{-1}$) (Calculated)
1	0 CMC	0.8720 ± 0.082	
2	0.25 CMC	0.765 ± 0.042	0.7630 ± 0.036
3	0.5 CMC	0.6625 ± 0.052	0.6526 ± 0.042
4	0.75 CMC	0.5320 ± 0.043	0.5512 ± 0.048
5	1 CMC	0.4250 ± 0.082	
6	2 CMC	0.3645 ± 0.042	
7	3 CMC	0.3625 ± 0.062	

Table 2: Solute permeability (ω) of various permeants in prescence of liquid membrane generated by venlafaxine alone and venlafaxine in prescence of lecithin-cholesterol mixture.

S. No.	Permeant	Initial concn.	$\omega_0 x 10^6$ ($moleS^{-1}N^{-1}$)	ω_1	ω_2 ($mole\ S^{-1}N^{-1}$)	ω_3
1	Potassium (Chloride)	10.430 mg ml^{-1}	425.31 ± 0.32	61232± 0.06	520.44 ± 0.4	360.33±0.2
2	Sodium (Chloride)	5.382 mg ml^{-1}	154.83 ± 0.64	244.32± 0.36	174.42± 0.36	122.42±0.36
3	Calcium (Chloride)	10 mg ml^{-1}	94.32 ± 0.36	168.36± 0.33	104.22± 0.42	84.33 ± 0.28
4	Adrenaline	10 µg ml^{-1}	0.330 ± 0.28	0.642± 0.32	0.302±0.34	0.27±0.42
5	Nor-adrenaline	10 µg ml^{-1}	1.04 ± 0.48	1.46 ± 0.32	1.02 ± 0.31	0.62 ± 0.31
6	Dopamine	10 µg ml^{-1}	1.046 ± 0.36	1.72 ± 0.28	1.03 ± 0.36	0.91 ± 0.28
7	Serotonin	10 µg ml^{-1}	1.342 ± 0.71	2.36 ± 0.81	1.437± 0.36	1.001 ± 0.34

All values of ω are reported as arithmetic mean ± standard deviation of 10 determinations. Values of ω when no drug was used, ω_1; values of ω is the prescence of lecithin-cholesterol mixture, ω_2; values of ω in presence of venlafaxine, ω_0 ; values of ω in prescence of venlafaxine and lecithin cholesterol mixture, ω_3.

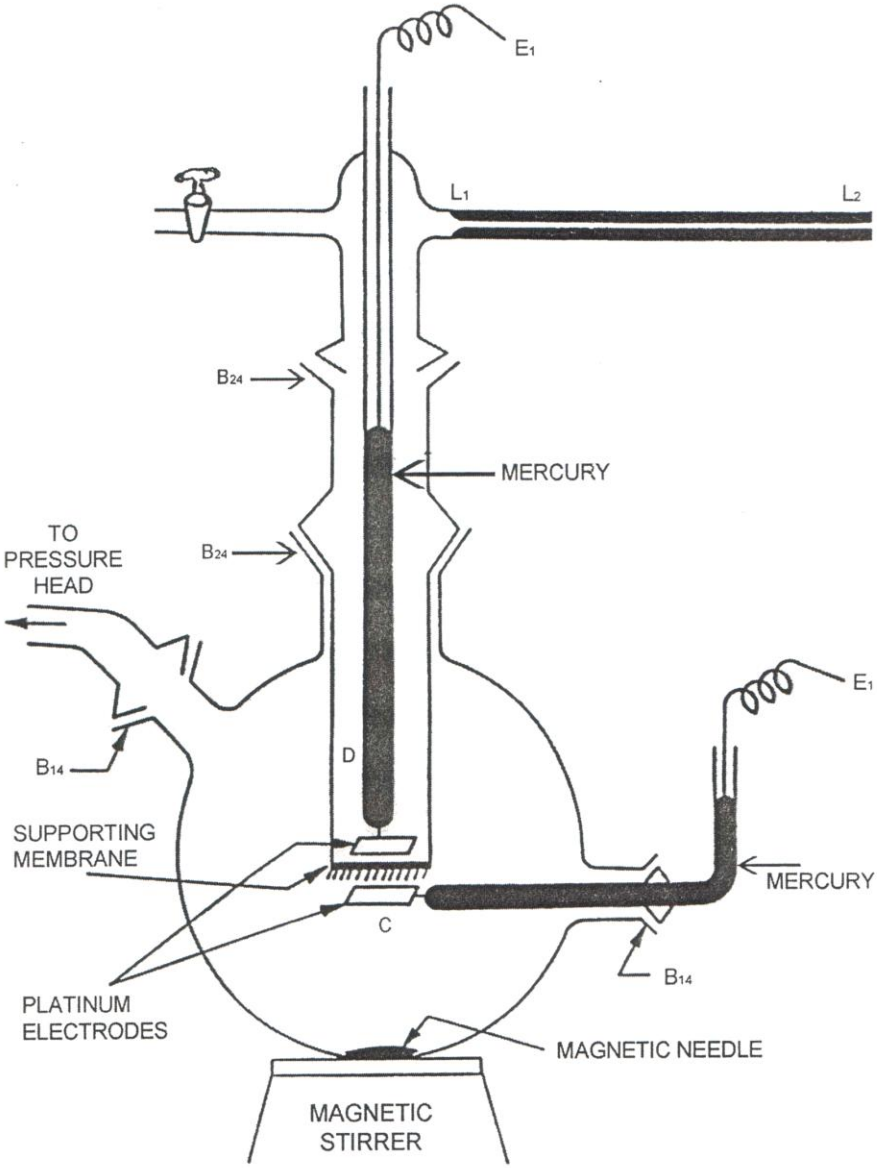

Fig. 1

REFERENCES

1. R.C.Srivastava, S.B.Bhise, S.S.Mathur, Liquid membrane phenomena and drug action, Adv. Coll. Interlace Sci. 20 (1984) 131-161
2. R.C.Srivastava, Transport through liquid membrane generated by lecithin, cholesterol, and lecithin-cholesterol mixtures in the presence of prostaglandin, J.Mem.Sci. 58 (1991) 211.
3. R.C.Srivastava, S.B.Bhise, Liquid membrane phenomenon in reserpine action, J.Pharm, Sci. 72 (1983) 599.
4. R.C.Srivastava, R.P.S. Jakhar, Transport through liquid membranes generated by lecithin-cholesterol mixtures, J.Phys. Chem. 85 (1982) ,1441-1445.
5. R.C.Srivastava, A.N. Nagappa, D.B.Raju, Role of liquid membrane phenomenon in the biological actions of prostaglandin: studies on prostaglandin E, and prostaglandin $F_2\alpha$, Indian J.Biochem, Biophys. 26 (1991) 172.
6. R.C. Srivastava, Liquid membrane phenomena in local anesthetics, J.Coll.Interlace Sci. 94 (1983) 456.
7. R.C. Srivastava, R.K.Sharma, S.B.Bhise, Liquid membrane phenomena in diazepam action, J.Coll. Interface Sci. 93 (1983) 72.
8. R.C.Srivastava, S.B.Bhise, C.V.S. Subrahmanyam, Liquid membrane phenomenon in antithistamines, Int. J.Pharm. 17(1983) 263.
9. R.C. Srivastava, S.B.Bhise, Biological implications of liquid membrane phenomena, Indian J.Chem. Soc. 60 (1983) 1135.
10. R.C. Srivastava, On the reduced furosemide response in the presence of diphenylhydantoin, Coll. Surf. 19 (1986) 83.
11. R.C.Srivastava, S.B.Bhise, C.V.S.Subrahmanyam and A.K.Malhotra ,1985,Liquid membrane phenomenon in antiepileptic drugs, Int.J. of Ph.,24,297-305..
12. R.C. Srivastava, R.K.Sharma, S.B.Bhise, Liquid membrane phenomenon in steroidal drugs, Coll.Surf.14 (1985) 1.

13. R.E. Kesting, W.J.Subeasky, J.D.Paton, Liquid membrane at the cellulose acetate membrane/saline solutions interface in reverse osmosis, J.Coll. Interface Sci. 28 (1968) 156.
14. R.C. Srivastava, R.P.S. Jakhar, S.B. Bhise, Liquid membrane phenomenon in imipramine action, J.Coll, Interface Sci. 87 (1982) 60.
15. A.N. Nagappa, H.L. Kole, P.V.Pandi, K.Girish, P.K.Rahul, L.Shanmukha, Role of liquid membrane hypothesis in the biological actions of β-blockers, J.Mem. Sci. 211 (2003) 349.
16. H.M. Willard, Instrumental Methods of Analysis, CBS Publishers and Distributors, New Delhi, 1990.
17. R.C.Srivastava,A.Tandon,S.B.Bhise,R.K.Sharma,1983,Photo-osmosis through liquid membrane bilayers, J. C. Int. Sc.,93,568
18. G.H.Jeffery, Vogel's Textbook of Quantitative Chemical Analysis, Addison Wesley Longman Ltd, New York, 1990,705ensen, Dynamical order and disorder in lipid bilayers, chem. Phys. Lipids 73 (1994) 3.
19. O.G.Mouristen, J.Risbo, K.Jorgensen, M.M.Sperotto, Phase behavior and permeability properties of phospholipids bilayer containing a short-chain phospholipids permeability enhancer, Biochim, Biophys, Acta 1331 (1997) 235.
20. R.E.Kesting,W.J.Subcasky,J.D.paton,liquid membrane at the cellulose acetate membrane/saline solution interface in reverse osmosis ,J. Coll. Int, Sc.28(1968)156-160 .
21. V.Singh, B.Malhotra, D.B.Raju, A.N.Nagappa, R.C.Srivastava, Liquid membrane phenomenon in the actions of digitalis, Indian J.Biochem. Biophys. 28 (1991) 34.
22. T.K.Sherwood, P.L.T.Brain, R.E.Fisher, Ind.Eng. Chem. Fund. 6 (1967) 2.
23. L.Dixon, G.W.Halbert, A.B.Mullen,G.A.Magee, Mono layer studies of tricyclic antidepressants, British Pharmaceutical Conference, 23-26 September, Glasgow, Scotland, 2001,.193.
24. R.C.Srivastava and R.P.S Jakhar,Transport through liquid membranes generated by Cholesterol,J.Phy. Ch.,1981,85,1457.
25. K.D.Tripathi, Essentials of Medicinal Pharmacology, Jaype Brothers Medicinal Publishers (P) Ltd, New Delhi, 1999 (Chapter 12).
26. J.G.Hardman, L.E.Limbird, A.G.Gilman (Eds.). The Pharmacological Basis of Therapeutics, Mc-Graw-Hill, Medical Publishing Division, New York, 2001 (Chapter 19).
27. D.Cloin,Therapeutic Drugs,Churchill livingstone,new York,1992,(chapter 9).
28. R.E.kesting,A.Vincent,J.Eberlin,R.OSW,Drug repot(1964) 117.