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DESIGN, DEVELOPMENT AND *IN VITRO* EVALUATION OF CONTROLLED RELEASE GEL FOR TOPICAL DELIVERY OF QUETIAPINE USING BOX- BEHNKEN DESIGN

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ABSTRACT

The purpose of this work was to develop and characterize a vesicular drug carrier for topical delivery of Quetiapine to overcome the problems related with oral route that is high first pass metabolism and fluctuating drug plasma concentration. The effects of key formulation variables on entrapment efficiency (EE %), vesicle size and *in vitro* drug permeation were studied using a Box- Behnken design. Liposomes bearing Quetiapine were prepared by using saturated lipids like 1, 2-Dipalmitoyl-sn-glycero-3-phosphocholine (DPPC) and 1, 2-Distearoyl-sn-glycero-3-phosphocholine (DSPC) with relatively less stability problems through rotary evaporation method. The liposomal formulation was characterized for various parameters including EE %, vesicles shape, size distribution, lamellarity, *in vitro* release study, skin permeation and stability studies. Firstly liposomal suspension was prepared and then previously prepared suspension was incorporated in carbopol 940P gel with an objective of enhancing stability of liposome by avoiding aggregation of vesicles and for better skin permeation. The encapsulation efficiency of drug was found to be ranging from 60.59±4.54% to 83.56±2.97%. Nano liposomes were found to have mean particle size of 405.8±1.1 nm and zeta potential of −10.9±1.54 mV. The optimized liposomal gel showed the desired controlled release of drug uptil 12 h and *J* flux was also found to be higher than the plain gel of drug. The stability studies proved that both liposome suspension and gel were stable uptil 6 months. Finally, from the research work it could be concluded that the liposome accentuates the transdermal flux of Quetiapine and could be used as an effective carrier for transdermal delivery.

INTRODUCTION: In last two decades, number of innovative microparticulate carrier systems viz. microemulsion, nanoemulsion, nanoparticles, liposomes, ethosomes etc. have been reported for improving delivery of drug to the skin. Yet in the dermatological field, liposomes were used initially because of their moisturizing and restoring action. Later their capability of enclosing many different biological materials and of delivering them to the epidermal cells or even deeper cell layers was investigated [1].

Liposomes are enclosed spherical vesicles that are organized in one or several concentric phospholipid bilayers with an internal aqueous phase. After development liposomal technology has made considerable progress. Several important liposomal formulations for the treatment of different diseases are now available commercially or are in advanced

clinical trials. Because of their structure, liposomes can entrap hydrophilic pharmaceutical agents in their internal aqueous compartment or lipophilic drugs within the lipid membrane. The particle size of liposomes ranges from 20 nm to 10 µm in diameter. Pharmaceutical researchers use the tools of biophysics in evaluating liposomal dosage forms. Liposomes have covered predominantly medical, albeit some non-medical areas like bioreactors, catalysts, cosmetics and ecology [2, 3].

Potential applications of liposomes as pharmaceutical carriers are: Liposomes are biocompatible, completely biodegradable, non-toxic, flexible and non-immunogenic for systemic and non-systemic administration. It supply both a lipophilic environment and aqueous "milieu interne" in one system and are therefore suitable for delivery of hydrophobic, amphipathic and hydrophilic drugs. It has the ability to protect their encapsulated drug from the external environment and to act as sustained release depots (Propranolol, Cyclosporine).

Liposomes can be formulated as a suspension, as an aerosol, or in a semisolid form such as gel, cream and lotion, as a dry vesicular powder (proliposome) for reconstitution or they can be administered through most routes of administration including ocular, pulmonary, nasal, oral, intramuscular, subcutaneous, topical and intravenous.It could encapsulate not only small molecules but also macromolecules like superoxide dismutase, haemoglobin, erythropoietin, interleukin-2 and interferon-g. Liposomes can reduce toxicity and increase stability of entrapped drug via encapsulation (Amphotericin B, Taxol).Liposomes can increase efficacy and therapeutic index of drug (Actinomycin-D). It has flexibility to couple with site-specific ligands to achieve active targeting (Anticancer and Antimicrobial drugs) [1].

Recently, liposome based formulations for topical delivery has been shown to be extremely promising for; enhancement of drug penetration and improve pharmacological effect, decreased side effects, controlled drug release and drug photoprotection. It can also be used as an alternative delivery system for patients who cannot tolerate oral dosage forms. It is of great advantage in patients who are nauseated or unconscious.

First pass metabolism, an additional limitation to oral drug delivery, can be avoided through transdermal liposomal formulation and it also allows continued drug administration permitting the use of a drug with short biological half-life [1, 2].

Quetiapine is an atypical antipsychotic; it is a selective monoaminergic antagonist with high affinity for the serotonin Type 2 ($5HT_2$) and dopamine type 2 (D_2) receptors. Quetiapine is used in the treatment of schizophrenia or manic episodes associated with bipolar disorder.Steady state concentration (C_{ss}) of Quetiapine at the therapeutic level is 0.924 µg/ml and total clearance (CL_T) is 1.5 ml/min/kg [4]. Thus, the aim of this research work was to optimize the liposomal formulation for enhanced skin delivery of Quetiapine, a lipophilic drug having low oral bioavailability of about 9%. It has low molecular weight (383.507) and melting point (170-174°C) with a log partition coefficient of 2.8; there are no reports of skin irritation attributed to Quetiapine.

MATERIALS AND METHODS:

Materials: Quetiapine was received as a gratis sample from Alembic Research Center (Baroda, India). DPPC, DSPC were received as a gift sample from LIPOID GmbH (Nattermannallee, Germany). Soya lecithin and cholesterol were purchased from Spectro chem Pvt Ltd (Mumbai, India). Carbopol 940P was purchased from S. D. Fine Chemicals Ltd, Mumbai, India. Methanol and Chloroform were purchased from Merck (Darmstadt, Germany). All other chemicals used were of reagent grade and were used as received.Double-distilled water was used for all experiments.

Calculation of J_{flux} for Quetiapine: The target flux is calculated using the following equation [5];

$$J_{flux} = \frac{C_{SS}\, CL_T\, BW}{A}$$

A represents the surface area of the transdermal gel application (i.e. 4 cm^2). BW, the standard human body weight of 60 kg, Css is 0.924 µg/ml and the CL_T is 1.5 ml/min/kg [4]. The calculated target flux value for Quetiapine was 20.79 µg/min/cm^2.

Preparation of Liposomal Formulation: Quetiapine liposomal formulations (QTFs) were prepared by conventional thin-layer hydration or rotary evaporation technique [6, 7] using Box- Behnken design. A four-factor, three-level Box- Behnken design was used for constructing a second-order polynomial models using Design Expert (Version 8.0.6.1; Stat-Ease Inc, Minneapolis, Minnesota). A design matrix comprising 29 experimental runs was constructed, for which the nonlinear computer-generated quadratic model is defined as:

$$Y = b_0 + b_1X_1 + b_2X_2 + b_3X_3 + b_4X_4 + b_{12}X_1X_2 + b_{13}X_1X_3 + b_{14}X_1X_4 + b_{23}X_2X_3 + b_{24}X_2X_4 + b_{34}X_3X_4 + b_{11}X_1^2 + b_{22}X_2^2 + b_{33}X_3^2 + b_{44}X_4^2 \quad \ldots\ldots(1)$$

where Y is the measured response associated with each factor level combination; b_0 is constant; b_1, b_2, b_3, b_4 are linear coefficients, b_{12}, b_{13}, b_{14}, b_{23}, b_{24}, b_{34} are interaction coefficients between the four factors, b_{11}, b_{22}, b_{33}, b_{44} are quadratic coefficients computed from the observed experimental values of Y from experimental runs; and X_1, X_2, X_3 and X_4 are the codes of independent variables.

The terms X_1X_2 (i = 1, 2, 3 or 4) represent the interaction effect. The independent variables selected were the amount of the Soya lecithin or Soya phosphotidylcholine (SPC) (X_1), 1, 2-Dipalmitoyl-sn-glycero-3-phosphocholine (DPPC) (X_2), 1, 2-Distearoyl-sn-glycero-3-phosphocholine (DSPC) (X_3) and Cholesterol (X_4). The dependent variables were EE % (Y_1), vesicle size (Y_2) and percentage cumulative drug permeated (Y_3) with constraints applied on the formulation of liposome. The concentration range of independent variables under study is shown in **Table 1** along with their low, medium and high levels, which were selected based on the results from preliminary experimentation. The concentration range of soya lecithin (X_1), DPPC (X_2), DSPC (X_3) and Cholesterol (X_4) used to prepare the 29 formulations and the respective observed responses are given in **Table 2.**

Preparation of Liposome by Thin Film Hydration Technique: The liposome dispersions were prepared by the conventional film method. Drug (15 mg) was dissolved in the methanol: chloroform (2:1 v/v) solution of phospholipids (DSPC, DPPC, Soya lecithin and cholesterol). This mixture was dried to a thin film at 50°C by slowly reducing the pressure using a rotary evaporator. The film was kept at under vacuum (1 mbar) for 2 h at room temperature, flushed with nitrogen, and then hydrated with the appropriate amount of phosphate buffer saline (PBS) pH 7.4 for 30 min above phase transition temperature.

After complete lipid hydration and formation of liposomes, the vesicle dispersion was placed in a probe sonicator for 30 min at 4-5°C under water bath, for vesicle size reduction. Finally, the liposomal dispersions were left in peace for annealing structural defects, at a temperature above the lipid transition temperature for 1–2 h [8-11]. Separation of liposomes from non-encapsulated molecules was achieved by centrifugation (three spins at 15,000 rpm for 40 min) at +4°C [12].

Characterization of Liposomes:

Vesicles shape, Size, and Size Distribution: Liposome vesicles were visualized using optical microscope. Digital micrograph and soft imaging viewer software were used for image capture and analysis. The vesicles size and size distribution were determined using a computerized inspection system with zetasizer (dynamic light scattering method, HAS 3000; Malvern Instruments, Malvern, United Kingdom) [13].

Lamellarity: The lamellarity of the liposomes was determined by CLSM (Confocal laser scanning microscopy) study.

Encapsulation Efficiency: Liposome encapsulation efficiency was determined from the amount of entrapped drugs using the ultracentrifugation technique. Briefly, total amount of drug was determined after having dissolved and disrupted drug-loaded liposomes in ethanol or Triton X-100 using an ultrasound bath for 10 min. Then, sample was centrifuged at 50,000 rpm for 50 min at +4°C. The free drug was determined in the supernatant at 290 nm with a UV- Visible spectrophotometer [1, 8, 9, 14].

The drug encapsulation efficiency (EE %) was calculated as follows:

$$\text{EE \%} = \frac{\text{Total amount of drug incorporated} - \text{Free amount of drug (supernant)}}{\text{Total amount of drug}} \times 100$$

TABLE 1: VARIABLES IN BOX- BEHNKEN DESIGN FOR PREPARATION OF QUETIAPINE LIPOSOME

Code	Independent variables	Low (-1)	Medium (0)	High (+1)
X_1	Soya lecithin (SPC) (mg)	50	100	150
X_2	DPPC (mg)	50	80	120
X_3	DSPC (mg)	30	50	80
X_4	Cholesterol (mg)	5	15	25
Dependent variables:			**Goal**	
Y_1	Entrapment efficiency % (EE %)		Maximum	
Y_2	Mean vesicle size (nm)		In range	
Y_3	% Cumulative drug permeated		Maximum	

TABLE 2: MATRIXING OF BOX- BEHNKEN DESIGN

Formulation Code	Independent variables				Dependent variables		
	X_1	X_2	X_3	X_4	Y_1	Y_2	Y_3
F1	0	-1	-1	0	76.74 ±3.19	748.2± 2.6	65.72±0.87
F2	0	0	0	0	80.74±3.52	459.7±3.2	90.80±2.19
F3	-1	-1	0	0	63.94 ±5.78	298.2±4.7	65.43±2.33
F4	-1	0	+1	0	62.71 ±3.38	851.3±1.5	95.81±3.28
F5	0	0	+1	+1	65.23±6.61	274.6±4.7	44.94±4.63
F6	-1	0	0	+1	75.23±2.13	467.3±6.1	91.04±2.05
F7	0	0	+1	-1	67.57 ±4.27	552.1±5.4	69.19±0.77
F8	-1	+1	0	0	69.76 ±3.55	467.3±8.2	91.81±0.89
F9	0	0	0	0	78.62 ±5.25	354.2±9.5	37.55±2.66
F10	0	0	0	0	77.56±6.48	586.3±7.2	65.81±3.20
F11	-1	0	0	-1	60.59±4.54	331.4±5.1	62.44±4.84
F12	0	+1	-1	0	62.13±5.25	602.2±2.5	73.53±2.48
F13	+1	0	0	-1	83.56±2.97	393.9±6.7	30.93±4.85
F14	0	0	-1	-1	71.33±4.63	493.7±3.6	92.00±3.97
F15	0	+1	+1	0	71.63±3.17	521.5±4.3	77.17±2.78
F16	0	0	-1	+1	70.36±2.45	310.0±3.7	43.88±2.60
F17	0	-1	0	+1	79.88±3.74	637.2±2.3	61.60±3.74
F18	+1	-1	0	0	77.98±4.57	732.4±1.4	67.95±2.48
F19	0	+1	0	-1	72.17±5.35	269.1±2.2	49.80±2.96
F20	+1	0	+1	0	67.44±2.24	653.6±7.6	71.99±3.98
F21	0	+1	0	+1	79.46±3.85	306.7±5.4	71.55±3.15
F22	0	-1	+1	0	73.45±2.62	208.6±3.9	42.02±2.63
F23	+1	0	-1	0	75.34±1.40	614.3±6.1	67.84±1.85
F24	+1	0	0	+1	81.84±4.02	485.7±8.3	86.49±2.76
F25	-1	0	-1	0	65.12±2.36	476.5±7.2	76.47±1.53
F26	0	-1	0	-1	78.45±1.65	235.2±5.3	69.04±2.90
F27	0	0	0	0	76.14±3.40	769.1±2.4	77.97±3.55
F28	0	0	0	0	79.21±5.81	405.8±1.1	97.64±1.34
F29	+1	+1	0	0	71.62±4.29	799.3±2.7	81.17±2.93

X1, Soya lecithin(mg) ; X2, DPPC(mg); X3, DSPC(mg); X4, Cholesterol(mg); Y1, EE %; Y2,Mean vesicle size (nm); Y3, % Cumulative drug permeated in 12 h. Results are expressed as mean ± SEM.

***In vitro*- Drug Release Study:** *In vitro* drug release of drug from the liposomal formulation was evaluated using the dialysis tube technique. 5 ml aliquot of liposomal suspension was placed in the dialysis bag and hermetically tied. Perfect sink conditions prevailed during the drug release studies and the entire system was kept at 37±2^{o}C under continuous magnetic stirring at 70 rpm. Samples (1 ml) of the dialysate was taken at various time intervals and assayed for drug concentration by spectrophotometric method. [15]

Preparation of Liposomal Gel: The appropriate amount of carbopol 940P was weighted and added slowly in a citrate buffer solution (pH 5.0), under constant stirring by a paddle stirrer. After addition of the full amount of solid material, the gel was allowed to swell under moderate stirring for at least 24 h or until fully swollen and transparent. Other ingredients, such as 15% w/v polyethylene glycol-400 (PEG-400) and triethanolamine (0.5% w/v), were added to obtain homogeneous dispersion of gel and sodium benzoate (0.5% w/v) was added in the buffer used for gel preparation.

Liposomal gel formulations were prepared by mixing the liposome dispersions with the gels in the ratio of 1:5 (w/w) (liposome dispersion/gel). Plain gel was also prepared with addition of plain drug [1, 8].

Evaluation of Liposomal Gel:

Physical Examination: The prepared gel formulations were inspected visually for their color, homogeneity, consistency and spreadability. Clarity was determined by using clarity chamber with black and white background [16, 17].

pH: The pH values of 1% aqueous solutions of the prepared gels were measured by a pH meter [16].

Viscosity: Viscosity of prepared gels was measured by Brookfield Viscometer. Apparent viscosity was measured at 25°C and rotating the spindle at 1.5 rpm [11, 18-21].

Content uniformity: Gel formulation (100 mg) was dissolved in methanol and filtered. The volume was made to 100 ml with methanol. The resultant solution was suitably diluted with methanol and absorbance was measured at 290 nm of drug using Shimadzu – 1700 UV Visible spectrophotometer [21, 22].

***In-vitro* Drug Permeation Study:** An essential parameter in the evaluation of drug delivery is the rate at which the drug is released from the carrier. Skin permeation study with drug-containing liposomal formulation was carried out using modified Franz diffusion cell. Full thickness abdominal skin of male Wister albino rats weighing 140 to 200 g was used for the skin permeation**.** Briefly, to obtain skin, animal was sacrificed. Hair from the abdominal region was carefully removed and an excision in the skin was made.

The dermal side of the skin was thoroughly cleaned of any adhering tissues. Dermis part of the skin was wiped 3 to 4 times with a wet cotton swab soaked in isopropanol to remove any adhering fat. The skin specimen was cut into appropriate size after carefully removing subcutaneous fat and washing with normal saline. Skin was mounted in a modified Franz diffusion cell, kept at 32±0.5^{o}C. The known quantity of gel equivalent to 15 mg of drug was spread uniformly on the skin on donor side.

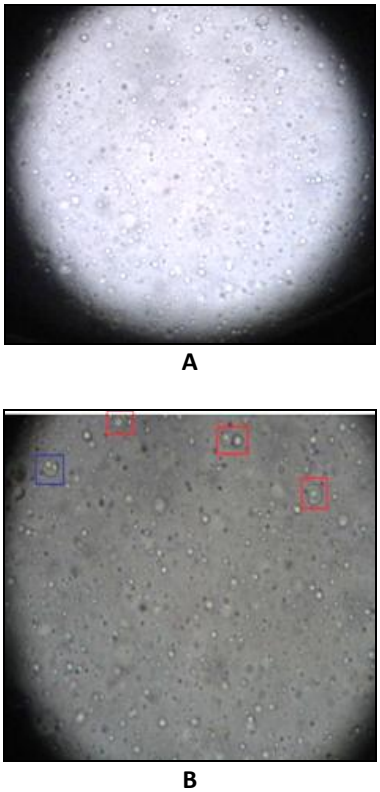
pH 7.4 phosphate buffer was used as the acceptor medium, from which samples were collected at regular intervals and were estimated with UV spectroscopy [23-28].

Kinetic Modeling: In order to understand the kinetics and mechanism of drug release, the results of *in vitro* drug release were fitted into various kinetic equations like zero order (cumulative% release *vs.* time), first order (log% drug remaining *vs.* time), Higuchi's model (cumulative% drug release *vs*. square root of time), Korsmeyer peppas plot (log of cumulative% drug release *vs*. log time). R^2 (coefficient of correlation) and *n* (Diffusion exponent) values were calculated for the linear curve obtained by regression analysis of the in vitro drug permeation plots [8, 11, 27, 28].

Stability Study: Stability studies of liposomal suspension and gel was done for 6 months under conditions required by guidelines of the ICH. Accelerated stability studies were performed by keeping the temperature 25±0.5^{o}C and 60±5 %RH (relative humidity). The stability was evaluated by comparing the particle size, zeta potential, encapsulation efficiency, viscosity and percentage cumulative permeation of drug [1, 11].

RESULTS AND DISCUSSION: Liposomes have represented a milestone in the field of innovative drug delivery systems for the encapsulation, prolonged and controlled delivery of active molecules to the site of action. Their attraction lies in their composition, which makes them biocompatible and biodegradable. Also, their structure and colloidal size along with a lack of immune system activation or suppression may be useful in various applications [29, 30]. Considering all these desirable properties as well as the necessity for improving hemocompatibility, we attempted to develop and optimize the Quetiapine loaded nano-liposomes to prevent unwanted first pass metabolism.

Microscopy: It was observed from optical microscopical determination that liposome suspension showed a mixture of different types of liposomes **(Figure 1). Figure 1(A)** shows the whole field of liposome suspension whereas **Figure 1(B)** shows the MLV (Red boxes) and MVV (Blue box) type of liposome.



A

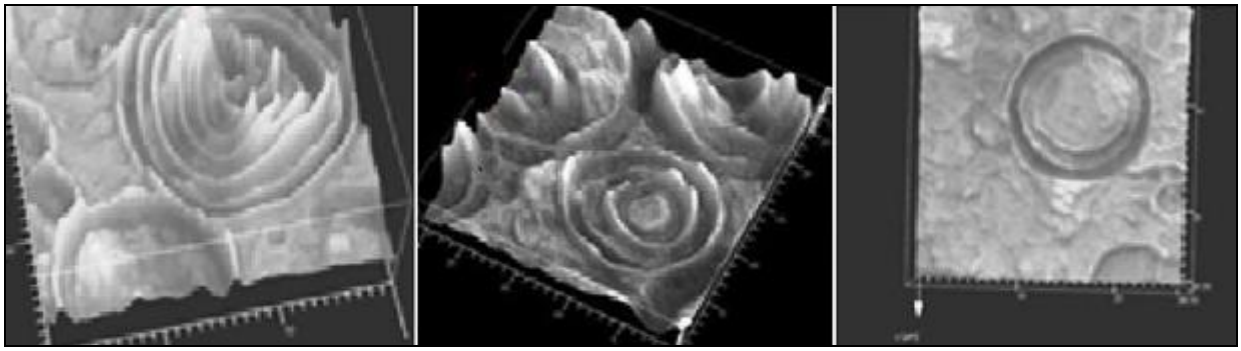
B

FIGURE 1: PHOTOMICROGRAPHS FROM OPTICAL MICROSCOPE SHOWING MICROSTRUCTURE OF LIPOSOMES (MAGNIFICATION ×400)

Lamellarity: In this work, we have demonstrated that it is possible to determine both, the outer morphology and the lamellarity of vesicle systems by means of CLSM. Transmitted light CLSM pictures showed a main distribution of MLVs in all the systems studied, with a number of concentric bilayers ranged between 6 **(Figure 2A)** and 3 **(Figure 2B)**. Sonicated vesicles showed a clear tendency to decrease their lamellarity, finding a representative number of unilamellar vesicles in the formulations, as it is shown in **Figure 2C.**

Entrapment efficiency: In the field of nanotechnology, EE % is an important index to characterize drug delivery systems. A high EE % would be beneficial in incorporating the required dose in the minimum volume, facilitating local administration. Here, the EE % was found in the range of 60.59±4.54% to 83.56±2.97% **(Table 2).** Effects of independent variables on EE % are presented by three-dimensional graph in **Figure 3.** It was observed that when SPC was increased from low to high level, the EE% was found to be highest (83.56%), whereas increase in DPPC from low to high level had only 60.59% drug incorporation. The effect of phospholipid concentration on EE % is shown by the equation (2) as shown below;

$$\mathbf{Y_1} = 78.45 + 5.04X_1 - 1.97X_2 - 1.09X_3 + 1.53X_4 - 3.05X_1X_2 - 1.37X_1X_3 - 4.09X_1X_4 + 3.2X_2X_3 + 1.46X_2X_4 - 0.33X_3X_4 - 4.15X_1^2 - 1.39X_2^2 - 7.41X_3^2 - 0.34X_4^2 \quad \text{.....(2)}$$



A B C

FIGURE 2: MICROPHOTOGRAPHS CORRESPONDING TO MULTILAMELLAR LIPOSOMES BY CLSM USING TRANSMITTED CHANNEL: (A) FIVE-SIX LAMELLAE; (B) THREE LAMELLAE (C) UNILAMELLAR

The variables like concentration of X_1(SPC) and X_4(cholesterol) have synergistic effect on EE% that means the increase in amount of X_1,X_4 can increase the EE %.Whereas, the variable X_2, X_3 have negative impact on EE % that indicate increase in DPPC,DSPC concentration will reduce the EE %.When DSPC was increased from lower to higher level, the EE% was found to be reduced upto 62.13%. Whereas, the DPPC was not significantly affect EE%. It was revealed that, SPC had more prominent enhancing effect on EE%. The entrapment of drug occurs in both the bilayers and the aqueous compartment of the vesicles [31]. When the lipid compartment and aqueous phase became saturated with the drug, the vesicles provided limited entrapment capacity [32]. Lipids are the major structural components of liposomes and therefore have great influence on fluidity characteristics of liposomal membranes. Depending on the chain length and the degree of saturation, lipids show different T_m values. DSPC contains a saturated C18 fatty acid and forms

rigid membranes. Liposomes composed of this lipid are in the gel state, whereas SPC liposomes have a mixture of phospholipids of different chain lengths and varying degrees of saturation and are in the liquid crystalline state; hence, regions of high bilayer disorder exist. Because the characteristics of these lipid compositions differ widely; thus, there was significant influence of the lipid on the amount of drug incorporated. Increasing the content of cholesterol (X_4) from 5 to 25 mg had significantly affected the EE %. The upper level for the cholesterol proportion was considered to be 25 mg because the higher cholesterol level markedly affected the stability of drug liposomes and resulted in rapid aggregation of vesicles in the trial runs in our preliminary studies. It was observed that the high level of cholesterol significantly interfere with the close packing of lipids in the vesicles, thereby reducing the encapsulation of the hydrophobic drug, Quetiapine. Moreover, Cholesterol is known to increase membrane rigidity and packing density by accumulating in the molecular cavities formed by the phospholipid molecules assembled into bilayer vesicles [31], which may result in decreased bilayer partitioning and hydrophobic space available for the incorporation of hydrophobic drugs like paclitaxel [32] and nystatin [33].

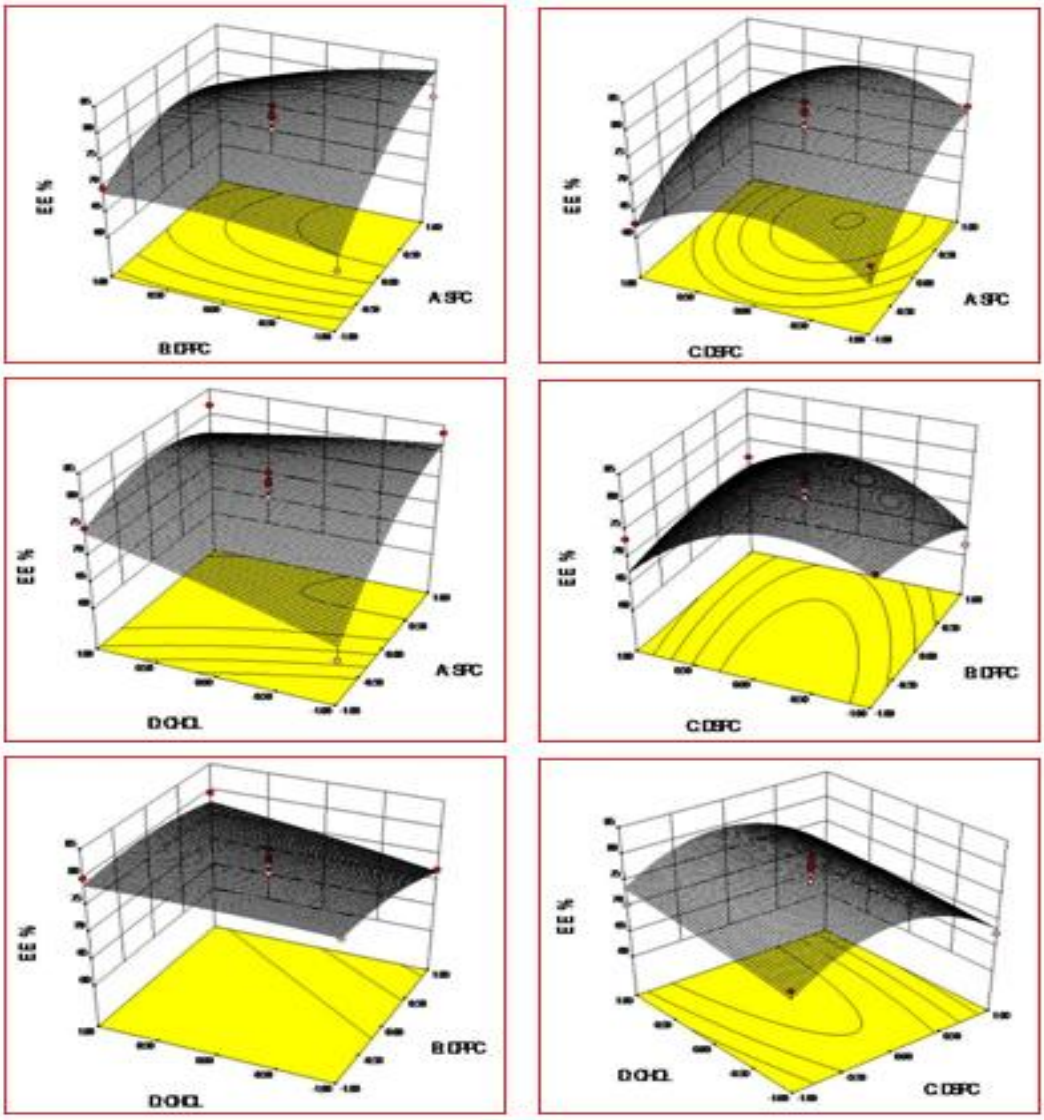


FIGURE 3: RESPONSE SURFACE PLOT SHOWING EFFECT OF INDEPENDENT VARIABLES ON PERCENT ENTRAPMENT EFFICIENCY

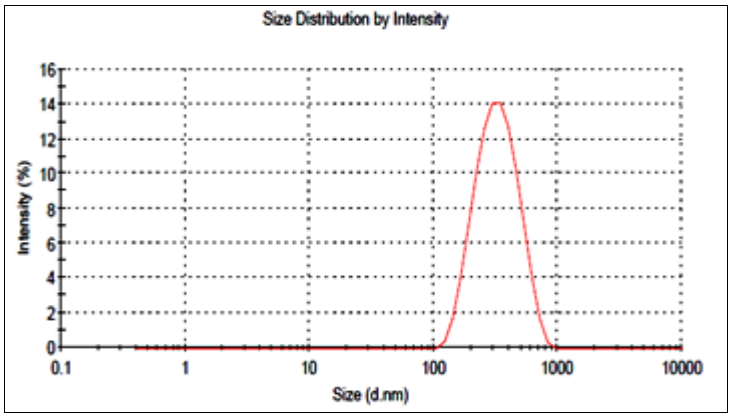
Vesicle size, Size distribution and Zeta Potential Analysis: Polydispersity index (PDI) of all the formulations is shown in the **Table 3**. The PDI was observed in the range of 0.140 - 0.452. Most of formulation had PDI lower than 0.2. Since PDI is less than 0.2, it can be concluded that the formulations were relatively monodispersed. The vesicle size distribution of liposomal formulation is shown in the

Figure 4 (A). The zeta potential of drug loaded liposomes was found to be in the range of −43.7 to −10.9 mV as shown in **Table 3** and **Figure 4 (B)**. Zeta potential is the electric charge on the surface of particles, which creates an electrical barrier and acts as a 'repulsive factor' and prevent the aggregation of the spheres. The aggregation of neutral liposomes is brought about by Van der Waals interactions. Small concentration of charged lipids can provide sufficient electrostatic repulsion to prevent the aggregation of the particles upon the addition of hydrophobic drugs to the membrane [34].

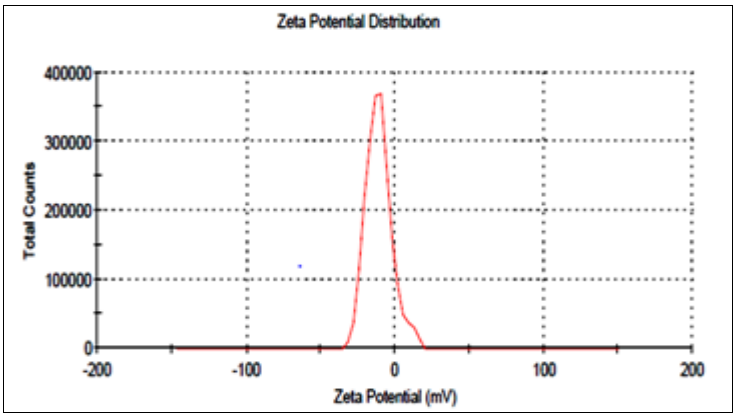
TABLE 3: EVALUATION OF LIPOSOMAL SUSPENSION

Formulation code	PDI	Zeta Potential (mV)	%CDR for 12 h	Desirability Function
F1	0.384±0.014	-15.90±1.32	62.18±1.34	0.739
F2	0.307±0.070	-26.99±5.21	84.32±2.57	0.882
F3	0.452±0.002	-13.30±0.43	59.83±1.23	0.667
F4	0.199±0.060	-17.80±4.83	96.82±2.34	0.848
F5	0.180±0.020	-22.62±4.35	47.41±1.82	0.673
F6	0.145±0.003	-17.18±1.44	93.25±2.78	0.853
F7	0.157±0.017	-43.70±0.70	61.52±1.94	0.705
F8	0.321±0.054	-23.00±0.60	94.59±2.47	0.867
F9	0.178±0.012	-18.10±3.29	41.55±3.65	0.653
F10	0.140±0.031	-16.80±3.60	69.65±2.34	0.744
F11	0.346±0.140	-31.41±5.48	64.86±2.75	0.689
F12	0.197±0.080	-23.13±2.36	79.95±1.86	0.797
F13	0.254±0.110	-10.97±0.53	37.42±2.44	0.646
F14	0.183±0.013	-18.11±3.29	90.29±1.23	0.837
F15	0.310±0.030	-28.42±4.15	80.63±1.76	0.766
F16	0.234 ±0.017	-34.17±2.83	49.30±1.09	0.725
F17	0.165±0.012	-28.41±1.08	64.86±2.76	0.751
F18	0.319±0.064	-13.92±2.46	72.58±2.37	0.768
F19	0.174±0.008	-17.64±1.16	57.14±1.86	0.657
F20	0.285±0.020	-21.73±2.74	77.67±3.56	0.718
F21	0.146±0.037	-24.80±1.21	77.97±2.58	0.782
F22	0.259±0.054	-19.74±3.70	43.38±1.43	0.686
F23	0.182±0.017	-21.80±1.78	79.05±2.01	0.741
F24	0.308±0.015	-12.59±2.34	84.32±3.62	0.867
F25	0.153±0.076	-16.83±3.61	87.92±1.75	0.728
F26	0.224±0.021	-33.29±3.14	72.58±2.19	0.764
F27	0.191±0.023	-14.35±2.89	84.27±1.14	0.795
F28	**0.169±0.016**	**-10.90±1.54**	**97.84±1.89**	**0.943**
F29	0.178±0.025	-25.09±4.42	88.69±2.56	0.786

Results are expressed as mean ± SEM.



(A) VESICLE SIZE DISTRIBUTION



(B) ZETA POTENTIAL MEASUREMENT

FIGURE 4: VESICLE SIZE DISTRIBUTION; (A) AND ZETA POTENTIAL DETERMINATION; (B) USING ZETASIZER ANALYZER REPORT (MALVERN ANALYZER) OF BATCH F28

The size distribution of vesicles was determined by zetasizer (dynamic light scattering). The mean vesicle sizes of various formulations are presented in **Table 2.** The vesicle size was found to be in the range of 235.2±5.3 nm to 851.3±1.5 nm. These variations in vesicles size were highly significant ($P < 0.001$). We conclude that small amount of phospholipids in liposomal membranes increases the flexibility of vesicles. To understand the effect of lipid composition on vesicle size, coefficient observed for drug loaded liposomes size was fitted in Eq. (1) to generate Eq. (3)

$$\mathbf{Y_2} = 515.16 + 64.89X_1 + 8.1X_2 - 15.28X_3 + 17.23X_4 - 27.68X_1X_2 - 83.88X_1X_3 - 11.03X_1X_4 + 114.72X_2X_3 - 90.95X_2X_4 - 23.48X_3X_4 + 74.32X_1^2 - 18.77X_2^2 + 42.32X_3^2 - 151.32X_4^2 \quad \ldots.(3)$$

In the equation, positive sign of coefficient shows the synergistic effect on the response, whereas the negative sign indicate the antagonistic effect on response. Positive correlation was observed for variables X_1, X_2 and X_4 on vesicle size of drug loaded liposomes. The variable X_3 had negative impact on vesicle size which means that increase in X_3 concentration will retard the vesicle size. Among all variables, effect of X_1 was more prominent on vesicle size than the effect of other variables as indicated in equation. It was revealed that enhancement of SPC showed the increasing in vesicle size. Effects of independent variables on vesicles size are also presented by three-dimensional graph in **Figure 5**. It was observed that use of only DSPC retards the vesicle size, but use of DSPC along with SPC showed the significant retarding effect on vesicle size.

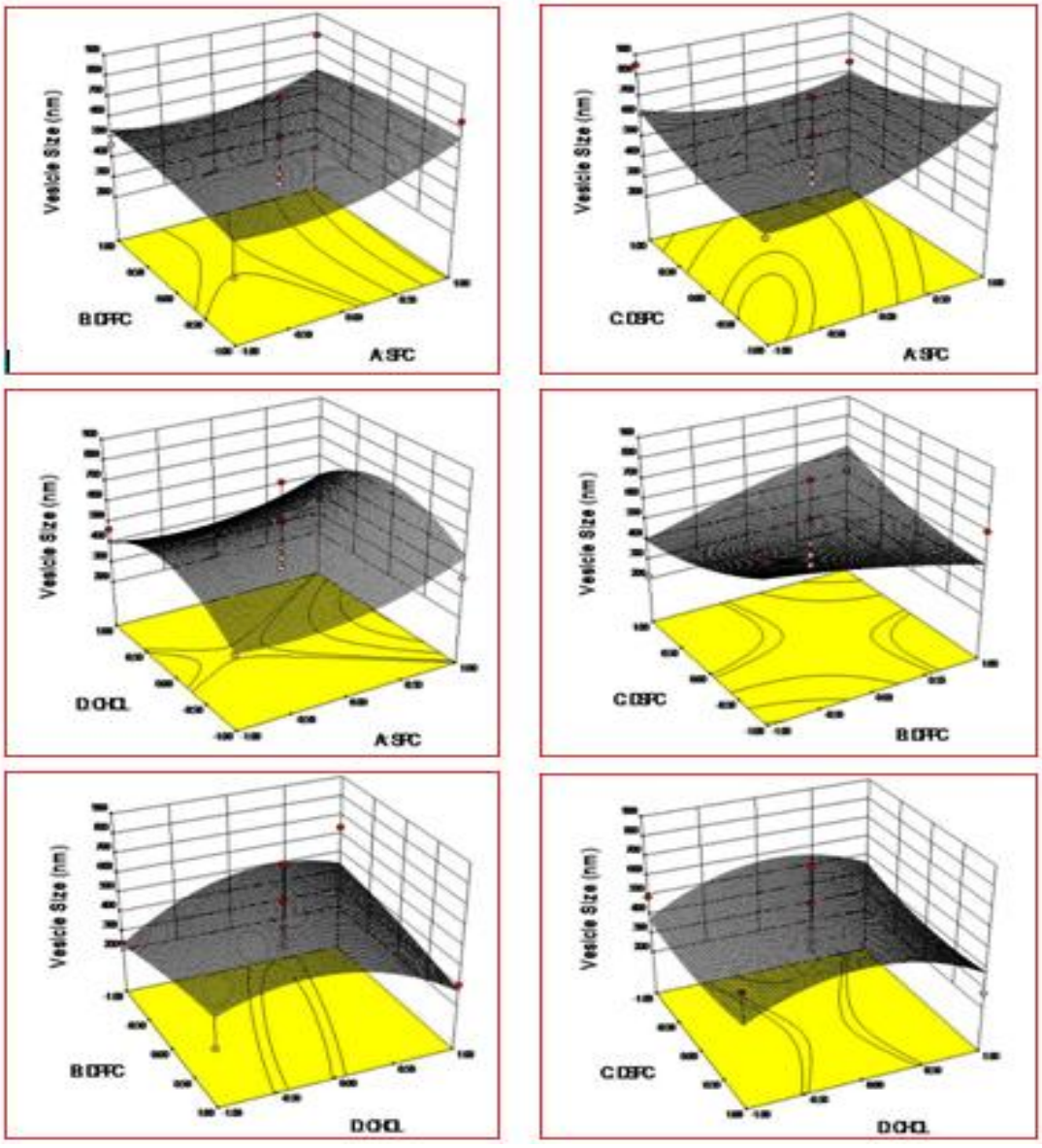


FIGURE 5: RESPONSE SURFACE PLOT SHOWING EFFECT OF INDEPENDENT VARIABLES ON VESICLE SIZE

***In vitro* Drug Release Studies from Liposomal Suspension:** The amount of drug release from the different liposomal suspension was found to be ranging from 37.42±1.48% to 97.84±2.65%. The %CDR of all formulations is shown in the **Table 3.**

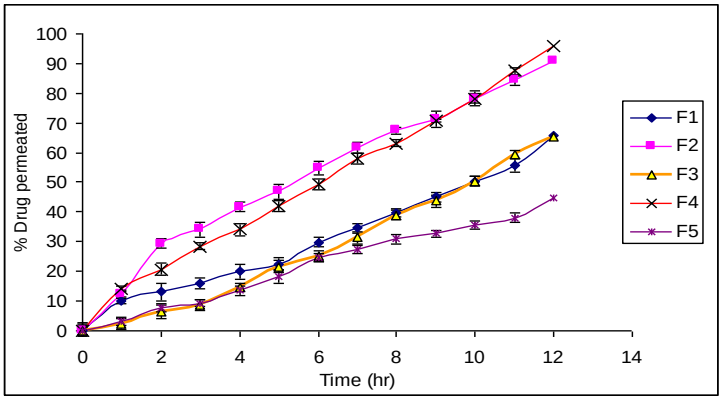
Physical examination, pH, Viscosity, Content uniformity of Gel: All liposomal gel formulations were found to be clear and transparent. The pH of the liposomal gel was found to be in the range of 6-7. The range of content uniformity was from 85.15% to 96.44%. The results of pH, viscosity and content uniformity of good formulations are shown in **Table 4.**

TABLE 4: EVALUATION OF LIPOSOMAL GEL

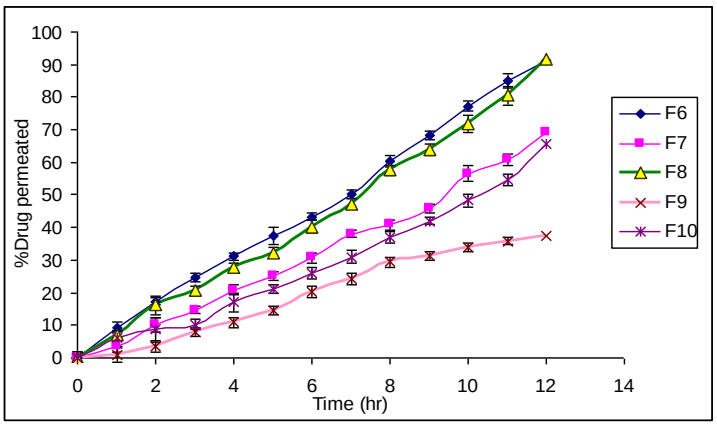
Formulation code	pH	Content uniformity %	Viscosity (Cps)	Clarity
F2	6.35 ±0.17	90.29 ±1.43	5654 ±218	Clear and transparent
F4	6.22 ±0.05	88.17 ±2.36	6765 ±347	Clear and transparent
F6	6.94 ±0.02	94.75 ±0.86	5380 ±215	Clear and transparent
F8	6.77 ±0.09	93.69 ±1.71	6317 ±481	Clear and transparent
F14	6.48 ±0.04	91.56 ±0.28	6125 ±518	Clear and transparent
F28	**6.71±0.24**	**96.44±1.47**	**4873±830**	**Clear and transparent**

Results are expressed as mean ± SEM.

***In vitro* drug permeation studies from Liposomal Gel:** **Figure 6** shows the *in-vitro* permeation profile of the Quetiapine from the different liposomal gel formulations in 7.4 pH phosphate buffer. *In vitro* permeation of Quetiapine from the liposomal gel formulation was found to be in the range of 30.93 ±4.85% to 97.64±1.34% during a period of 12 h. Thus, the liposomal gel formulations release the drug for prolonged period. No lag phase was observed in any of the formulations.



A



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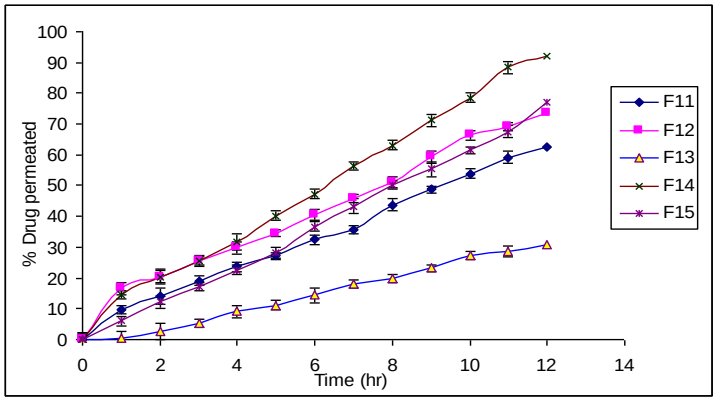

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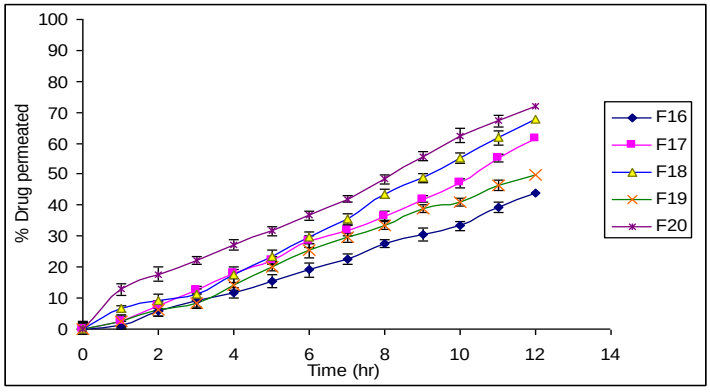
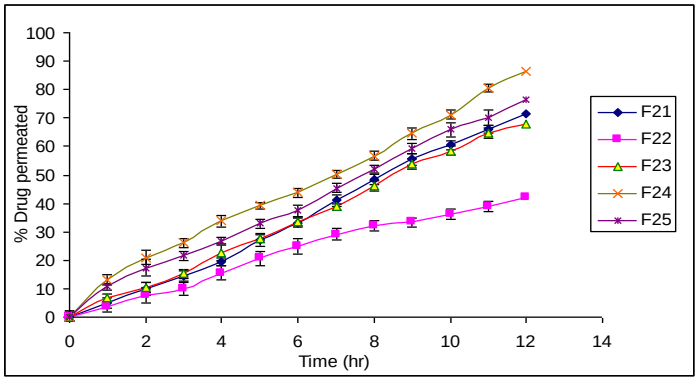
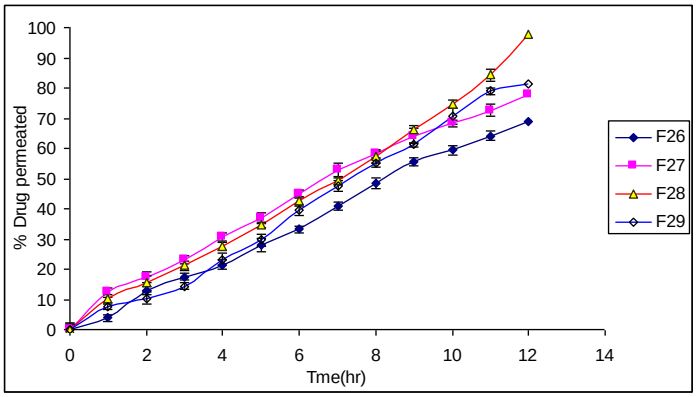


D



E



F

FIGURE 6: DRUG PERMEATION PROFILE OF LIPOSOMAL GEL FORMULATIONS

A. Percentage cumulative drug permeated from liposomal gel batches F1 to F5.
B. Percentage cumulative drug permeated from batches F6 to F10.
C. Percentage cumulative drug permeated from batches F11 to F16.
D. Percentage cumulative drug permeated from batches F17 to F20.
E. Percentage cumulative drug permeated from batches F21 to F25.
F. Percentage cumulative drug permeated from batches F26 to F29.

Effect of different concentration of lipid on permeation of quetiapine is estimated from the Eq. (4) as shown below;

$$Y_3 = 72.55-6.39X_1 + 6.36X_2 -1.28X_3 +2.17\ X_4-3.29X_1X_2 -3.79X_1X_3+6.74X_1X_4+6.08X_2X_3+7.3X_2X_4+5.97X_3X_4+6.27X_1^2 -3.17X_2^2-2.7X_3^2-8.28X_4^2 \quad \ldots\ldots(4)$$

It was confirmed that variables X_1 and X_3 reduce the drug release from lipidic bilayer. Whereas, variable X_2 and X_4 enhance the drug permeation. DSPC is saturated lipid which forms the rigid bilayer of liposome. Due to lipophilic nature of drug, it was incorporated in the intralamellar spaces of vesicular bilayer which may also reduce the release of drug and provide prolong release of drug from vesicle.

Among all the variables, DPPC has prominent effect on the drug permeation. Effects of independent variables on drug permeation are presented by three-dimensional graph in **Figure 7.**

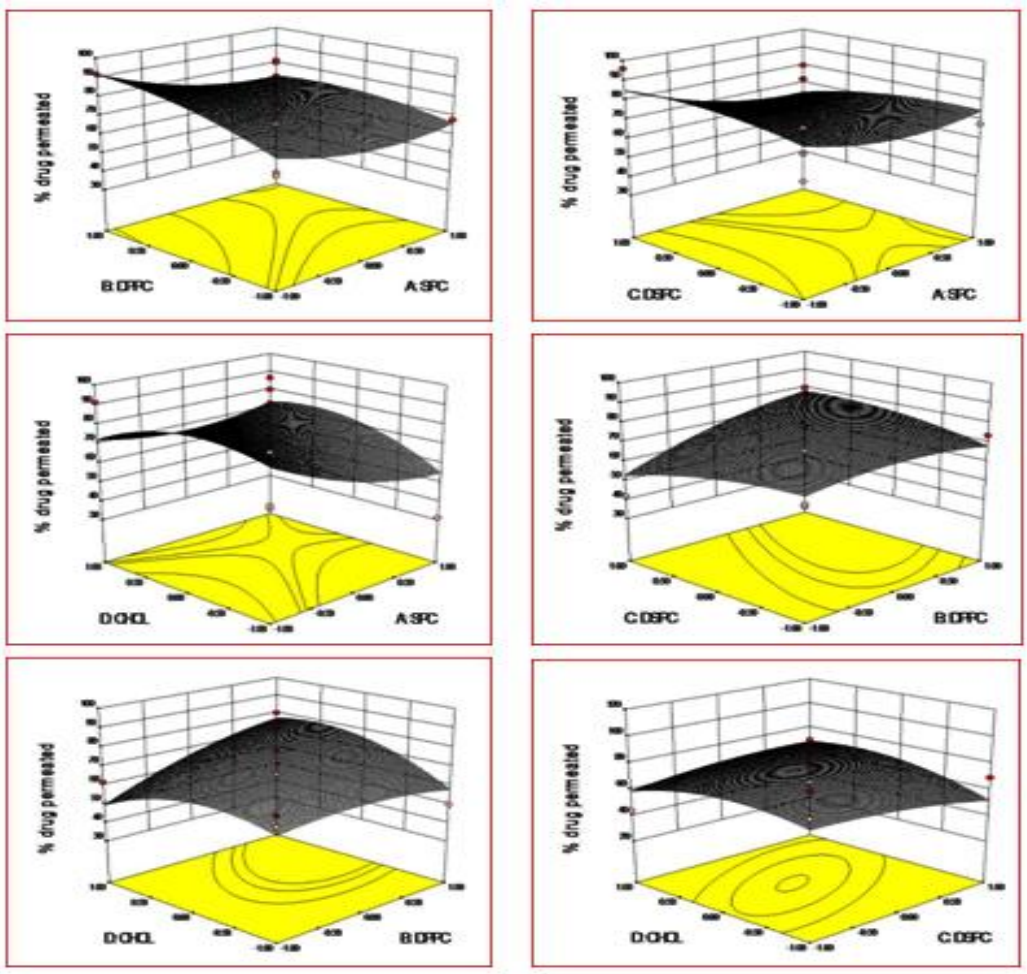


FIGURE 7: RESPONSE SURFACE PLOT SHOWING EFFECT OF INDEPENDENT VARIABLES ON PERCENT DRUG PERMEATION

From the figure, it was revealed that as concentration of DPPC was increase from the lowest to highest, the maximum drug permeation was found to be 91.81%.

The enhancement of SPC was not significantly affect the drug permeation When cholesterol concentration was increased from low to high level, it had positive impact on the drug permeation.

Kinetic Modeling: The release study data of Quetiapine loaded liposomes analyzed using rate constant equations such as zero order, first order, Higuchi and Korsmeyer peppas equations showed that liposomal formulations had the tendency to follow zero order diffusion pattern of release. Drug transport mechanism was found to be non-anomalous diffusion based zero order **(Table 5).**

TABLE 5: KINETIC FITTING RESULTS OF QUETIAPINE RELEASED FROM DRUG ENTRAPPED LIPOSOMES

Formulation code	Correlation coefficient (R2)			Peppas model	
	Zero order	First order	Higuchi	n^b	Correlation coefficient (R^2)
2	0.9760	0.6051	0.9765	1.5493	0.6545
4	0.9966	0.6669	0.9384	1.3902	0.6653
6	0.9972	0.7210	0.9183	1.3955	0.7627
8	0.9939	0.7593	0.8967	1.4133	0.7988
14	0.9960	0.6783	0.9306	1.3609	0.6560
28	**0.9907**	**0.7488**	**0.8885**	**1.3582**	**0.7533**

b: Diffusion exponent calculated based on the Peppas model

Optimization of Formulation: After formulating all the batches, the effect of independent variables on the response like Y_1, Y_2 and Y_3 was estimated from the equation of individual response. The optimum formulation of Quetiapine-loaded liposome system was selected based on the criteria of attaining the maximum value of in vitro skin permeation and EE %, minimizing the vesicles size by achieving the equation (2),(3),(4) for dependent variables. Upon "trading off" various response variables and comprehensive evaluation of feasibility search and exhaustive grid search, the formulation (F28) composition with SPC (100 mg), DPPC (80 mg), DSPC (50 mg), and Cholesterol (15 mg) was found to fulfill requisites of an optimum formulation (QTF-OPT).

The optimized formulation (F28) showed the EE % of 79.21±5.81% with vesicles size range and permeation across rat skin is 405.8±1.1 nm and 97.64±1.34% in 12 h respectively. The relation between observed and predicted values of the responses for the optimized Quetiapine liposomal gel is shown in **Table 6.** The optimized formulation has the highest (0.943) desirability function. The desirability of each formulation is shown in **Table 3.** The *J* flux value of drug for optimized formulation (F28) was 42.33 µg/min/cm^2.

Whereas, it was only 23.2727 µg/min/cm^2 for plain gel. **Figure 8** shows the comparison of drug permeation from plain gel and optimized liposomal gel (F28).

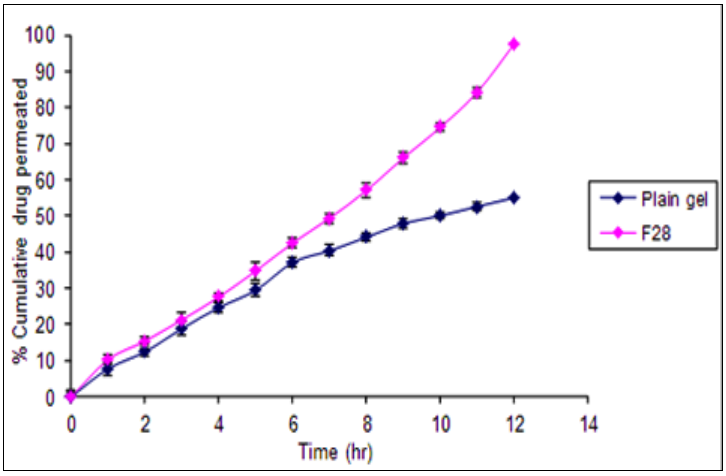


FIGURE 8: COMPARISON OF DRUG PERMEATION OF PLAIN AND LIPOSOMAL GEL OF QUETIAPINE (F28)

The calculated F value was found to be 3.26 which was greater than the tabulated F value, hence there was significance difference in drug permeation between liposomal and plain gel. This research work revealed that liposomal formulation enhances the drug permeation than the plain gel of drug. **Figure 9** quantitatively compares the resultant experimental values of the responses with those of the predicted values.

TABLE 6: OBSERVED AND PREDICTED VALUES OF THE RESPONSES FOR THE OPTIMIZED QUETIAPINE LIPOSOMAL GEL

Response	Observed	Predicted	Residual
Y_1: Entrapment efficiency % (EE %)	79.21	78.45	0.76
Y_2: Mean vesicle size (nm)	405.8	515.16	109.36
Y_3: % Cumulative drug permeated	97.64	95.27	2.37

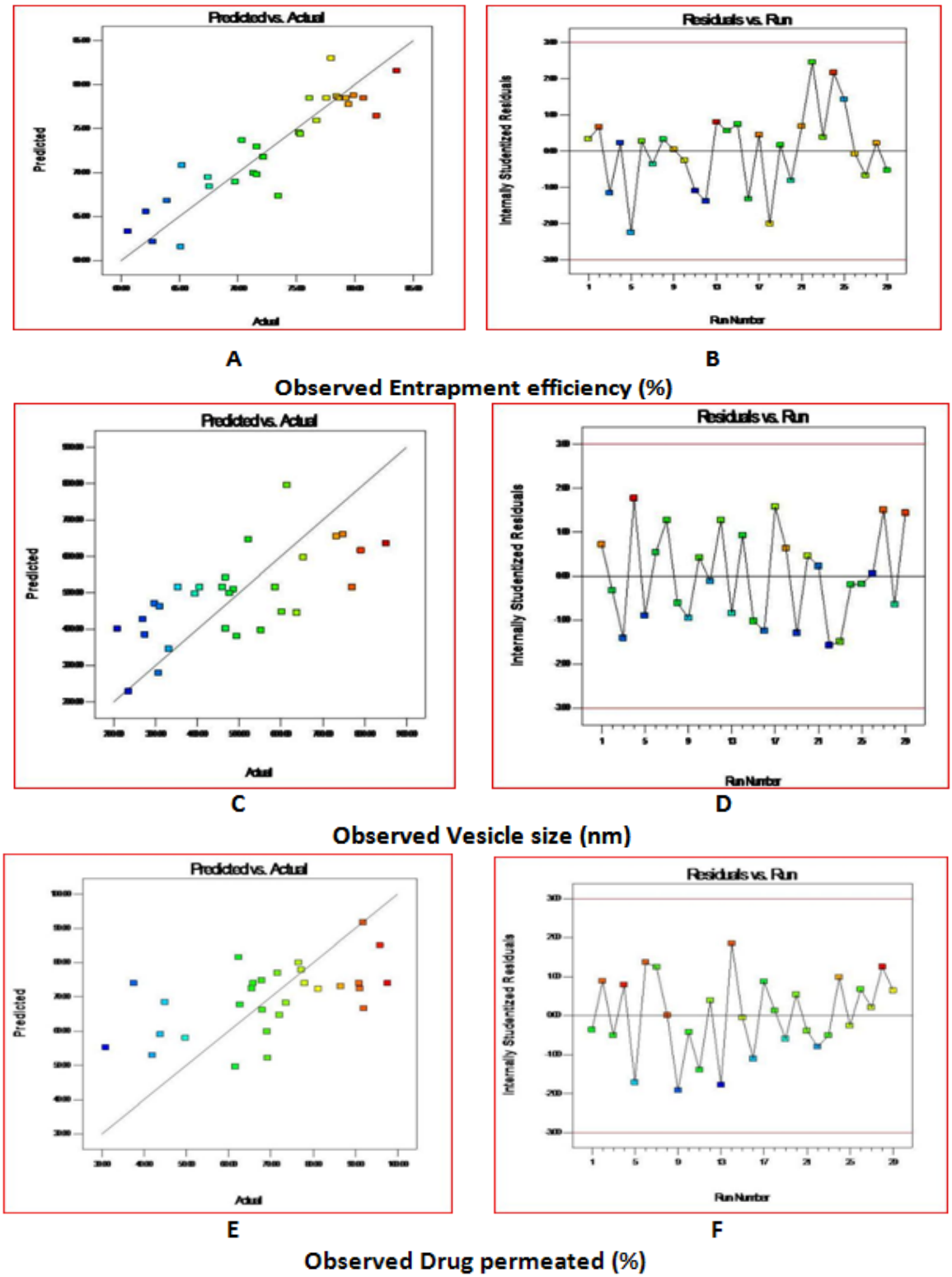


FIGURE 9: LINEAR CORRELATION PLOTS (A, C, E) BETWEEN ACTUAL AND PREDICTED VALUES AND CORRESPONDING RESIDUAL PLOTS (B, D, F) FOR DIFFERENT RESPONSES

Stability of formulation: The liposomal suspension and gel were stable for at least 6 months at 25 ±0.5 ºC temperature, with negligible change in EE %, size and zeta potential. There was negligible change in the viscosity and drug permeation rate from liposomal gel after stability studies of optimized formulation**.** The results of physical and chemical stability of liposomes in gel as well as suspension formulation are indicated in **Table 7,** which revealed that the formulation exhibits sufficient stability.

TABLE 7: STABILITY STUDY OF F28 AT 25±0.5°C

Evaluation parameters	F28			
	Initial	1 month	3 month	6 month
Liposomal suspension				
EE%	79.21±5.81	78.87±3.15	76.26±2.57	77.48±1.7
Vesicle size (nm)	405.8±1.1	443.7± 3.8	469.2±1.6	494.1±2.5
Zeta potential (mV)	-10.90±1.54	-9.5±1.9	-10.43±1.7	-10.37±2.6
Liposomal gel				
Viscosity (cps)	4873±830	4526±115	4963±592	4357±647
% cumulative drug permeated in 12 h.	97.64±1.34	96.11±1.52	96.21±1.46	97.38±1.91

Results are expressed as mean ± SEM.

CONCLUSION: The results of the present study showed that deformable lipid vesicles, improve the transdermal delivery of the lipophilic drug, Quetiapine. The formulation-optimizing study using statistical experimental design showed that optimum concentrations of each phospholipid are required to provide the maximum value of transdermal flux or skin permeation, gaining maximum EE %, minimizing the vesicles size.

The results of the present study demonstrated that introduction of liposome as a vesicular drug carrier overcomes the limitation of low penetration ability of Quetiapine across the skin. Hence, it could be concluded that liposomes are a potentially suitable carrier for transdermal delivery of Quetiapine. Further studies are needed to establish their therapeutic utility in human beings.

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