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Research Article

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The Role of Liquid Membrane Phenomenon in Antiplatelet Aggregation of Quercetin-3-O-Fatty Acid Esters

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ABSTRACT

Quercetin-3-O-fatty acid esters showed antiplatelet aggregation activity. The role of surface activity in the mechanism of action of quercetin-3-O-fatty acid esters was studied. Quercetin-3-O-fatty acid esters canform a liquid membrane by itself, and can also form a liquid membrane together with L-α-phosphatidylcholine and cholesterol. Transport of platelet aggregation inducer, adenosine diphosphate and the calcium ion across the liquid membrane formed by quercetin-3-O-fatty acid esters and quercetin-3-O-fatty acid esters with L-α-phosphatidylcholine-cholesterol was determined. The liquid membranes changed the transport of adenosine diphosphate and calcium, and indicated that the liquid membrane formed by quercetin-3-O-fatty acid esters had the effect on the mechanism of action of antiplatelet aggregation of quercetin-3-O-fatty acid esters.

Keywords: Surface activity; Quercetin-3-O-fatty acid esters; Liquid membrane hypothesis; Transport; Antiplatelet aggregation

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INTRODUCTION

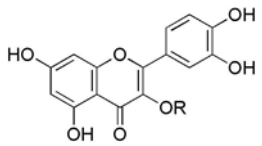
The liquid membrane hypothesis was propounded by Kesting et al. [1], and there are two novel features: (1) the surfactant layer forms spontaneously at the interface and acts as a liquid membrane, and (2) the complete liquid membrane is obtained at the critical micelle concentration (CMC) of the surfactant.

A wide variety of drugs are known to have surface active [2-7], and the excellent correlations between surface activity and biological effects have been demonstrated [8-15].

These drugs are expected to show the pharmacological active by changing the permeability of cell membranes. The investigations of research group of Nagappa and Srivastava on a wide variety of drugs have revealed that the modification of the accesses to the sites of action of the relevant molecules is an important step common to the mechanism of the surface-active drugs and play an important role in drug action [16-22]. Based on these studies and the liquid membrane hypothesis propounded by Kesting et al., they proposed for "a liquid membrane hypothesis of drug action" surface-active drugs, that is, the surface-active drugs may form the liquid membranes at the sites of action, and may act as the barriers to modify the transport of the relevant molecules to these sites.

Four derivatives of quercetin, quercetin-3-O-acetate (Q-ac), quercetin-3-O-propionate (Q-pr), quercetin-3-O-N-butyrate (Q-bu) and quercetin-3-O- N-valerate (Q-va), prepared by esterification of the C3-OH of quercetin with the acid chlorides (seen in Figure 1) showed antiplatelet aggregation activity [23,24], and they have both hydrophilic (phenolic hydroxyls and ester carbonyl) and lipophilic (2-phenyl chromone and aliphatic side-chain) domains in their structure. Saija et al. [25] and Montenegro et al. [26] reported that quercetin-3-O-fatty acid esters with short aliphatic acyl chains were able to interact with phospholipid bilayers. Pignataro et al. [27] and Sardone et al. [28] reported that quercetin-3-O-palmitate was able to be spread at air-water interface to form monolayer and interact with phospholipid membranes mainly at level of the lipid domains of the bilayers. Therefore, the four quercetin derivatives have surface active in TT Nature. They are capable of forming liquid membrane. In present study, the results demonstrated that these four quercetin derivatives themselves and these four quercetin derivatives with L-α-phosphatidylcholine and cholesterol could force the liquid membranes on the supporting membrane.

Platelet activation, induced by binding of platelet agonists such as adenosine diphosphate to membrane receptors, is an important process for platelet adhesion and aggregation and associated with the increase of intracellular calcium ion concentration [29]. Our early research revealed that quercetin-3-O-fatty acid esters inhibited platelet aggregation induced by adenosine diphosphate, increased the generation of cyclic adenosine monophosphate (cAMP) and decreased free calcium concentration in platelet [23,24]. Could the mechanism of quercetin-3-O-fatty acid esters on antiplatelet aggregation be related with their surface activity and explained with "a liquid membrane hypothesis of drug action"? The present study also designed to discuss the transport of platelet aggregation inducer, adenosine diphosphate, and calcium ion across the liquid membrane formed by quercetin-3-O-fatty acid esters.



R = H, Quercetin;
$COCH_3$, Q-ac;
$COCH_2CH_3$, Q-pr;
$COCH_2CH_2CH_3$, Q-bu;
$COCH_2CH_2CH_2CH_3$, Q-va

Figure 1. Chemical structure of quercetin and quercetin-3-O-fatty acid esters

MATERIALS AND METHODS

Materials

Quercetin (≥ 98%) was obtained from Nanjing TCM Institute of Chinese Materia Medica (Nanjing, China). L-α-phosphatidylcholine, cholesterol, adenosine diphosphate was supplied by Sigma-Aldrich Co. LLC (St. Louis, MO, USA). Calcium chloride was analytical reagent and purchased from Aladdin Industrial Corporation (Shanghai, China). Adenosine diphosphate colorimetric/fluorometric assay kit was obtained from BioVision Inc. (Milpitas, California, USA). Distilled water was used in this study.

Quercetin-3-O-fatty esters were synthesized as previously reported [24,30]. Briefly, a two-step method combining both chemical and biological procedure was used. In the first step, quercetin was chemically transformed in a pentaester, which was selectively hydrolyzed, in the second step, by means of a lipase to obtain a 3-O-acyl ester. Esters containing an aliphatic acyl chains ranging from C2 to C5 were prepared (seen in Figure 1) and characterized as previously reported [24,30].

Determination of CMC of Quercetin-3-O-Fatty Esters

The CMC of quercetin-3-O-fatty esters solutions were determined by surface tension method. The surface tension was measured by a surface tension meter (BZY-1, Huayang Instrument Co., Ltd., Foshan, China) with a Du-Nouy ring. pH of the solutions of quercetin-3-O-fatty esters remained constant. All the measurements were carried out at 37 ± 0.1°C.

Determination of Hydraulic Permeability (L_p)

Hydraulic permeability was used to demonstrate the formation of the liquid membrane on the supporting membrane. The glass transport cell imitated according to the transport cell using by the research group of Nagappa and Srivastava [16-22] was used for the transport studies, and is diagrammed in Figure 2. A Sartorius cellulose acetate microporous membrane (Cat. No. 11107, pore size 0.2 μm, thickness 1 × 10^{-4} m and area 2.55 × 10-5 m^2) was used as the support of the liquid membrane, and separated the transport cell into two compartments namely compartment C and compartment D. The determination of the hydraulic permeability was carried out referring to previously reported [16-22]. Briefly, the aqueous solutions of quercetin-3-O-fatty esters of various concentration range (below and above their respective CMC), were placed in the compartment C of the transport cell, whereas distilled water was placed in the compartment D. A series of pressures were applied on the compartment C by adjusting the pressure head, and the consequent volume flux were measured by noting the rate of advancement of liquid meniscus in the capillary L_1L_2 using a cathetometer of least count 0.001 cm. The magnitude of the applied pressure difference was also measured by noting the position of the pressure head with the cathetometer. An important caution in the measurement of volume flux was taken that allowing a sufficient time after the application of pressure on compartment C before measurement of liquid meniscus in the capillary L_1L_2. This ensures that the flow in the capillary was steady flow. The distance traveled by the liquid meniscus was plotted against time. If the graph was found to be straight line passing through origin,

the flow was taken as steady. During the volume flux measurement, the solution in the compartment C was well stirred, and the electrode E_1 and electrode E_2 were electrically short circuited. The volume flux J_v at various applied pressure differences (P) were calculated by the formula below,

$$J_V = \frac{\pi r^2 l}{\pi R^2 t} = \frac{l}{t}$$

Where "*r*" and "*R*" are radii of the capillary "L_1" and "L_2" respectively, and "*l*" is the distance traveled by liquid meniscus at time "*t*". Ten replicates were carried out and the values are presented as mean ± S.D. of 10 replicates.

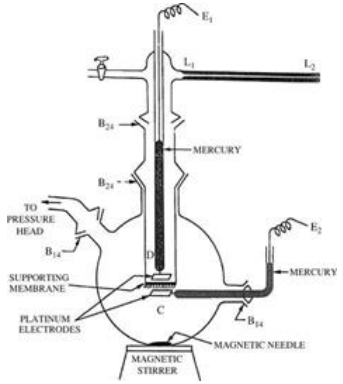


Figure 2. The glass transport cell. E_1, E_2: electrode terminals; L_1, L_2: capillary; B_{14}, B_{24} are ground glass joints; supporting membrane–cellulose acetate microporous membrane (Sartorius Cat. No 11107) [20].

Solute Permeability Studies

The permeability of calcium ion and adenosine diphosphate across the liquid membranes generated by quercetin-3-O-fatty esters was determined referring to previously reported [16-22]. In brief, compartment C of the transport cell was filled with the solution of the permeant (calcium ion and adenosine diphosphate) in the aqueous solutions of quercetin-3-O-fatty filled acid esters. The compartment D was with the distilled water. In control experiments, no quercetin-3-O-fatty esters were used in compartment C. After 2 h, the concentration of the permeant in the compartment D was measured. All measurements were carried out at 37 ± 0.1°C. Total 10 replicates were carried out and the values are presented as mean ± SD of 10 replicates.

The permeability (ω) of the relevant permeant in presence of liquid membranes formed by quercetin-3-O-fatty acid esters were calculated by the formula below,

$$\left(\frac{J_S}{\Delta\pi}\right)_{J_V=0} = \omega$$

Where J_S is the permeant flux, J_v is the volume flux per unit area of the membrane, and $\Delta\pi$ is the osmotic pressure difference.

The condition $J_V = 0$ was imposed on the system, and the amount of permeant transported to the compartment D (filled with distilled water) in a known period of time was estimated. For permeant permeability experiments the concentrations of quercetin-3-O-fatty acid esters chosen was above their CMCs.

Solute Permeability Studies Using L-A-Phosphatidylcholine-Cholesterol Mixture

Solute permeability was studied referring to previously reported [16-22]. L-α-phosphatidylcholine-cholesterol mixture was used to simulate the *in vivo* condition. The L-α-phosphatidylcholine-cholesterol mixture was dissolved in phosphate buffer solution at pH=7, and the final concentrations of L-α-phosphatidylcholine and cholesterol were 1.1750×10^{-6} mol/L and 1.9191×10^{-5} mol/L, respectively. The ethanol solution of test solute was added into the above phosphate buffer solution with stirring, and the final volume of added ethanol never exceeded 0.4%. The control experiments showed that adding not exceeded 0.4% volume of ethanol in water did not affect the surface tension of water. Also, a Sartorius cellulose acetate microporous membrane was chosen as supporting membrane to highlight passive transport through the liquid membrane formed by quercetin-3-O-fatty esters. The concentration of drugs used in these studies was also above CMCs of quercetin-3-O-fatty esters. Control experiments of only L-α phosphatidylcholine-cholesterol mixture without drug were also carried out. The experimental procedure carried out in similar way as that of solute permeability studies as described above. In liquid membranes formed by quercetin-3-O-fatty esters, it is postulated that, the hydrophobic parts of quercetin-3-O-fatty esters preferentially oriented towards the hydrophobic part of the supporting membranes and hydrophilic parts were drawn outwards i.e. away from it. So, the permeants faced the hydrophilic surface of the liquid membranes formed by quercetin-3-O-fatty esters.

Estimation of Calcium Ion and Adenosine Diphosphate

Determination of calcium ion by atomic absorption spectrophotometer (Shimadzu, AA-6300). The amount of adenosine diphosphate was estimated by adenosine diphosphate colorimetric/fluorometric assay kit.

RESULTS AND DISCUSSION

There are many examples that drugs can insert into membranes and change the microstructure and function of cell membranes [31-39]. So, the surface-active drugs are logically expected to interact with cell membranes and alter the permeability of membranes. This plays an important role in the mechanism of action of drugs. In order to clarify this hypothesis, the research group of Nagappa and Srivastava has studied on a wide variety of drugs belonging to different pharmacological categories and correlated their ability of forming film at the interface and modifying accesses of relevant molecules to the action sites with the mechanism of action [16-22].

Quercetin-3-O-fatty acid esters showed antiplatelet aggregation activity and inhibited the increase of intracellular calcium ion concentration [23,24]. Their surface activity can be speculated from their amphiphilic molecular structure. In fact, our studies confirmed that these four esters were capable of reducing the surface tension of water to some extent, and established CMC of Q-ac, Q-pr, Q-bu and Q-va are 2.5×10^{-4}, 2.0×10^{-6}, 3.0×10^{-7}, 5.0×10^{-8} mol/L,

respectively. We tried to associate the liquid membrane hypothesis with the mechanism of antiplatelet aggregation of quercetin-3-O-fatty acid esters In present study, the values of hydraulic permeability of all the four quercetin-3-O-fatty acid esters at different concentrations were found to be consistent with the formula,

$$J_V = L_p \Delta p$$

Where, J_v is the volume flux per area of the membrane, Δp is the applied pressure difference, and L_p is the hydraulic conductivity co-efficient. The values of L_p recorded at different concentrations of the drugs, and estimated from the slopes of J_v versus Δp plots that are given in Tables 1-4. The progressive decrease of L_p values were observed with increase of the concentrations of all the four quercetin-3-O-fatty acid esters up to their CMC, and then L_p values were more or less constant when the concentrations were beyond the CMC.

Table 1. Values of L_p at various concentrations of Q-ac [a] ([a] values are presented as mean ± standard deviation (SD) of 10 replicates)

Q-ac concentration (× 10^5 mol/L)		L_p × 10^5 ($m^3 \cdot s_{-1} \cdot N^{-1}$) (Experimental)	L_p × 10^5 ($m^3 \cdot s^{-1} \cdot N^{-1}$) (Calculated)
0	**-**	**3.81 ± 0.02**	**-**
6.25	(0.25CMC)	3.63 ± 0.04	3.59 ± 0.03
12.5	(0.5CMC)	3.39 ± 0.05	3.39 ± 0.04
18.5	(0.74CMC)	3.18 ± 0.06	3.13 ± 0.07
25	(CMC)	2.83 ± 0.02	-
50	(2CMC)	2.80 ± 0.05	-
75	(3CMC)	2.70 ± 0.04	-

Table 2. Values of L_p at various concentrations of Q-pr[a] ([a] values are presented as mean ± SD of 10 replicates)

Q-pr concentration (× 10^7 mol/L)		L_p × 10^5 ($m^3 \cdot s^{-1} \cdot N^{-1}$) (Experimental)	L_p × 10^5 ($m^3 \cdot s^{-1} \cdot N^{-1}$) (Calculated)
0	**-**	**3.81 ± 0.02**	**-**
5	(0.25CMC)	3.42 ± 0.03	3.38 ± 0.03
10	(0.5CMC)	2.82 ± 0.04	2.69 ± 0.02
15	(0.74CMC)	2.40 ± 0.04	2.38 ± 0.03
20	(CMC)	2.06 ± 0.05	-
40	(2CMC)	2.01 ± 0.04	-
60	(3CMC)	1.99 ± 0.05	-

Table 3. Values of L_p at various concentrations of Q-bu[a] ([a] values are presented as mean ± SD of 10 replicates)

Q-bu concentration(× 10^8 mol/L)		L_p × 10^5 ($m^3 \cdot s^{-1} \cdot N^{-1}$) (Experimental)	L_p × 10^5 ($m^3 \cdot s^{-1} \cdot N^{-1}$) (Calculated)
0	**-**	**3.81 ± 0.02**	**-**
7.5	(0.25CMC)	3.02 ± 0.02	2.95 ± 0.04
15	(0.5CMC)	2.43 ± 0.05	2.29 ± 0.06
22.5	(0.74CMC)	2.03 ± 0.04	1.90 ± 0.04
30	(CMC)	1.57 ± 0.03	-
60	(2CMC)	1.59 ± 0.03	-
90	(3CMC)	1.55 ± 0.05	-

Tables 4. Values of L_p at various concentrations of Q-va[a]([a] values are presented as mean ± SD of 10 replicates)

Q-va concentration (× 10^8 mol/L)		L_p × 10^5 ($m^3 \cdot s^{-1} \cdot N^{-1}$) (Experimental)	L_p × 10^5 ($m^3 \cdot s^{-1} \cdot N^{-1}$) (Calculated)
0	**-**	**3.81 ± 0.02**	**-**
1.25	(0.25CMC)	2.92 ± 0.04	2.88 ± 0.03
2.5	(0.5CMC)	2.22 ± 0.03	2.18 ± 0.02
3.75	(0.74CMC)	1.54 ± 0.06	1.46 ± 0.05
5	(CMC)	1.33 ± 0.04	-
10	(2CMC)	1.33 ± 0.03	-
15	(3CMC)	1.32 ± 0.06	-

This trend indicated the progressive coverage of the supporting membrane with the liquid membrane formed by quercetin-3-O-fatty acid esters and was in accordance with the Kesting's hypothesis [1]. At CMC, the liquid membrane formed by quercetin-3-O-fatty acid ester completely covered the support membrane. According the mosaic model [16,38], when the concentration of the surfactant is "n" times of CMC ($n \leq 1$), the values of L_p can be calculated by the formula,

$$\left[(1-n)\cdot L_p^S + n\cdot L_p^C\right]$$

L_p^S and L_p^C are the values of of hydraulic conductivity co-efficient for the supporting membrane and surfactant layer liquid membrane, respectively. Where Functionally L_p^S and L_p^C would be the values of L_p at 0 and 1 CMC of the surfactant.

As seen in Tables 1-4, for all the four quercetin-3-O-fatty acid esters, The calculated Lp values were consistent with the experimentally determined values. Analysis of the in the light of the also furnishes further evidence in favor of the formation of liquid membrane on The permeability values of calcium ion and adenosine diphosphate across L-α-phosphatidylcholine-cholesterol and quercetin-fatty acid esters liquid membrane were presented in Table 5.

Table 5. Solute permeability (ω) of various permeants across liquid membrane formed by quercetin-fatty acid esters alone and quercetin-fatty acid esters in presence of L-α-phosphatidylcholine-cholesterol mixture [a, b] ([a]all the values of ω are are presented as mean ± SD of 10 replicates: [b] ω_0: values of ω of control; ω_1: values of ω across L-α-phosphatidylcholine-cholesterol mixture; ω_2: values of ω with using drug; ω^3: values of ω in presence of drug and L-α-phosphatidylcholine-cholesterol mixture)

Permeants	Initial concentration	$\omega_0 \times 10^6$ ($mo^l s^{-1} \cdot N^{-1}$)	$\omega_1 \times 10^6$ ($mo^l s^{-1} \cdot N^{-1}$)	$\omega_2 \times 106$ ($mo^l s^{-1} \cdot N^{-1}$)				$\omega_3 \times 106$ ($mo^l s^{-1} \cdot N^{-1}$)			
				Q-ac (2CMC, 5×10^{-4} mol/L)	Q-pr (2CMC, 4×10^{-6} mol/L)	Q-bu (2CMC, 6×10^{-7} mol/L)	Q-va (2CMC, 1×10^{-7} mol/L)	Q-ac (2CMC, 5×10^{-4} mol/L)	Q-pr (2CMC, 4×10^{-6} mol/L)	Q-bu (2CMC, 6×10^{-7} mol/L)	Q-va (2CMC, 1×10^{-7} mol/L)
Adenosine diphosphate	5 μmol/L	129.43 ± 0.65	107.32 ± 1.03	83.24 ± 0.29	76.24 ± 0.64	65.46 ± 0.17	53.82 ± 0.29	64.56 ± 0.79	48.29 ± 0.58	34.42 ± 0.26	23.92 ± 0.34
Calcium ion	10 μg/ml	547.22 ± 3.67	475.76 ± 2.37	453.27 ± 4.23	404.72 ± 8.31	372.89 ± 6.23	327.96 ± 3.95	337.88 ± 2.59	303.21 ± 3.24	282.76 ± 4.37	210.43 ± 1.72

A marked decrease of the permeability of various permeants was observed. The drug liquid Membrane offered resistance to the transport of these permeants. This reduction in the passive transport of adenosine diphosphate and calcium ion is likely to be accompanied by a reduction in their active transport. This occurs because the access of these permeants to the active carrier site of the biological membrane is likely to be effectively reduced due to the resistance of the drug liquid membrane. Platelet aggregation is a result of agonists binding to the receptor located in platelet membrane and then causing a series of intracellular signal transduction. The liquid membrane of quercetin-3-O-fatty acid esters inhibit penetration of adenosine diphosphate (Table 5), and may reduce the access of these agonists to the relevant sites of action and certainly decrease binding of these agonists with their receptors. This in turn may be responsible for the antiplatelet aggregation effect of quercetin-3-O-fatty acid esters. The increase of free calcium concentration in platelet is considered to be the precipitating factor of platelet secretion, deformation and aggregation. Our previous studies have shown that quercetin-3-O-fatty acid esters decreased free calcium concentration in platelet [23,24]. In present study, a marked decrease in the calcium ion transport across the liquid membrane of quercetin-3-O-fatty acid esters was showed. Thus, the effects of quercetin-3-O-fatty acid esters to decrease the calcium ion concentration in platelet may be due to inhibition of calcium ion transport in the site of calcium channel located in platelet membrane.

Thus, it appears that reduction of the permeability of adenosine diphosphate and calcium ion across the liquid membranes formed by quercetin-3-O-fatty acid esters may be one of the contributing factors for the antiplatelet aggregation activity of quercetin-3-O-fatty acid esters. The role of liquid membrane phenomenon in the mechanism of action of quercetin-3-O-fatty acid esters was studied.

CONCLUSION

It was observed that liquid membranes formed by quercetin-3-O-fatty acid esters could decrease the transport of the platelet aggregation agonists, adenosine diphosphate, and calcium ion. This may be closely associated with the mechanism of action of quercetin-3-O-fatty acid esters. Further studies of effects on binding of adenosine diphosphate with its receptor located in platelet membrane and in vivo studies are required to confirm the hypothesis.

Acknowledgements

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REFERENCES

[1] RE Kesting; WJ Subcasky; JD Paton. *J Colloid Interface Sci*. **1968**, 28(1), 156-160.
[2] AT Florence. *Adv Colloid Interface Sci*. **1968**, 2(2), 117-149.
[3] PS Guth; MA Spirtes. *Int Rev Neurobiol*. **1964**, 7(4), 231-278.
[4] A Felmeister. *J Pharm Sci*. **1972**, 61(2), 151-164.
[5] D Attwood; J Gibson. *J Pharm Pharmcol*. **1978**, 30(3), 176-180.
[6] D Attwood. *J Pharm Pharmcol.***1972**, 24(9), 751-752.
[7] D Attwood. *J Pharm Pharmcol.***1976**, 28(5), 407-409.
[8] PM Seeman; HS Bialy. *Biochem Pharmacol.* **1963**, 12(10), 1181-1191.
[9] JM Ritchie; P Greengard. *Annu Rev Pharmacol.* **1966**, 6(1), 405-430.
[10] FA Vilallonga; EW Phillips. *J Pharm Sci*. **1980**, 69(1), 102-104.
[11] D Hellenbrecht; B Lemmer; G Wiethold; H Grobecker. *Naunyn-schmiedeberg's Arch Pharmacol.* **1973**, 277(2), 211-226.
[12] K Thomas; K Albert. *Pharm Acta Helv.* **1979**, 54, 324-330.
[13] A Gesher; WPA Li. *J Pharm Pharmcol.* **1978**, 30(6), 353-358.
[14] EK Rideal; JH Schulman. *Nature.* **1939**, 144(3637), 100-102.
[15] JC Skou. *Acta Pharmacol Toxicol.* **1954**, 10(4), 280-284.
[16] V Singh; B Malhotra; DB Raju; AN Nagappa; RC Sirvastava. *Indian J Biochem Biophys.* **1991**, 28(1), 34-39.
[17] RC Srivastava; AN Nagappa. *Surface Activity in Drug Action*, 1st Edition, Science Press, Beijing, **2007**, 124-208.
[18] C Nagesh; JS Venkatesh; MM Shankraiah; I Shanmukha; L Narasu; SS Ramachandra. *Orient J Chem.* **2008**, 24, 271-276.
[19] AN Nagappa; RT Patil; V Pandi; K Ziauddin. *India J Biochem Biophys.* **2001**, 38(6), 412-416.
[20] AN Nagappa; PL Kole; D Srinivas; D Vivek; DP Ullas; RC Srivastava. *Colloid Surfaces B.* **2005**,43(1), 21-27.
[21] AK Jain; M Srivastava; SK Gupta. *Int J Pharm Sci Res.* 2014, 5, 2890-2895.
[22] MRP Kumar. Liquid membrane phenomenon in the biological action of novel antidepressants, Ph. D. thesis, Shri Jagdish Prasad Jhabarmal Tibrewala University, Jhunjhunu, **2010**.
[23] XL Wang; Y Duan. *J Chem Pharm Res*. **2014**, 6, 599-602.

[24] Y Duan; N Sun; M Xue; XL Wang; H Yang. *Mol Med Rep*. **2017**, 16(6), 9735-9740.
[25] A Saija; A Tomaino; D Trombetta; ML Pellegrino; B Tita; C Messina; FP Bonina; C Rocco; G Nicolosi; F Castelli. *Eur J Pharm Biopharm.* **2003**, 56(2), 167-174.
[26] L Montenegro; C Carbone; C Maniscalco; D Lambusta; G Nicolosi; CA Ventura; G Puglisi. *Int J Pharm.* **2007**, 336(2), 257-262.
[27] B Pignataro; L Sardone; G Marletta. *Mater Sci Eng C.* **2002**, 22(2), 177-181.
[28] L Sardone; B Pignataro; F Castelli; MG Sarpietro; G Nicolosi; G Marletta. *J Colloid Interf Sci.* **2004**, 271(2), 329-335.
[29] L Leung; R Nachman. *Annu Rev Med.* **1986**,37(1), 179-186.
[30] MT Gatto; S Falcocchio; E Grippa; G Mazzanti; L Battinelli; G Nicolosi; D Lambusta; L Saso. *Bioorg Med Chem.* **2002**, 10(2), 269-272.
[31] A Sajia; M Scalese; M Lanza; D Marzullo; F Bonina; F Castelli. *Free Radical Bio Med.* **1995**, 19(4), 481-486.
[32] L Movileanu; I Neagoe; ML Flonta. *Int J Pharm.* **2000**, 205(1-2), 135-146.
[33] K Ioku; T Tsushida; Y Takei; N Nakatani; J Terao. *Biochim Biophys Acta, Biomembr.* **1995**, 1234(1), 99-104.
[34] SV Verstraeten, CL Keen; HH Schmitz; CG Graga; PI Oteiza. *Free Radical Bio Med.* **2003**, 34(1), 84-92.
[35] A Arora; TM Byrem; MG Nair; GM Strasburg. *Arch Biochem Biophys.* **2000**, 373(1), 102-109.
[36] F Ollila; K Halling; P Vuorela; H Vuorela; JP Slotte. *Arch Biochem Biophys.* **2002**, 399(1), 103-108.
[37] AB Hendrich; R Malon; A Pola; Y Shirataki; N Motohashi; K Michalak. *Eur J Pharm Sci.* **2002**, 16(3), 201-208.
[38] OG Mouritsen; K Jorgensen. *Chem Phys Lipids.* **1994**, 73(1-2), 3-25.
[39] TK Sherwood; PLT Brain; RE Fishe. *Ind Eng Chem Fund.* **1967**, 6(1), 2-10.