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Original Article

Formulation, Development and Characterization of Liposome-based Gel of Eberconazole Nitrate for Topical Delivery

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ABSTRACT

Objectives: The present investigation was aimed to develop Liposome-Based Gel (LBG) for Eberconazole nitrate. The purpose was to administer the drug at a sustained rate through skin to improve bioavailability for longer period of time. **Materials and Methods:** Thin film hydration technique was used for the preparation of EBZ loaded liposomes. Preliminary trials were conducted for the selection of type of lipid and its ratio with cholesterol along with the lipid:drug ratio. EBZ loaded liposomes was optimized by using central composite design with amount of phospholipid, cholesterol and lipid:drug ratio as independent variables along with vesicle size and %Entrapment Efficiency (%EE) as dependent variables. Each formulation was evaluated for vesicle size, polydispersity index, % entrapment efficiency, pH and zeta potential. Further, with an aim to provide enhanced patient compliance the optimized batch of EBZ loaded liposomes was transformed into Liposomal Based Gel (LBG). **Results and Conclusion:** Results of all design batches showed nano size of the liposomal vesicles with good dispersion. The optimized batch of EBZ loaded liposome showed a vesicle size of 183.4 nm and 92.4% entrapment efficiency. TEM images of the optimized liposomal formulation showed well separated vesicles with narrow size distribution. The results of *ex vivo* skin permeation study of the optimized batch of EBZ loaded LBG revealed a remarkable improvement in the dissolution as compared to its conventional formulation. All these concluded LBG as one of the suitable approaches for developing topical formulation of poorly water-soluble drugs like eberconazole nitrate.

Keywords: Liposomes, Central composite design, Liposomal based gel, Eberconazole nitrate.

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INTRODUCTION

The skin, which is the body's outermost layer, serves as a barrier against pathogens and other foreign objects entering the body as well as shielding the wearer from hazardous environmental stimuli including light, heat, and radiation. The skin's protective nature makes topical drug administration particularly hard. Hence, a number of unique ways have been adopted to boost percutaneous absorption and improve medication permeability over the skin.[1] As a result, carrier systems must be developed to improve penetrability. These innovative drug delivery systems' key tenets include localization, sustaining a release profile for a predetermined amount of time, and preserving stability at the site of action. Many formulations, including nanovesicles, liposomes, niosomes, micelles, and nanocrystals, were created for this aim. The targeted distribution of drugs to the dermal layer using liposomal formulations offers advantages for the controlled release of molecules with low permeability.[2]

Liposomes are spherical, nanoscale vesicles made up of a lipid bilayer and water molecules. As a result, a diverse range of active moieties may be encapsulated in liposomes due to their hydrophilic or hydrophobic nature. Liposomes have the advantage of being biocompatible due to their similarity to cellular membranes.[3] The liposomes are dispersed in a gel matrix to create liposomal gel, which is semisolid dosage form for external use. A medication can be dispersed well in liposomal gel owing to its highly hydrophilic Three-Dimensional (3-D) network structure. The liposome gel has the benefit of being a liposome carrier in addition to having a distinct solution-gel transition property that makes it simple to create, simple to use, and have a strong affinity for skin tissue.[4]

Eberconazole Nitrate (EBZ) is an imidazole derivative, used topically in the treatment of superficial fungal infections. EBZ, 1-(2, 4-dichloro-10, 11-dihydro-5H-dibenzo [a, d] cyclohepten-5-yl)-1H-Imidazole nitrate, acts by inhibition of fungal lanosterol 14α-demethylase. This causes changes to its structure and

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Table 1: Selected level of independent and dependent variables.

Independent variables	Name of the variables	-α	-1	0	1	+α
X_1	Amount of Phospholipid (PL 97%) (mg)	200	341.49	550	758.11	900
X_2	Amount of Cholesterol (mg)	20	34.19	55	75.81	90
X_3	Lipid: drug ratio	10	18.11	30	41.89	50
Dependent variables		Constrains				
R_1	Entrapment efficiency (%EE)	Maximum				
R_1	Vesicle size (nm)	Minimum				

PL 97%: Soya phosphatidylcholine grade with 97% purity.

activity, which prevents the fungus from developing. Since the infection is frequently restricted to the outermost layers of skin, topical antifungal medications are useful in the treatment of dermatophytoses. The most popular and preferred first line treatments for localized dermatophytoses are topical medicines with strong local bioavailability.[5,6] The main aim was to enhance the permeation rate and improve the stability of EBZ when delivered through the skin.

MATERIALS AND METHODS

Materials

Eberconazole Nitrate was received as gift sample from Precise Chemipharma Pvt. Ltd., Navi Mumbai, India. PL 35% (Soya phosphatidylcholine grade with 35% purity), PL 97% (Soya phosphatidylcholine grade with 97% purity) and Lipoid (PL 90H, PL 90G Germany) were purchased from Himedia, Mumbai, India. Isopropyl alcohol and Chloroform were purchased from Chemdyes Corporation, Rajkot. Cholesterol was purchased from Suvidhinath Laboratories, Vadodara. HPLC grade Methanol and ethanol were purchased from Purvi Enterprises, Ahmedabad. Carbopol 940® was purchased from Alpha chemika, Mumbai. Commercially available Eberconazole cream was procured from local pharmacy for research purpose. Ultrapure distilled water was used all over the procedure. All other chemicals were used of HPLC or analytical grade.

Methods

Drug and Excipient compatibility studies

The compatibility between pure drug, cholesterol, and phosphatidylcholine was detected by FTIR analysis. The aim of this study was to test whether there is any interaction between the lipid carriers and drug. The compatibility was evaluated using an IR spectrophotometer (Bruker ALPHA-II, Japan.) The IR spectra was obtained from 4000 cm^{-1} to 400 cm^{-1} after approximately 5 mg of sample was fully mixed with 100 mg of potassium bromide (KBr) IR powder and compressed under vacuum for three minutes at a pressure of around 12,000 psi.[7]

Preparation of EBZ loaded liposomes

Eberconazole liposomes were prepared by thin film hydration technique using (Buchi, Rotary evaporator). Initially preliminary trials were conducted for the selection of the type of phospholipid, its ratio with cholesterol and also for the selection of the lipid:drug ratio. Accurately weighed amount of drug (EBZ), Phospholipid (PL) and Cholesterol (CH) were dissolved in a solvent mixture (chloroform: ethanol, 2:1 v/v) in 250 mL round-bottom flask. A thin layer of lipid film was formed on the wall of round-bottom flask by evaporating the organic solvent using a rotary film evaporator under reduced pressure. During the preparation process, the instrumental conditions such as temperature (55°C) and rotation speed (100 rpm) were maintained. The flask was kept under vacuum in desiccator to ensure complete removal of residual solvents. The obtained thin lipid film formed on the wall of the flask was then hydrated using phosphate buffer saline pH 7.4 solution at 55 ± 2°C. The resulting vesicle dispersion was vortexed for 5 min and then left undisturbed for 2–3 hr for complete process of hydration. The developed formulations were characterized with respect to carrier-specific parameters.[8]

Experimental design

In this study, central composite design was employed to optimize Eberconazole Nitrate Liposomes (EBZLs). In order to optimize, the amount of Phospholipid (PL 97%) (mg) (X_1), Amount of Cholesterol (mg) (X_2), and Lipid: drug ratio (X_3) was selected as independent variables. Each factor was set at five levels +α, +1, 0, −1, and −α. fifteen formulations of EBZLs (B1–B15) were designed using Design Expert® software version 7.1.5. % Entrapment efficiency (R_1) and vesicle size (R_2) were taken as dependent response parameters. The actual values and coded values of different variables are given in Table 1.[9] Fifteen formulations of EBZLs (B1–B15) were designed and given in Table 2.

Evaluation Parameters

Vesicle size, polydispersity index and zeta potential

The average liposome diameter and the Polydispersity Index (PDI) of liposomes were measured by Malvern (Zetasizer, MALVERN (ZEN1690), UK) at a fixed scattering angle of 90°

at 25°C. All formulations were diluted with distilled water (1:10 ratio) to ensure intensity adjustment. The same instrument was used to measure the zeta potential. A measurement of the repelling forces between vesicles is the zeta potential. Larger repulsive forces would prevent vesicles from aggregating and make them more stable.[9,10]

Percentage Entrapment Efficiency (% EE)

Centrifugation method was used to measure the entrapment efficiency of liposomes dispersions. Liposomes were centrifuged (REMI Elektrotechnik Ltd., India) at 20000 rpm for 1 hr at controlled temperature of 4°C. Supernatant containing unentrapped EBZ was withdrawn and measured UV spectrophotometrically (Shimadzu UV-1800i, Japan) at 208 nm against phosphate buffer (pH 7.4). The amount of EBZ entrapped in liposome was determined as follow:

$$\% \, EE = \frac{Ci - Cf}{Ci} X \, 100 \qquad (1)$$

Where C_i is initial concentration of drug used in formulating the liposomes and C_f is concentration of EBZ in supernant. The entrapment efficiency was obtained by repeating the experiment in triplicate and the values were expressed as mean standard deviation.[11]

Transmission Electron Microscopy (TEM)

A drop of liposomes dispersion was placed and adsorbed on microscopic carbon coated grids. These grids were subsequently negatively stained with 1% (w/v) aqueous solution of phosphotungstic acid, dried, and viewed under TEM (Talos F200i FEGTEM, Thermofisher, USA) at suitable magnifications, operating at an acceleration voltage of 200 kV.[10]

Preparation of drug loaded liposomes based gel

The viscosity played a vital role in increasing the drug retention on the skin since liposomes have a very low viscosity and cannot be held at the site of application for an adequate amount of time. Carbopol has been used in this study to increase the viscosity of liposomes, resulting in created liposomes-based gel that offers higher formulation application. Carbopols is regarded as a secure and non-irritating gelling agent, and there have been no reports of human sensitivity to it when applied topically. For this, Carbopol-940 was first dissolved in water and left aside for a sufficient amount of time to allow the polymer chains to fully hydrate and expand. EBZ loaded liposomal gel formulations were prepared by incorporation of optimized liposome dispersions into the structured vehicle of carbopol 940 with gentle mechanical mixing at 25 rpm for 10 min. Triethanolamine was added for neutralization. The same procedure was followed to prepare conventional gel containing EBZ.[12,13]

Evaluation of liposomes based gel

Physical examination

The EBZ Liposomal gel was prepared by the procedure mentioned and evaluated for color, odor and transparency.[13]

pH

The pH of the gel was recorded using digital pH meter (EZODO, Taiwan). Initially the pH electrode was calibrated and 1% aqueous solution of liposome-based gel was taken and electrode was immersed into the formulation till the fluctuation in readings stopped and a constant reading was obtained. This procedure was repeated three times and the result were recorded.[14]

Drug content

Liposomes based gel (1 g) was suspended in 100 mL solution of chloroform: methanol (2:1). The resultant dispersion was kept for 20 min for complete mixing with continuous agitation and filtered through a 0.45 µm membrane filter. The drug content was determined spectrophotometrically at 208 nm. Results were based on triplicate determination.[15]

Viscosity

Viscosity of prepared gel was measured by using rheometer (Brookfield Engineering laboratories, USA). Approximately 1g of liposome-based gel was taken and rotated at 5 rotations per minute with the help of spindle S62. The temperature was maintained at 25°C. Reading indicates the viscosity of the formulations in cps.[16]

Spreadability study

It was determined by wooden block and glass slide apparatus. Weights about 20g were added to the pan and the time were noted for upper slide (movable) to separate completely from the fixed slides. Spreadability was then calculated by using the formula:

$$S = ML / T \qquad (2)$$

Where,

S = Spreadability

M = Weight tide to upper slide

L = Length of glass slide

T = Time taken to separate the slide completely from each other

Lesser the time taken for the separation of two slides, better the Spreadability.[13,16]

Ex vivo skin permeation study

Ex vivo skin permeation study was performed by using Franz diffusion cell with an effective diffusion area of 2.0 cm^2. The excised goat skin sample was clamped between the donor and the receptor chamber of diffusion cell with the stratum corneum facing the donor chamber. Then, liposomal based gel containing

drug equivalent to 10 mg of EBZ was placed onto the donor chamber. The receptor chamber was filled with phosphate buffer pH 7.4 containing 20% v/v PEG 400. The receptor medium was maintained at 37°C ± 0.5°C and stirred at 100 rpm throughout the experiment. For each experiment, 1 mL sample was withdrawn from the donor compartment at pre-determined time intervals (0.25, 0.50, 0.75, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 24 hr) and same volume was replaced with fresh phosphate buffer pH 7.4. The absorbance of withdrawn sample was measured after appropriate dilution at 208 nm to estimate concentration of EBZ. Same procedure followed for conventional gel. The experiments were performed in triplicates, and the results were represented as the mean values of three runs. The Korsmeyer-Peppas model, the Higuchi model, the first-order and zero-order mathematical models were utilized to evaluate the kinetics and mechanism of drug release from the liposomes-based gel, and the best-fitted model was chosen based on high correlation coefficient (R) values for the release data.[17]

Stability study

The tendency of drug leakage, vesicle size, and extent of structural integrity of selected formulation was observed for a period of 3 months by storing the sealed formulation in glass vials (n=3) at two different temperatures, 4-8°C (Refrigerator; RF), 25±2°C (Room temperature; RT). To accomplish this, samples were taken out periodically and their drug content, vesicle size and physical changes were monitored by visual observations.[18]

RESULTS AND DISCUSSION

Compatibility study

FTIR (Fourier transform infrared) spectra were constructed to check the possible interaction between pure drug and liposomal components. The interpreted data were shown in Table 3. All the major peaks representing the characteristic functional groups seen in the spectra of pure drug (Figure 1a) were also remain present in its physical mixture (Figure 1b) which confirms the compatibility of the EBZ with cholesterol and phospholipid.

Preliminary trials

EBZ loaded liposomes were prepared by the thin-film hydration method at different Phospholipid to cholesterol (PL: CH) ratio and lipid: drug ratio as shown in Table 4. This method is one of the simplest methods to prepare liposomes on a small scale in a research laboratory. For the selection of a main lipid for further liposome preparation, four different types of phospholipids (PL 35%, PL 97%, PL 90H and PL 90G) were taken in a ratio of 50:50 and 70:30 with Cholesterol (CH). Further to study the effect of lipid: drug ratio, the liposomes were prepared with lipid: drug ratio of 10:1 and 30:1. Likewise total 16 (P1 to P16) batches were prepared and evaluated for visual appearance, shape, and vesicle diameter and % entrapment efficiency. From the results shown in Table 4 it was found that PL 97% in a ratio of 70:30 with cholesterol and lipid: drug ratio of 30:1 form a stable small size vesicle with highest entrapment efficiency (78.8%) compare to all other formulations.

Further four batches (P17 to P20) were prepared to study the effect and select the range of lipid: drug ratio. Here PL 97% in a ratio

Table 2: Central composite design layout.

Batch	X_1	X_2	X_3
B1	-1	-1	-1
B2	1	-1	-1
B3	-1	1	-1
B4	1	1	-1
B5	-1	-1	1
B6	1	-1	1
B7	-1	1	1
B8	1	1	1
B9	-1.68	0	0
B10	1.68	0	0
B11	0	-1.68	0
B12	0	1.68	0
B13	0	0	-1.68
B14	0	0	1.68
B15	0	0	0

X_1: Amount of Phospholipid (PL 97%); X_2: Amount of Cholesterol; X_3: Lipid: drug ratio.

of 70:30 with cholesterol was kept constant for all four batches. From the evaluation results it was observed that as the lipid: drug ratio was increased, % entrapment efficiency was increased, and this effect was significant up to lipid: drug ratio of 50:1. Further increase in the lipid: drug ratio (60:1) lead to decrease in the %EE and above that (70:1) there was a complete phase separation in the liposomal formulation. Hence it was decided to optimize the lipid: drug ratio between 10:1 to 50:1.

PL 35% (Soya phosphatidylcholine grade with 35% purity); PL 97% (Soya phosphatidylcholine grade with 97% purity); PL 90H (Phospholipon 90H with 90% purity); PL 90G (Phospholipon 90G with 90% purity); PL: Ch (ratio of Phospholipid to Cholesterol); %EE (Entrapment efficiency); P1 to P20 (Preliminary trial batch 1 to 20).

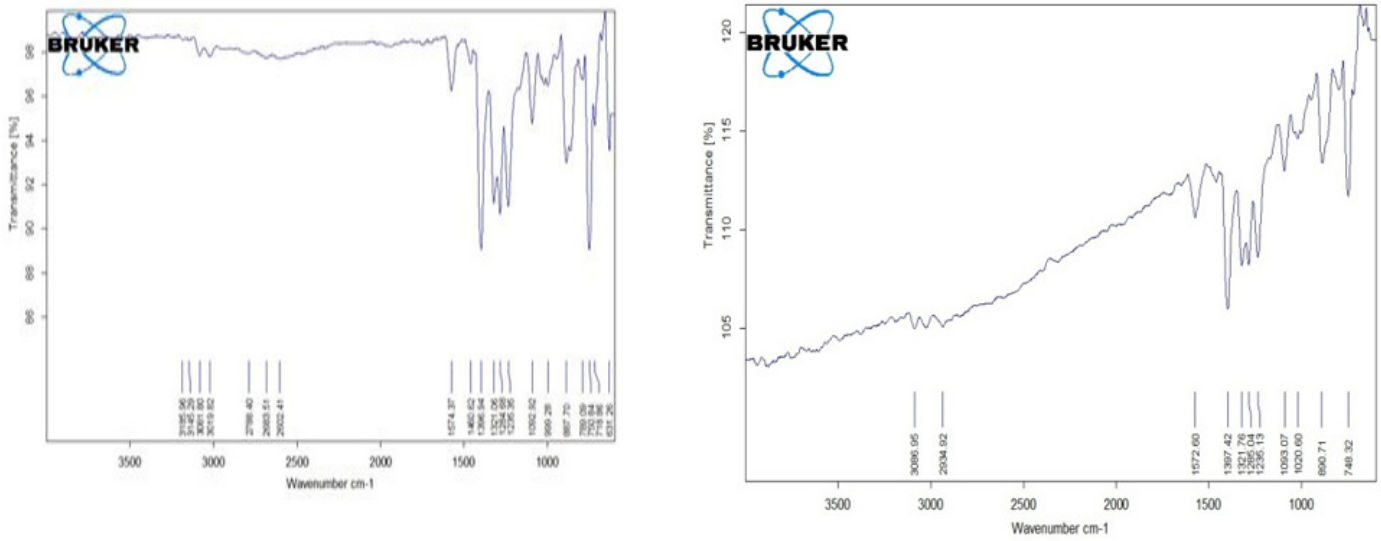
Table 3: Interpretation of FTIR spectra.

Functional Group	Pure drug (cm^{-1})	Physical mixture (cm^{-1})
Aromatic C-H stretching	3081.80	3086.80
Aromatic C-H bending	750.84	748.32
Methyl group C-H bending	1460.62	1397.42
Aromatic amine C-N stretching	1284.68	1285.04
Cyclic alkenes C=C stretching	1574.37	1572.60
Cyclic alkenes C=C bending	887.70	890.71
Aryl benzene (chloro benzene)	1092.92	1093.07

Experimental design

Preliminary investigations of the process parameters revealed that factors such as Amount of Phospholipid (mg) (X_1), Amount of Cholesterol (mg) (X_2) and Lipid: drug ratio (X_3) exhibited significant influence on response variables; hence, they were utilized for further systematic studies. Formulation of liposomes was performed while the amount of Phospholipid, Cholesterol and Lipid: drug ratio was changed as independent operational variables, according to a three-factor central composite design. Five different parameters of liposomes were examined: Vesicle size (nm), %EE, Polydispersity index (PDI), Zeta Potential (mV) and pH. Results are shown in Table 5.

The data clearly indicate the strong influence of X_1, X_2 and X_3 on selected responses (vesicle size and %EE). The polynomial equations can be used to draw conclusions after considering the magnitude of coefficients and the mathematical sign carried: positive or negative. For vesicle size, coefficient b_1, b_3, b_{12}, b_{13}, and b_{23} were found to be insignificant, as p values were more than 0.05 and hence removed from the full model. For %EE, values of b_1, b_2, b_3, b_{13} and b_{23} were insignificant and hence removed from the full model (Table 6). Table 7 shows the results of Analysis of Variance (ANOVA) performed to justify the removal of insignificant factors. The high values of correlation coefficients for vesicle size and %EE indicate a good fit. The tabulated (critical) values of F for vesicle size and %EE were found to be 0.606 (df = 5, 5) and 2.167 (df 5, 5) respectively. Moreover, calculated F value [4.093 (vesicle size) and 5.192 (%EE)] was found to be less than tabulated (critical) value, which suggests no significant difference between the full and reduced model.



A B

Figure 1: FTIR spectra of (a) EBZ (b) Physical mixture.

Table 4: Preliminary trials for the selection of components and its levels.

Batch	Type of lipid	PL: Ch	Lipid: drug	Visual Appearance	Shape	%EE	Vesicle diameter
P1	PL 35%\	50:50	10:1	Stable	Vesicular	46.3	Small
P2			30:1	Stable	Irregular, some Vesicular	53.9	Small to large medium
P3		70:30	10:1	Stable	Vesicular	55.3	Small to medium
P4			30:1	Phase separation	---	----	----
P5	PL 97%	50:50	10:1	Stable	Vesicular	62.5	Small
P6			30:1	Stable	Vesicular	63.4	small
P7		70:30	10:1	Stable	Vesicular	70.1	small, few medium
P8			30:1	Stable	Vesicular	78.8	Very small
P9	PL 90H	50:50	10:1	Stable	Vesicular	54.4	small
P10			30:1	Phase separation	---	----	----
P11		70:30	10:1	Stable	Vesicular	59.2	Small to medium
P12			30:1	Stable	Non-Vesicular	61.1	Large medium
P13	PL 90G	50:50	10:1	Stable	Vesicular	60.8	small
P14			30:1	Phase separation	---	----	----
P15		70:30	10:1	Stable	Irregular, Few Vesicular	67.3	Small
P16			30:1	Stable	Vesicular	69.9	Small
P17	PL 97%	70:30	40:1	Stable	Vesicular	78.1	Very small
P18			50:1	Stable	Vesicular	81.6	Very small
P19			60:1	Stable	Irregular, some Vesicular	77.2	Small to large medium
P20			70:1	Phase separation	---	---	---

The data of all the 15 batches of central composite design were used to generate interpolated values using Design Expert® software version 7.1.5. Medium levels of X_1, X_2 and X_3 were found to be favorable for lower value of vesicle size and high value of %EE. Multiple linear regression analysis (Table 6) also revealed positive value of coefficient b_2 for vesicle size. This indicated that as amount of cholesterol (X_2) was increased, there was a significant increase in vesicle size. Similarly positive values were obtained for coefficient b_1 and b_3 for %EE indicating increasing in entrapment efficiency with increasing in amount of phospholipid and lipid: drug ratio.

Influence of formulation composition factor on vesicle size and %EE

A strong influence of all the independent variables (amount of phospholipid, amount of cholesterol and lipid: drug ratio) was observed on vesicle size and %EE. The lowest value of vesicle size and highest value of %EE was observed with batch B15 which clearly indicates the number of liposomal components decreases the vesicle size and increase the %EE up to some level but after that level there is a negative effect on the required results for liposomes preparation. Response surface plot [Figures 2(a) and 2(b)] for %EE and vesicle size illustrated influence of all three factors.

Optimized batch analysis

Optimized batch was selected on the basis of following criteria: maximum % EE and minimum vesicle size. The result of vesicle size and %EE was compared with that of computed values (Table 8). Overlay plot was generated (Figure 3) by using Design Expert® software version 7.1.5. To confirm the validity of design, optimized batch of liposomes was prepared experimentally using the same procedure. The result of vesicle size and %EE was compared with that of computed values. When both (experimentally obtained and theoretically computed) values were compared, % error was found to be less than <8% for both responses which suggested suitability of design applied.

Evaluation of liposomes

Vesicle size analysis, polydispersity index, pH and Zeta potential measurement

The vesicle size of the liposomes is a crucial factor because it determines the rate and extent of the drug release as well

Table 5: Evaluation parameters of design batches of EBZ loaded liposomes.

Batch	Vesicle size (nm)	%Entrapment Efficiency	Polydispersity index (PDI)	Zeta Potential (mV)	pH
B1	284.3±5.1	70.6±2.7	0.049 ± 0.05	-22.1±1.5	6.8±0.4
B2	327.2±4.6	82.1±1.5	0.095 ± 0.06	-27.0±1.2	6.9±0.2
B3	349.6±9.2	74.5±2.1	0.113 ± 0.04	-26.5±1.6	6.6±0.2
B4	318.9±8.4	70.9±1.1	0.214 ± 0.05	-32.4±1.3	6.9±0.3
B5	293.2±4.1	66.3±1.2	0.335 ± 0.05	-26.8±2.0	6.7±0.3
B6	274.1±5.2	80.4±2.3	0.072 ± 0.06	-29.1±1.1	7.0±0.2
B7	370.3±6.9	74.2±1.3	0.342 ± 0.04	-31.5±2.7	6.9±0.1
B8	331.7±6.7	72.7±1.4	0.091 ± 0.06	-23.3±2.7	6.6±0.4
B9	359.1±9.4	73.7±1.7	0.083 ± 0.05	-19.3±2.2	7.0±0.3
B10	371.6±7.2	70±1.1	0.264 ± 0.03	-28.3±1.7	6.9±0.2
B11	272.3±8.2	81.4±1.8	0.090 ± 0.06	-25.3±2.1	6.7±0.2
B12	358.4±6.3	73±2.5	0.191 ± 0.03	-26.8±2.5	7.1±0.3
B13	286.5±4.4	72±1.6	0.345 ± 0.05	-31.7±1.5	6.8±0.3
B14	249.8±8.7	75.8±1.1	0.078 ± 0.04	-28.5±2.0	7.0±0.2
B15	181.6±3.2	92.5±1.1	0.048 ± 0.02	-31.3±1.8	6.9±0.4

Table 6: Summary of Multiple linear regression analysis.

Coefficient	%EE		Vesicle size (nm)	
	FM	RM	FM	RM
B_0	91.0767	91.0765	182.809	182.8090
B_1	0.6437	---	-1.7955	---
B_2	-0.5347	---	24.650	24.6502
B_3	0.3359	---	-5.3028	---
B_{12}	-3.4	-3.4	-11.637	---
B_{13}	0.925	---	-8.737	---
B_{23}	0.35	---	9.7125	---
B_{11}	-6.5895	-6.5895	63.4697	63.4697
B_{22}	-5.7924	-5.7923	45.7543	45.7543
B_{33}	-5.8632	-5.86323	29.0310	29.0310

FM (full model), RM (reduced model), %EE (entrapment efficiency).

permeation of drug through the skin. The vesicle size of the liposomes of all design batches was shown in Table 1. The vesicle size of optimized batch of liposome dispersion was found to be 183.4 ± 4.16 nm (Figure 4). This low value of vesicle size would definitely provide larger surface area leading to ease in drug transfer through the SC (Stratum Corneum) of skin. The Polydispersity Index (PDI) indicates the uniformity of vesicle size distribution within the formulation. The PDI value of optimized batch of liposome dispersion was found to be 0.459 indicating uniformity of globule size within the liposomes and also indicate that the liposomes were monodispersed to polydispersed. The zeta potential of all batches was found to be in the ranges between -19.3±2.2 mV and -32.4±1.3 mV. Since there were no positively charged molecules present, the negative charge of each formulation demonstrated the relationship of soya lecithin molecules at the liposomal surface. The zeta potential of optimized batch of liposome dispersion was found to be -30.5 ± 0.14 mV which justify the stability of the optimized formulation.[19] pH values of all batches were found to be nearer to the skin pH (7.4) which is very important in accordance to the skin irritation in topical formulation. The pH of optimized batch of liposome dispersion was found to be 6.8±0.3.

%EE

The determination of %EE is a crucial factor when it comes to liposomes because it might have an impact on the medication release and skin penetration. In the present study, the observed %EE for all design batches were in the range of 66.30–92.5%. The

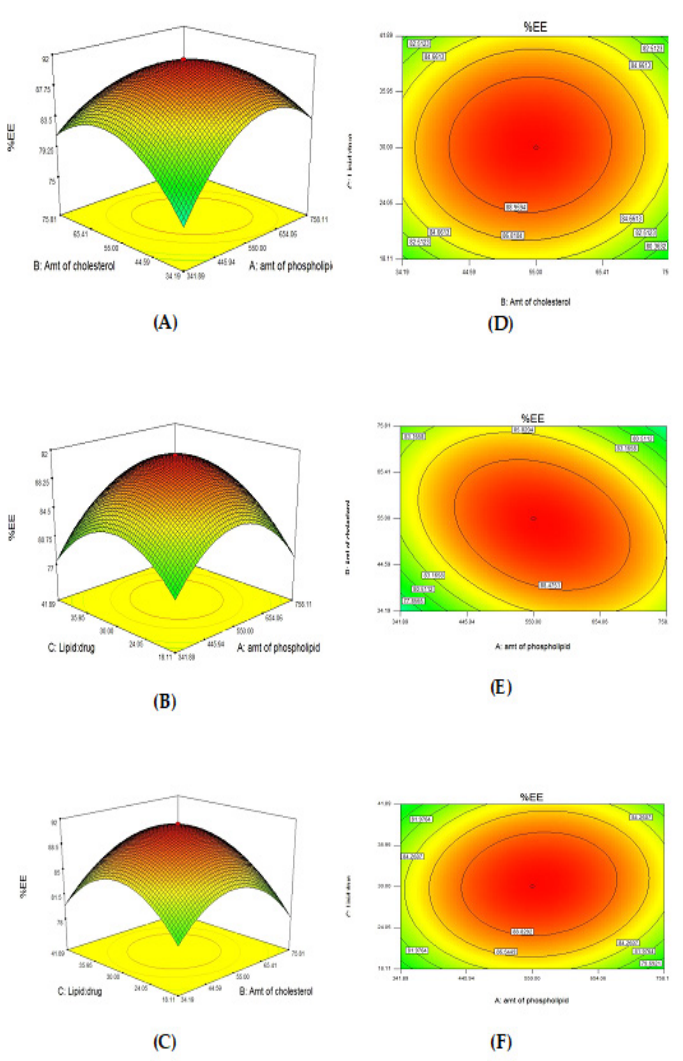


Figure 2(a): Influence of formulation composition factors represented by response surface plots showing effect of variables A) X_1 and X_2, B) X_1 and X_3, C) X_2 and X_3; contour plots showing effect of variables D) X_1 and X_2, E) X_1 and X_3, F) X_2 and X_3 on %EE (Entrapment efficiency).

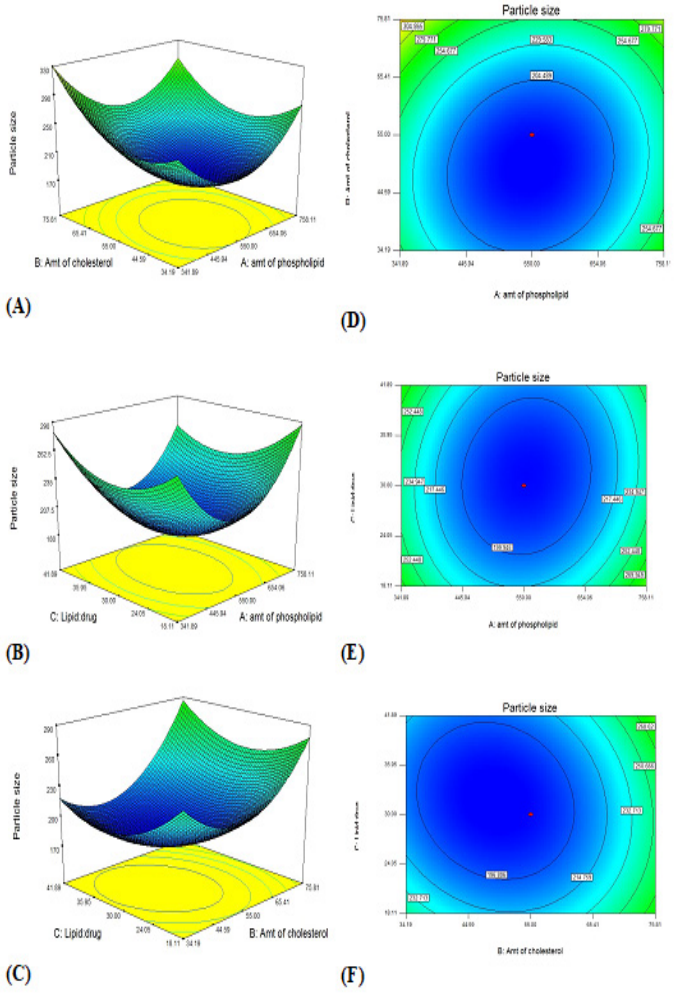


Figure 2(b): Influence of formulation composition factors represented by response surface plots showing effect of variables A) X_1 and X_2, B) X_1 and X_3, C) X_2 and X_3; contour plots showing effect of variables D) X_1 and X_2, E) X_1 and X_3, F) X_2 and X_3 on %EE (Entrapment efficiency).

%EE of optimized batch of liposomes dispersion was found to be 92.4 ± 0.14%.

Transmission Electron Microscopy (TEM)

To examine the shape of the optimized liposomal formulation, TEM study was performed. The outcomes are shown in Figure 5. The well-separated vesicles with narrow size distribution are visible in the TEM image of the optimized liposomal formulation with EBZ loading. The absence of aggregation is depicted in Figure 5. All of the visible vesicles were smaller than 200 nm, making them very well suited for topical application.

Preparation of drug loaded liposomes based gel

Liposomes are challenging to apply to skin because of their decreased viscosity. Therefore, they are tried to be gelled using appropriate gelling agent for the ease of application. The amount of gelling agent used in gel preparation was also optimized. The effects of three different Carbopol 940 concentrations were examined. Carbopol 940 was found to make a very hard, stiff gel at a concentration of 1.5% but was unable to produce an acceptable gel at a concentration of 0.5%. Only Carbopol 940 at a concentration of 1% w/w was able to produce gel (Figure 6) consistency without affecting the EBZ liposomes' architecture and thicken the liposomes.

Evaluation of Liposomes Based Gel (LBG)

The gels prepared were translucent and homogenous with absence of lumps. Due to the presence of gelling agent the viscosity for the LBG got enhanced as compared to liposomes. The optimized batch of EBZ loaded LBG showed the value of viscosity as 174.41 cps and that of conventional gel was 261.21 cps. The percentage drug content of the LBG was found to be 98.92 ±0.54% and that of conventional gel was 86.78 ±1.25%. pH measurements of LBG system were 6.6 ±0.28 and that of conventional gel was 6.9±0.4. Therefore, the pH of both prepared gels was within the required range and was considered to be safe and non-irritant for topical

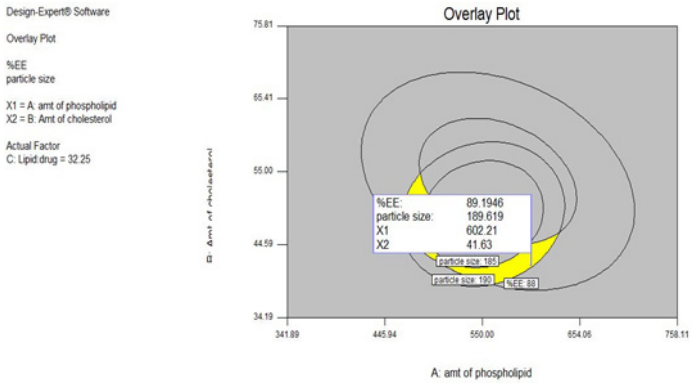


Figure 3: Overlay plot for optimization of Liposomes dispersion.

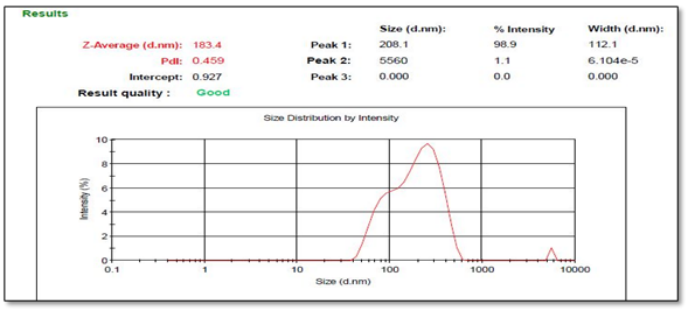


Figure 4: Vesicle size analysis of optimized liposomal formulation.

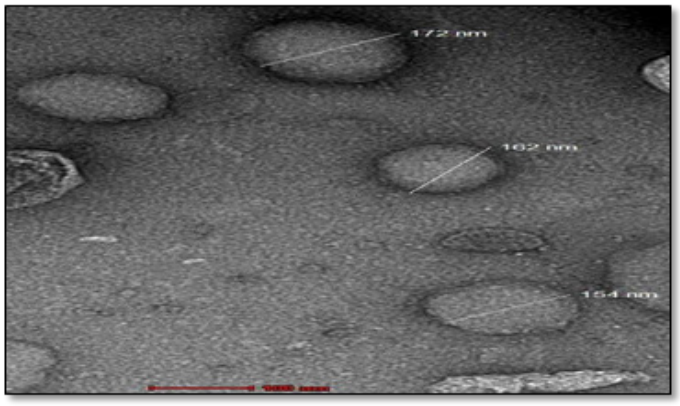


Figure 5: TEM study of EBZ loaded liposomal formulation

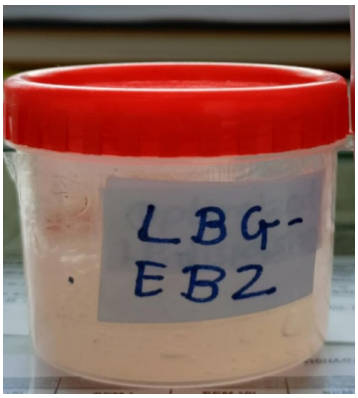


Figure 6: 1% w/w Liposome Based Gel of Eberconazole Nitrate.

Table 7: Analysis of variance (ANOVA).

	DF	SS	MS	R^2	F
Entrapment efficiency (%EE)					**DF=(5,5)**
Regression					F_{cal} =2.167
FM	9	416.733	46.303	0.9066	F_{tab} =5.192
RM	4	397.812	99.453	0.8654	F_{cal} <F_{tab}
Error					
FM	5	42.910	8.582		
RM	10	61.831	6.183		
Vesicle size (nm)					**DF=(5,5)**
Regression					F_{cal} =0.606
FM	9	37794.16	4199.35	0.9719	F_{tab} =4.093
RM	4	34917.62	8729.405	0.8980	F_{cal} <F_{tab}
Error					
FM	5	1089.10	217.820		
RM	10	3965.649	396.56		

FM (full model), RM (reduced model); F_{cal} (F calculated); F_{tab} (F tabulated)

application. The Spreadability is important for uniform and ease of application of topical preparation from patient compliance point of view. Spreadability of LBG was 33.96 gm.cm/sec and that of conventional gel was 14.88 gm.cm/sec. It indicates that LBG was easily spreadable with small amount of shear as compared to conventional gel.[4]

Table 8: Results of optimized batch of liposome dispersion.

Responses	Predicted value	Experimental Value[a]	Relative error (%)
Vesicle size (nm)	189.62	183.44 ± 4.16	-3.28
%EE	89.20	92.44 ± 0.14	3.59

[a] All data are shown in mean ± SD (n =3).

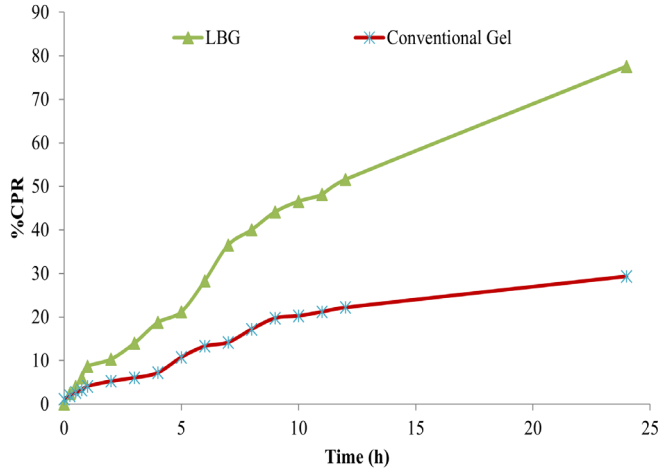


Figure 7: Percentage cumulative amount of Eberconazole nitrate permeated through goat skin over a period of 24 hr.

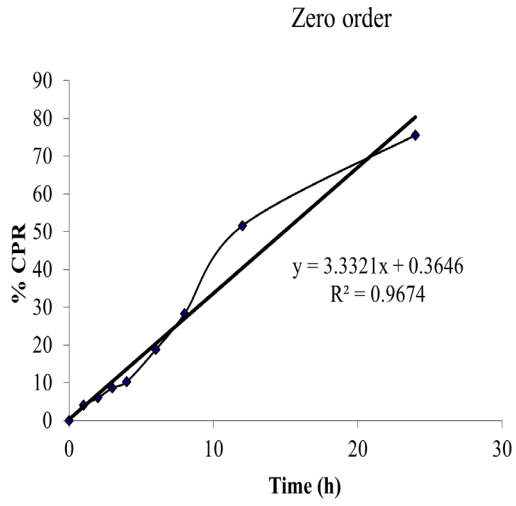


Figure 8: Kinetic modeling of drug release data of Eberconazole nitrate loaded LBG.

Ex vivo skin permeation study

The *ex vivo* skin permeation profiles of EBZ loaded liposomal based gel and conventional gel performed through excised skin of goat were shown in Figure 7. A steady increase of EBZ concentration in the receptor chambers with time was observed which support the sustained release of drug at site of application. The significantly higher value of drug release after 24 hr 77.55% for the optimized batch of EBZ loaded LBG as compared to its corresponding conventional gel 29.35% suggested marked improvement in release rate. The better penetration associated with LBG was might be due to the presence of phospholipid-constituents which modified the skin layer and enhanced the drug permeation, whereas in the case of conventional gel this form of interaction was not present. Thus, this *ex vivo* study for 24 hr revealed that the prepared LBG was better in facilitating the drug penetration and sustained release of EBZ.[8,20,21] Kinetic modelling of drug release data was investigated to analyze the drug release mechanism from the dosage form. The R^2 value of zero-order (Figure 8), first order, Higuchi and Korsmeyer-Peppas model were found to be 0.967, 0.714, 0.894 and 0.957 respectively. The zero-order shows the best fit model with the highest R^2 value.[22]

Stability study

The point of stability testing is to give proof on how the medicinal product contents Active Pharmaceutical Fixing (API) changes regarding time affected by various environmental variables. Optimized liposomes and LBG were found to be stable as both the formulations do not exhibit concentration change in drug, aggregation, vesicle disruption, phase separation and organoleptic characteristics.

CONCLUSION

The present study concluded the suitability of experimental design for design and development of liposomes as carriers for topical delivery of poorly water-soluble drug, EBZ. Independent variables of process parameters such as amount of phospholipid, cholesterol and lipid: drug ratio had an intense effect on the vesicle size and entrapment efficiency. The optimized batch of liposomal formulation was found to have nano sized vesicles with good morphological characters. The EBZ loaded liposomal formulation was successfully converted to gel using Carbopol 940 as gelling agent. The marked improvement in the *ex vivo* investigation compared to its conventional gel formulation is highly attributed to the reduction in the size of vesicles with increase in effective surface area and high entrapment efficiency. However, it further requires *in vivo* investigations before commercialization of the formulation.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

ABBREVIATIONS

LBG: liposome based gel; **EBZ:** Eberconazole nitrate; **%EE:** % entrapment efficiency; **PL 35%:** Soya phosphatidylcholine grade with 35% purity; **PL 97%:** Soya phosphatidylcholine grade with 97% purity; **PL 90H:** Phospholipon 90H with 90% purity; **PL 90G:** Phospholipon 90G with 90% purity; **PL:** Ch : ratio of Phospholipid to Cholesterol; **PDI :** Polydispersity index; **TEM:** Transmission Electron Microscopy; **%CPR:** Cumulative percentage release.

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