Contents lists available at UGC-CARE

International Journal of Pharmaceutical Sciences and Drug Research

[ISSN: 0975-248X; CODEN (USA): IJPSPP]

Available online at www.ijpsdronline.com

Research Article

Topical Delivery of Eberconazole Nitrate Loaded Microemulsion: Formulation, Design and Evaluation

Nidhi Shah[1*], Khushbu Patel[1], Indrajeet Singhvi[2]

[1]*Department of Pharmaceutics, Nootan Pharmacy College, Sankalchand Patel University, Visnagar, Gujarat, India.*
[2]*Faculty of Pharmacy, Nootan Pharmacy College, Sankalchand Patel University, Visnagar, Gujarat, India.*

ARTICLE INFO

Article history:
Received: 29 May, 2023
Revised: 10 July, 2023
Accepted: 12 July, 2023
Published: 30 July, 2023

Keywords:
Microemulsion, Eberconazole nitrate, Central composite design, Topical delivery.

DOI:
10.25004/IJPSDR.2023.150412

ABSTRACT

The objective of the present research work was to develop a microemulsion for the transdermal delivery of eberconazole nitrate (EBZ). Initially, oil, surfactant and co-surfactant were selected based on their solubility and emulsification study. A pseudoternary phase diagram was constructed to optimize the surfactant-co surfactant (S_{mix}) ratio. Eberconazole nitrate (EBZ) loaded microemulsion was optimized using central composite design (CCD) with amount of Capmul MCM (X_1), tween 80 (X_2) and transcutol (X_3) as independent variables along with the cumulative amount of drug release (Q_{24}) (Y_1), flux (Jss) (Y_2) and lag time (t_L) (Y_3) as dependent variables. Drug release study of all the design batches showed successfully increased permeation of drug which might be due to the compositional characteristics of ME. The globule size of the optimized batch of EBZ loaded ME (153.6 nm) confirms the micrometer size of the formulation. Zeta potential and polydispersity index (PDI) of the optimized batch was found to be -30.5 mV and 0.253, respectively, proving stability and uniform distribution of dispersed systems. The optimized batch of MEs has a pH value of 6.96 ± 0.21, indicating no chance of skin irritation. Further morphological and structural examination of the optimized batch of EBZ loaded ME was done by transmission electron microscope (TEM) and images illustrated the spherical micelles with size range of 100 to 200 nm which evidently may support the high absorption and results into the enhancement of drug permeation which may increase the therapeutic effect, decrease the dose frequency and improving the patience compliance for topical drug delivery.

Introduction

The pharmaceutical industry's topical drug delivery system has grown to represent a significant class. Even though this path was already found, new discoveries in this area continue to be made. Topical dosage forms are designed to conveniently distribute medications to a specific area of skin.[1] The primary benefit of a topical administration system is its capacity to administer medications more precisely to a particular spot. It enables the use of medications with a brief biological half-life and a restricted therapeutic window to lengthen the duration of effect. About 40% of new chemical entities have low water solubility, which poses a significant challenge to contemporary drug delivery systems and causes poor absorption, poor bioavailability, and other problems. Skin is a natural barrier for topical drug administration, making it challenging. To overcome such limitation, novel drug delivery systems like microemulsion, nanoparticles and vesicular systems are currently being castoff by investigators to expedite drug transportation through the skin.[2]

A microemulsion is anticipated to pass through the stratum corneum when applied to the skin, altering both the lipid and polar routes in its structure.[3] In the case of topical distribution, microemulsion improves the drug's skin transport due to its greater penetration rate as a result of the surfactant's appearance and lipophilicity.[4]

***Corresponding Author:** Ms. Nidhi P. Shah

Address: Department of Pharmaceutics, Nootan Pharmacy College, Sankalchand Patel University, Visnagar, Gujarat, India.

Email ✉**:** nidhishah514@gmail.com

Tel.: +91-9429305640

Relevant conflicts of interest/financial disclosures: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Eberconazole nitrate is an antifungal drug useful in the treatment for dermatophytosis, candidiasis, yeasts and pityriasis. EBZ acts by inhibition of fungal lanosterol 14α-demethylase.[5] This leads to an alteration in its structure and function, thereby inhibiting the growth of the fungus.[6] Topical antifungal agents are helpful in the treatment of dermatophytoses since the infection is often limited to the superficial layers of skin. EBZ is clinically effective in treating fungal infections, with a good safety profile and tolerability. It has acceptable topical availability with no detectable systemic drug levels and does not appear to cause skin sensitivity. To achieve its better antifungal activity, it is suitable to incorporate it into topical formulation rather than formulating the oral dosage form. This makes it an ideal candidate for topical delivery.[7]

In light of this, the present investigation aimed to develop microemulsion based topical delivery of EBZ for successful drug delivery with targeted flux value to achieve therapeutic drug levels for desired pharmaceutical effects.

Materials and Methods

Materials

Eberconazole nitrate was generous gift sample from Precise Chemipharma Pvt Ltd, Navi Mumbai, India. The materials like; Capmul MCM, Capmul PG8 and Captex 200P were obtained as gift samples from Indchem International, Mumbai. Solutol HS 15, Transcutol were obtained from Zeel Pharmaceuticals, Mumbai. Acrysol k 140 was obtained as gift sample from Corel Pharma Chemical, Ahmedabad. Tween 20, Tween 80, Tween 40, Sodium alginate were purchased from Suvidhinath Laboratories, Vadodara. HPLC grade methanol was purchased from Purvi Enterprises, Ahmedabad. Almond oil, castor oil, oleic acid, olive oil, cinnamon oil, polyethylene glycol, PEG 200 and PEG 400 were purchased from Astron chem., Ahmedabad. Cellophane membrane (LA387-5MT) was purchased from Himedia, Mumbai, India. Ultrapure distilled water was used all over the procedure was obtained from Umiya sales corporation, Ahmedabad. All other chemicals were used of HPLC or analytical grade.

Methods

Identification of drug

An Fourier transform infrared (FTIR) spectrum of pure drug was carried out by FTIR spectrophotometer (Bruker, ALPHA-II, Japan). About 5 mg of sample was mixed thoroughly with 100 mg potassium bromide (KBr) IR powder and compacted under vacuum at a pressure of about 12,000 psi for 3 minutes and the IR spectrum was recorded from 4000 to 400 cm^{-1}. The resultant spectra were compared for any spectral changes.[8]

Selection of Microemulsion Components

Screening of Oils (solubility study)

The solubility of EBZ in various oils was measured using the shake flask method. An excess amount of EBZ was added into each oil (Olive oil, Captex 200P, Miglyol 810, castor oil sunflower oil, almond oil, Imwitor 740, cinnamon oil, IPA (Isopropyl alcohol), Caprol PGE860, IPM (Isopropyl myristate), Capryol 90, (Acconon E, Capmul MCM, Capmul PG8 and Oleic acid) (2 mL) followed by sealing in glass vials. A vortex mixer was used to facilitate the solubilization. Sealed vials were stirred in a water bath at 40°C for 24 hours and allowed to reach equilibrium at 30°C for 24 hours. Each vial was centrifuged at 3000 rpm for 10 minutes using a centrifuge, followed by the removal of undissolved EBZ by filtering with a membrane filter (0.45 µm). Samples were suitably diluted with methanol and a drug concentration was obtained via a spectrophotometric method using methanol as a blank using a double-beam UV-visible spectrophotometer (Shimadzu UV-1800i, Japan) at 208 nm. The experiment was repeated in triplicates.[9]

Screening of surfactants (Emulsification study)

Surfactant was screened on the basis of their potential to emulsify the selected oil phase. Briefly 10 mL of 10%v/v aqueous solution of various surfactants was titrated individually by selected oil phase until it becomes hazy and the total amount of oil consumed was noted down.[10]

Screening of co-surfactants (Emulsification study)

The screening of the co-surfactant was conducted on the basis of ease of emulsification. Seven different co-surfactants were evaluated for their potential to emulsify the oil phase.[10]

Pseudoternary phase diagram

Raw materials were selected based on their drug solubilization and emulsification capacity and ME-region of oil, surfactant, co-surfactant, and water was constructed by water titration method using CHEMIX School 10.0 software. Surfactant was mixed with co-surfactant in fixed weight ratios (3:1, 2:1, 1:1, 1:2 and 1:3). surfactant and co-surfactant mixture (S_{mix}) were then mixed with oil in different ratios as 9:1, 8:2, 7:3, 6:4, 5:5, 4:6, 3:7, 2:8, and 1:9 (w/w). Water was added drop wise to each mixture under vigorous stirring by using a magnetic stirrer. The resultant mixture was visually observed for transparency. The samples were marked as points in the phase diagram and the area covered by such points was considered as the microemulsion region of existence.[11]

Preparation of drug loaded microemulsions

Drug-loaded MEs were prepared by dissolving 1% w/w EBZ in oil phase followed by the addition of an optimized surfactant and co-surfactant blend (S_{mix}). The resultant

mixtures were continuously stirred for a period of 2 minutes on vortex mixer. Further, the aqueous phase (distilled water) was added slowly with continuous stirring using magnetic stirrer (EIE instrument Pvt. Ltd, Ahmedabad) at 300 rpm at 37˚C for 10 minutes.[12]

Experimental Design

On the basis of ternary phase diagrams the levels of oil, surfactant and co-surfactant were decided and central composite design (CCD) was employed to evaluate the joint influence of the effect of independent variables Amount of Capmul MCM (X_1), Amount of S_{mix} (Tween 80:trancutol 1:2) (X_2) and Amount of water (X_3)]. The critical responses such as the cumulative amount of drug release per unit area at 24 hours (Q_{24}), Flux (Jss) and Lag time (t_L) were selected as dependent variables. The design consists of 15 runs (8 factorial points, 6 star points and 1 center point), yielding 15 experiments in total. Each was formulated in triplicates to estimate the model's reproducibility. A third-order quadratic model incorporating interactive and polynomial terms was used to evaluate the responses.

$$Y = b_0 + b_1X_1 + b_2X_2 + b_3X_3 + b_{12}X_1X_2 + b_{23}X_2X_3 + b_{13}X_1X_3 + b_{11}X_1^2 + b_{22}X_2^2 + b_{33}X_3^2 + b_{123}X_1X_2X_3 \quad (1)$$

Where Y is the measured response associated with each factor level combination; b_0 is an intercept; b_1 to b_{123} are regression coefficients computed from the observed experimental values of Y; and X_1, X_2, and X_3 are the coded levels of independent variables. The terms X_1X_2 and X^2i (i=1, 2, or 3) represent the interaction and quadratic terms, respectively. All three independent variables with their five different levels and complete experimental plan using Design Expert software version 7.1.5 was shown in Tables 1 and 2, respectively.[13]

Table 1: Actual and coded values with their levels of independent variables

Level	Factors		
	X_1	X_2	X_3
-α (-1.68)	5	30	10
-1	8.04	36.08	16.08
0	12.5	45	25
1	16.96	53.92	33.92
+α (+1.68)	20	60	40

X_1: amount of Capmul MCM, X_2: amount of S_{mix} (tween 80: Transcutol), X_3: amount of water

Table 2: Central composite design layout

Batch	X_1	X_2	X_3
ME1	-1	-1	-1
ME2	1	-1	-1
ME3	-1	1	-1
ME4	1	1	-1
ME5	-1	-1	1
ME6	1	-1	1
ME7	-1	1	1
ME8	1	1	1
ME9	-1.68	0	0
ME10	1.68	0	0
ME11	0	-1.68	0
ME12	0	1.68	0
ME13	0	0	-1.68
ME14	0	0	1.68
ME15	0	0	0

X_1: amount of Capmul MCM, X_2: amount of S_{mix} (tween 80: Transcutol), X_3: amount of water

Evaluation Parameters

Particle size and polydispersity index

The average droplet size and polydispersity index of all batches of MEs were measured by using a Malvern (Zetasizer, MALVERN (ZEN1690), UK) at a fixed scattering angle of 90° at 25°C.[14]

Determination of drug content

An accurately weighed quantity of drug-loaded ME was transferred to 10 mL volumetric flask and diluted with methanol up to 10 mL. The sample was then vortexed for 10 minutes and then filtered. The drug content of each filtrate was estimated using UV spectrophotometer at 208 nm.[15]

Viscosity

The viscosity of optimized batch of EBZ-loaded ME was determined by using rheometer (Brookfield Engineering Laboratories, USA) with S62 spindle and 25°C temperature in triplicates.[16]

pH

The pH of an optimized batch of EBZ loaded ME was measured using digital pH meter (EZODO, Taiwan).[16]

In-vitro permeation study

In-vitro permeation study was done by using Franz diffusion cell (Orchid Scientifics, FDC-08, Nashik). A cellophane membrane as soaked overnight in phosphate buffer pH 7.4 and then mounted on Franz diffusion cells with a surface area of 2.0 cm^2 and receptor compartment with a capacity of 20 mL. The receptor compartment was filled with pH 7.4 phosphate buffer containing 20% v/v PEG 400 and the content was magnetically stirred. The donor compartment was filled with 1-mL of EBZ-loaded MEs to achieve desired drug concentration at the site. At predetermined time intervals (0.25, 0.50, 0.75, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 24 hours), 5 mL of aliquots were withdrawn, suitably diluted, filtered if needed and analyzed for drug content by UV-visible spectrophotometer

(Shimadzu UV-1800i, Japan) at 208 nm. Comparative *in-vitro* permeation study of EBZ loaded optimized batch of ME and pure drug solution also done by same procedure. 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 EBZ loaded optimized batch of ME and the best-fitted model was chosen based on high correlation coefficient (R) values for the release data.[17]

Analysis of permeation data

The cumulative amount of EBZ permeated per unit area was plotted against time and the steady state flux J_{ss} ($mg/cm^2/h$) was calculated from the slope of the linear portion of the graph and expressed as

$$Jss = dM / dT \qquad (2)$$

Where, M was the cumulative amount of drug permeated (mg) through skin per unit area (cm^2) within experimental time t (h).

The lag time (t_L) was calculated from the x-intercept of the linear portion of the plot of the cumulative amount of drug permeated vs. time in steady-state conditions.[13]

Transmission electron microscopy

The morphology of EBZ-loaded MEs was examined using transmission electron microscopy (Talos F200i FEGTEM, Thermofisher, USA). One drop of diluted sample with water (1:10) was deposited on a 3 mm carbon film-coated 300 mesh supported copper grid and later stained with one drop of 1% aqueous solution of phosphotungstic acid (PTA) and examined under the electron microscope.[18]

Stability study

The optimized batch of EBZ-loaded microemulsion formulation was subjected to a stability study at 40 ± 2°C/75 ± 5% RH over a period of 3 months in a stability chamber (Remi Electrotechnics Ltd. Mumbai, India). Parameters like pH, appearance, globule size, viscosity and drug content were taken into consideration as stability indicators.[18]

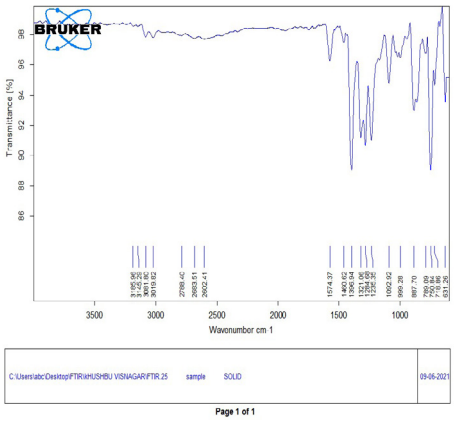


Fig. 1: FTIR spectrum of Eberconazole nitrate

Table 3: FTIR interpretation data of pure EBZ drug

Functional Group	*Reported Value*	*Observed Value*
Aromatic C-H stretching	3100–3000	3081.80
Aromatic C-H bending	900–675	750.84
Methyl group C-H bending	1470–1450	1460.62
Aromatic amine C-N stretching	1342–1266	1284.68
Cyclic alkenes C=C stretching	1650–1566	1574.37
Cyclic alkenes C=C bending	895–885	887.70
Aryl benzene (chloro benzene)	1096–1089	1092.92

Results and Discussion

FTIR study

FTIR study was performed for the identification of molecule as well as to evaluate the purity of drug. The FTIR spectrum of EBZ was shown in Fig. 1 and the interpreted data was depicted in Table 3**.** All the major peaks available in the spectra confirm the presence of characteristic functional groups in a molecule. Hence the drug sample was found to be eberconazole nitrate.

Selection of Microemulsion Components

Screening of oils (solubility study)

In this study, oil selection for the preparation of MEs was done on the basis of their capacity to solubilize the maximum amount of drug. The solubility of EBZ in various oils at 30°C was summarized in Table 4. Among all the oils EBZ has shown the highest solubility in capmul MCM. Thus, capmul MCM was selected as the oil phase for the development of the formulation.

Screening of surfactants

Selection of surfactants was governed by their emulsification efficiency for the selected oil rather than their ability to solubilize EBZ. Good solubility of EBZ in surfactant was considered as an additional advantage. In the present study, nonionic surfactants were selected since they are known to be less affected by pH change, generally regarded as safe and are biocompatible. Results inferred that the oily phase exhibited the highest emulsification efficiency with tween 80 for the homogenous emulsion formation, as depicted in Table 4.

Screening of co-surfactants

Adding a co-surfactant to the surfactant containing formulation was reported to improve dispersibility and drug absorption from the formulation as co-surfactant accumulates with surfactants at the interfacial layer, thereby increasing the microemulsion region's area. This is because they can further reduce the surface tension and

Table 4: Solubility of EBZ in oils and amount of oil emulsified by surfactants and co- surfactants

Oil	Solubility (mg/mL)	Surfactants	Amount of Oil emulsified (mL)
Olive oil	0.1546 ± 0.017	Gelucire 50/13	0.51
Captex 200P	0.213 ± 0.081	Acrysol K140	0.65
Miglyol 810	0.384 ± 0.046	Tween 80	0.85
Castor oil	1.647 ± 0.491	Tween 40	0.36
sunfloweroil	1.981 ± 0.815	Tween 20	0.29
Almond oil	2.261 ± 0.328	Tween 60	0.35
Imwitor 740	3.729 ± 0.631	Acconon CC400	0.32
cinnamon oil	3.218 ± 0.445	Solutol HS15	0.42
IPM	4.072 ± 0.648	Co- surfactants	Amount of Oil emulsified (mL)
Caprol PGE860	6.404 ± 0.964	Labrafil M2125CS	0.45
IPA	8.376 ± 1.174	PEG 200	0.5
Capryol 90	14.312 ± 2.194	PG	0.6
Acconon E	15.631 ± 2.186	PEG 400	0.5
Capmul PG8	15.706 ± 3.128	Labrafil M1944C	0.75
Oleic acid	26.682 ± 5.246	PlurolOleique CC497	0.8
Capmul MCM	38.904 ± 4.134	Transcutol	1.2

they tend to fluidize the interfacial surfactant film. The data clearly illustrated that (Table 4) transcutol underwent the highest emulsification with capmul MCM and tween 80 and hence selected as a co-surfactant.

Pseudo ternary phase diagram

Surfactant and co-surfactant ratio (S_{mix}) was determined due to their important role in microemulsion. For the pseudo ternary phase diagram different ratio of surfactant (Tween 80) and co-surfactant (transcutol) (S_{mix}) were taken (3:1, 2:1, 1:1, 1:2, 1:3) and are shown in Figs 2(A) to (E). These Smix ratios were chosen in increasing the concentration of surfactant with respect to co-surfactant and increasing concentration of co-surfactant with respect to surfactant for detailed study of the phase diagrams for the formulation of the microemulsion. Fig. 2(B) showed that the microemulsion area decreased as surfactant concentration with respect to co-surfactant increased. So, study was limited to 3:1 ratio. There was increasing in the microemulsion area with increasing concentration of co-surfactant with respect to surfactant up to 1:2 ratios. Further increase in the concentration of co-surfactant i.e. 1:3 ratio was lead to decreasing in the microemulsion region. The pseudo ternary phase diagram of S_{mix} ratio (1:2) had largest microemulsion region (Fig. 2C) and hence selected for further study. The optimized phase diagram had largest micro emulsification region with oil concentration 5–20% w/w, S_{mix} concentration 30–60% w/w and water concentration 10–40% w/w.

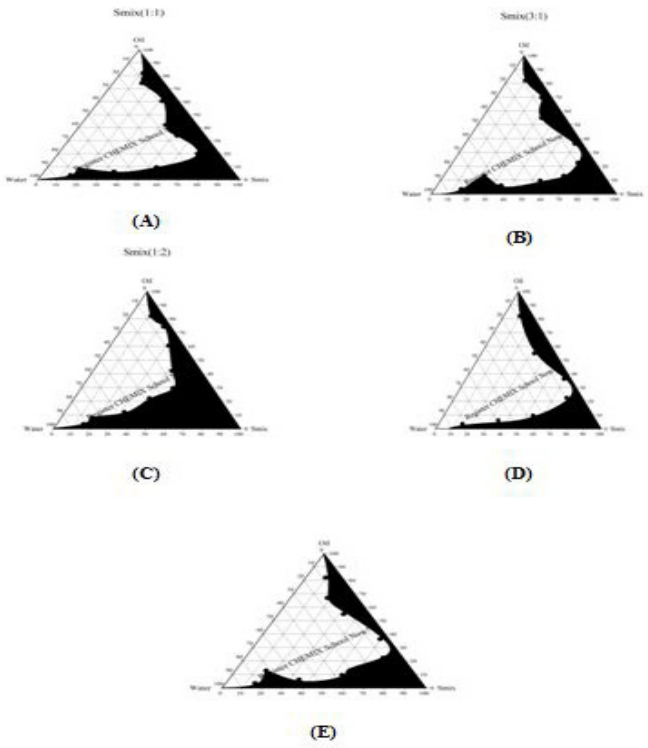


Fig. 2: Pseudo-ternary phase diagrams with different ratios of S_{mix} (A) 1:1, (B) 3:1, (C) 1:2, (D) 1:3, (E) 2:1 for EBZ loaded MEs

Experimental Design

Preliminary investigations of the process parameters using Design Expert software version 7.1.5 revealed that factors such as amount of Capmul MCM (X_1), amount of tween 80 (X_2) and amount of transcutol (X_3) exhibited significant influence on the cumulative amount of drug release/unit area Q_{24} ;Flux (Jss) Lag time (t_L) hence, they were utilized for the further systematic studies. The actual values of each of selected factor have been summarized against their respective coded values in Table 1. The results of all evaluated responses for experimental design batches of EBZ-loaded MEs have been summarized in Table 5. For all design batches selected dependent variables, Q_{24} (Y_1), Flux (Y_2) and lag time (Y_3) exhibited wide variations from 3100 to 4700 (µg/cm^2), 127 to 213 (µg/Cm^2/h) and 0.25 to 0.65 seconds, respectively (Table 5). The data clearly indicate strong influence of selected factors (X_1, X_2 and X_3) on all three responses (Y_1, Y_2 and Y_3). A stepwise multivariate linear regression was performed to evaluate the observations. The equations representing the quantitative effect of the independent variables on the measured responses are shown below;

$$Y_1 = 4359.25 + 51.71X_1 + 486.66X_2 + 68.22X_3 + 17.69X_1X_2 + 30.71_X1X_3 + 29.25X_2X_3\ 198.97X_1^2 - 159.02X_2^2 - 96.59X_3^2 \quad (3)$$

$$Y_2 = 178.08 + 3.09X_1 + 23.63X_2 + 3.63X_3 - 0.345X_1X_2 + 0.255X_1X_3 + 1.535X_2X_3 - 7.36X_1^2 - 2.66X_2^2 - 2.572X_3^2 \quad (4)$$

$$Y_3 = 0.468 - 0.0198X_1 - 0.1174X_2 - 0.0077X_3 + 0.0137X_1X_2 - 0.0037X_1X_3 - 0.0087X_2X_3 + 0.0022X_1^2 - 0.00487X_2^2 - 0.01019X_3^2 \quad (5)$$

The fitted polynomial equations (full and reduced model) relating the responses to the transformed factors are summarized. For Q_{24} (Y_1) coefficients X_1X_2, X_1X_3, X_2X_3 and X_3^2 were found to be insignificant, whereas for Flux Jss (Y_2) and for lag time (Y_3) coefficients X_1X_2, X_1X_3, X_2X_3, X_2^2 and X_3, X_1X_2, X_1X_3, X_2X_3, X_12, X_22 and X_3^2 respectively were found to be insignificant ($p>0.05$) and hence, these terms were deleted from their respective full model in order to develop reduced model. The removal of insignificant terms was further justified by executing ANOVA test (Table 6). The high value of correlation coefficients for Q_{24} (Y_1), Jss (Y_2) and t_L (Y_3) illustrates goodness of fit. The critical value of F for Y_1, Y_2 and Y_3 were found to be 5.192, 5.192 and 4.093. For all three responses, calculated F values [2.167 (Y_1), 2.08 (Y_2) and 0.606 (Y_3)] were less than their respective critical values which supported non-significant difference between full and reduced model. The final reduced model equations for both responses could be summarized as follows:

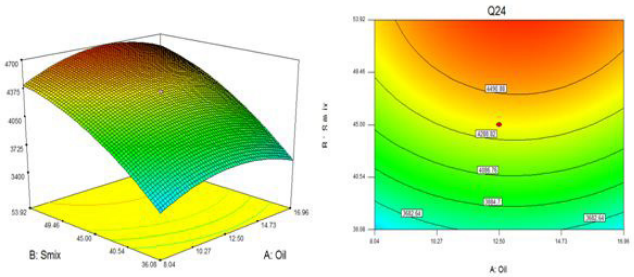
$$Y_1 = 4162.34 + 51.74X_1 + 486.86X_2 + 68.24X_3 - 139.27X_1^2 - 99.24\,X_2^2 \quad (6)$$

$$Y_2 = 172.66 + 3.097X_1 + 23.63X_2 + 3.63X_3 - 5.704X_1^2 - 0.919X_3^2 \quad (7)$$

$$Y_3 = 0.4566 - 0.0198X_1 - 0.1174X_2 \quad (8)$$

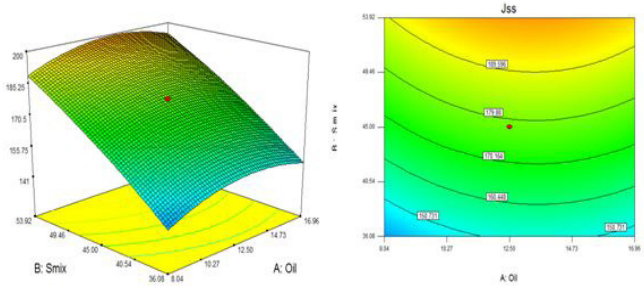
Influence of formulation composition factors on drug release Q_{24}; flux (Jss) & lag time (t_L)

All three independent variables (oil, S_{mix}, and water) significantly impacted the Q_{24} and flux (Jss). Among all experimental design batches, ME12 having the highest values of Q_{24} (4767.14 µg/cm^2) and flux Jss (212.5 µg/cm^2/h) (Table 5). Additionally, for Y_1 and Y_2, response surface and contour plots (Figs 3(a) and (b)) showed a significant impact from all three components (oil, S_{mix}, and water) that were investigated. The regression analysis results showed that coefficients b1, b2, and b3 had positive values, indicating that Q_{24} and flux (Jss) increased with increasing oil, S_{mix}, and/or water content. This may be ascribed to microemulsion globule size reduction with increased oil content, the potential of surfactants and co-surfactants to perturb the membranes, and increased hydrophilicity of the highly lipidic drug by the aqueous phase. All three independent variables significantly impacted lag time, with batch ME12 having the lowest tL value of 0.25 h (Table 5). Additionally, the response surface and contour plots (Fig. 3(c)) for Y_3 showed that the three components (oil, S_{mix}, and water) under study had a significant impact. The regression analysis results



(A) (B)

Fig. 3(a): (A) 3D Response surface plot, (B) Contour plot for Q_{24}



(A) (B)

Fig. 3(b): (A) 3D Response surface plot, (B) Contour plot for Flux Jss

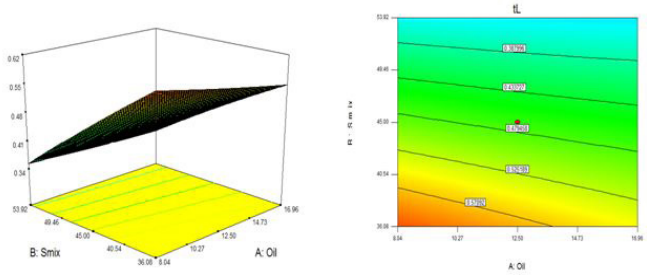


Fig. 3(c): (A) 3D Response surface plot, (B) Contour plot for t_L

showed negative values for the b1 and b2 coefficients, indicating that tL dropped when oil and/or S^{mix} levels increased. This might be caused by globule size decrease with increased oil content and diffusivity alteration with enhanced surfactant and co-surfactant content.[19,20].

Check point batch

The over lay graph was constructed (Fig. 4) and the checkpoint batch was selected by keeping the criteria as the maximum value of Q_{24} and Jss where as lowest value of lag time (t_L). This optimized batch of EBZ-loaded microemulsion was prepared practically according to the levels of factors illustrated in Table 7. The results illustrated no significant difference between the experimental values and predicted values which suggested the suitability of design applied. This optimized batch of EBZ-loaded microemulsion was evaluated for particle size, PDI, zeta potential, drug content, pH, *in-vitro* drug release and TEM.

Table 5: Evaluation parameters of experimental design batches

Batch	*Globule Size (nm)*	*PDI*	*% DC*	Q_{24} *(µg/cm²)*	*Jss (µg/cm²/h)*	t_L *(h)*	$_PH$
ME1	173.48 ± 5.24	0.253 ± 0.06	96.23 ± 0.17	3398.35	137.3	0.62	6.30 ± 0.21
ME2	161.5± 6.15	0.231 ± 0.07	97.27 ± 0.30	3321.25	144.82	0.56	6.78 ± 0.27
ME3	162.8 ± 7.15	0.213 ± 0.05	98.42 ± 0.45	4167.14	180.32	0.38	6.67 ± 0.25
ME4	151.5 ± 6.65	0.314 ± 0.06	99.43 ± 0.19	4379.9	185.35	0.37	6.92 ± 0.29
ME5	182.38 ± 5.57	0.235 ± 0.04	100.16 ± 0.18	3291.25	142.3	0.6	6.94 ± 0.21
ME6	206.5 ± 8.57	0.342 ± 0.05	97.53 ± 0.27	3556.09	149.73	0.52	6.72 ± 0.25
ME7	159.3± 5.22	0.252 ± 0.03	97.81 ± 0.25	4396.15	190.35	0.32	6.95 ± 0.24
ME8	173.24 ± 7.23	0.212 ± 0.02	97.58 ± 0.69	4512.65	197.51	0.3	6.77 ± 0.21
ME9	177.38 ± 4.14	0.273 ± 0.06	95.62 ± 0.52	3778.15	152.16	0.5	6.57 ± 0.27
ME10	183.39 ± 9.19	0.294 ± 0.04	96.44 ± 0.16	3890.65	161.16	0.44	6.69 ± 0.25
ME11	161.49 ± 6.15	0.271 ± 0.07	97.55 ± 0.57	3127.65	127.32	0.65	6.87 ± 0.29
ME12	170.38 ± 5.57	0.241 ± 0.02	99.95 ± 0.16	4767.14	212.5	0.25	6.96 ± 0.21
ME13	161.68 ± 5.33	0.265 ± 0.04	100.35 ± 0.13	3992.54	164.98	0.41	6.76 ± 0.25
ME14	153.5 ± 5.14	0.253 ± 0.05	97.65 ± 0.45	4255.43	175.35	0.46	6.96 ± 0.24
ME15	160.69 ± 5.27	0.263 ± 0.03	99.02 ± 0.52	4346.24	178.32	0.47	6.99 ± 0.24

Table 6: Analysis of variance (ANOVA)

	DF	SS	MS	R^2	F
		Q24 (µg/cm²)			DF=(4,5)
Regression					
FM	9	3622836.066	402537.341	0.98845136	
RM	5	3549432.958	709887	0.968424123	F_{cal} = F_{cal} = 2.167 F^{cal} = 5.192 F^{tab}_{cal}<F_{tab}
Error					
FM	5	42327.64974	8465.52995		
RM	9	115730.7576	12859		
Jss (µg/cm²/h)					DF=(4,5)
Regression					
FM	9	8333.659665	925.962185	0.99546762	
RM	5	8270.504997	1654.1	0.987923702	F_{cal} = 2.08 F^{cal} = 5.192 F^{tab}_{cal}<F_{tab}
Error					
FM	5	37.94330877	7.58866175		
RM	9	101.0979764	11.233		
Lag time (t_L)					DF=(7,5)
Regression					
FM	9	0.19790394	0.0219893	0.9742	
RM	2	0.193460999	0.0967	0.9523	F_{cal} = 0.606 F_{tab} = 4.093 F_{cal}<F_{tab}
Error					
FM	5	0.00522939	0.0010459		
RM	12	0.009672334	0.0008		

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

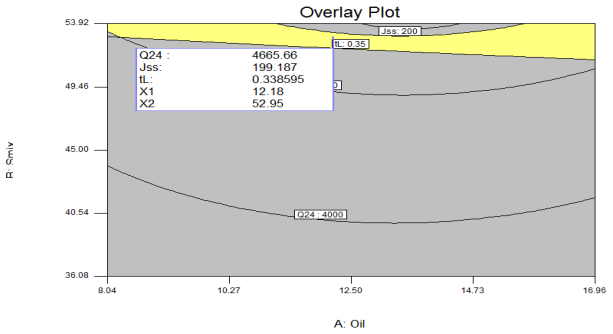


Fig. 4: Overlay plot for optimization of microemulsion

Table 7: Composition and results of checkpoint batch of EBZ loaded ME

Type of components	Name of components	Concentration (%)	
Oil	Capmul MCM	12.18	
Surfactant	Tween 80	17.65	
Co surfactant	Transcutol	35.30	
Water	Distilled water	34.52	
Responses	Predicted value	Experimental value	Relative error (%)
Q_{24} (µg/cm^2)	4665.66	4558.60	2.29
Jss (µg/cm^2/h)	199.18	203.8	2.32
t_L (h)	0.3385	0.327	3.25

Evaluation Parameters

Globule sizes of EBZ-loaded MEs for all design batches are summarized in Table 5. The globule size of the optimized batch of EBZ-loaded ME was found to be 153.6 nm which confirmed nanometer size of developed formulation (Fig. 5). However, this parameter was not included as a crucial response during the optimization just because of non-significant differences in their values with respect to the factors selected. The PDI of the optimized batch of EBZ-loaded ME was found to be 0.253 which confirms narrow size distribution of systems**.** The zeta potential value of the optimized batch of EBZ-loaded ME was found to be -30.5 mV (Fig. 6) which supported stability of dispersed systems. Generally, zeta potential values, which exceed 30mV regardless of the positive or negative prefix, are considered as optimum for creating enough dispersion in the formulation which can keep them stable.[21] For the penetration through skin bilayers, viscosity of the ME is a crucial parameter. The optimized batch of EBZ-loaded microemulsion has a viscosity value of 37.52 cps. The pH values of all the design batches were 6.32–6.99, which is within the specified range for topical formulations. The optimized batch of MEs has the pH value of 6.96 ± 0.21. The values of %drug content of all the design batches were more than 95% along with very low standard deviations which suggested uniform dispersion of drug in developed microemulsion. The value of %drug content of the optimized EBZ loaded microemulsion batch was 99.27%.

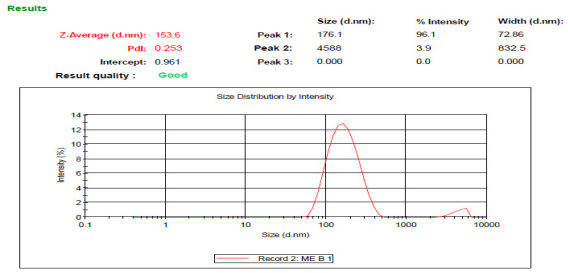


Fig. 5: Particle size graph of optimized batch of ME

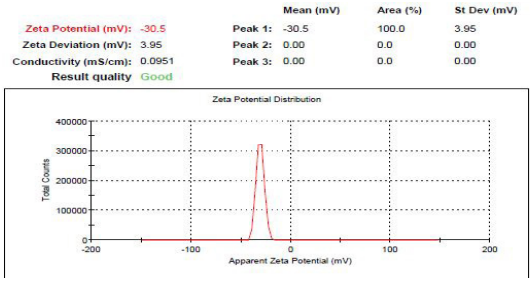


Fig. 6: Zeta potential graph of optimized batch of ME

In-vitro permeation study

The effects of formulation components of all the microemulsion batches on permeation through cellulose membrane were studied by in vitro permeation study. The detailed *in-vitro* permeation data are shown in Table 5 and permeation profiles are shown in Fig. 7. The permeation profile of all the batches showed that the microemulsion formulations successfully increased the permeation of the drug with increased time. Among all the formulations, ME12 formulation composed of 12.5 mL oil, 60 mL S_{mix} and 25 mL water has the highest permeation profile with flux value of 212.5 µg/cm^2/h. The permeation of EBZ was increased with the amount of oil to a certain extent. Above that further increase in the amount of oil resulted in decreased permeation of the drug. Those results might be due to the increase in the globule size of the internal (oil) phase of microemulsion as the amount of oil increases. The results of comparative permeation profile of EBZ loaded pure drug solution and optimized batch of microemulsion exhibited significant enhancement in *in-vitro* permeation by ME dosage form compared to their pure drug solution (Fig. 8).[13,22] The higher permeability of EBZ from the microemulsion formulations is mostly probably due to the presence of surfactant and co-surfactant which act as permeation enhancer. Kinetic modeling of drug release data was investigated to analyze the drug release mechanism from the optimized batch of ME dosage form. The R^2 value of zero-order (Fig. 9), first order, Higuchi and Korsmeyer-Peppas model were found to be 0.947, 0.564, 0.930 and 0.935, respectively. The zero-order shows the best fit model with the highest R^2 value.[23]

Transmission electron microscopy (TEM)

Morphological and structural examination of the optimized batch of EBZ-loaded ME was carried out using a

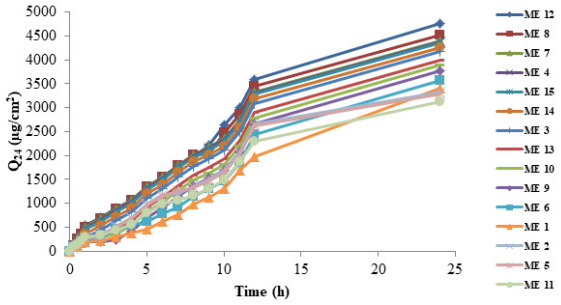


Fig. 7: *In-vitro* permeation profiles of EBZ-loaded microemulsions

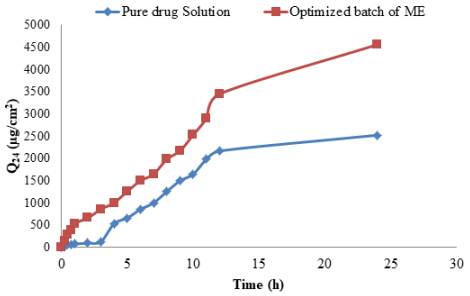


Fig. 8: Comparative in vitro permeation profiles of EBZ loaded pure drug solution and optimized batch of microemulsion over a period of 24 h

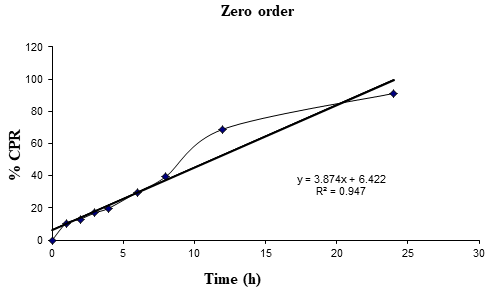


Fig. 9: Kinetic modeling of drug release data of Eberconazole nitrate loaded optimized batch of ME

transmission electron microscope. TEM images illustrated formation of spherical micelles with size range of 100 to 200 nm (Fig. 10). These results were in accordance to that of globule size analysis.

Stability Study

The optimized batch of microemulsion was examined for stability study and was evaluated for various parameters to detect any changes in pH, globule size, viscosity and drug content. Neither separation nor any significant changes in average globule size have been noted. The microemulsion was found to have remained clear and transparent with no any major changes observed in the pH, drug content and viscosity at the end of 3 months.

In the present work EBZ loaded microemulsion as a topical delivery was prepared successfully. The study revealed that all three dependent parameters cumulative amount of drug release per unit area at 24 h (Q_{24}), flux (Jss)

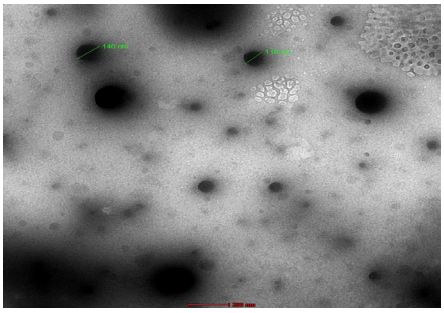


Fig. 10: TEM image of optimized batch of EBZ loaded microemulsion

and lag time (t_L) were highly important in determining the permeation study. The *in-vitro* enhancement in the permeation was due to the narrow globule size of the formulation. Microemulsion composition mainly surfactant and co-surfactant, were useful in loosening the intact and compact surface of the skin to enhance the permeation. It could be concluded from the present investigation that microemulsion could be an excellent approach for successful topical delivery of poorly water-soluble drugs like EBZ. However further, in vivo investigations are required to confirm improved antifungal efficacy of EBZ.

Acknowledgment

The authors would like to thank Precise Chemipharma Pvt Ltd,Navi Mumbai, India, for giving us an eberconazole nitrate sample as a gift and Nootan Pharmacy College, Faculty of Pharmacy, Sankalchand Patel University, Visnagar, Gujarat, India, for providing all the facilities for performing the experimental work.

References

1. Singh V, Sharma H, Veerma R, Javed A, Singh M. Topical non steroidal anti inflammatory drug (NSAIDs) microemulsions: Rationale, review and future prospective. Asian Journal of Pharmaceutics. 2013; 7: 1-7.
2. Sabale V, Vora S. Formulation and evaluation of microemulsion-based hydrogel for topical delivery. International journal of pharmaceutical investigation. 2012; 2(3): 140-149.
3. Payyal SP, Rompicherla NC, Sathyanarayana SD, Shriram RG, Vadakkepushpakath AN. Microemulsion based gel of sulconazole nitrate for topical application. Turkish Journal of Pharmaceutical Sciences. 2020; 17(3): 259-264.
4. Goswami P, Choudhury A, Dey BK. Microemulsion–A Potential Carrier for Improved Bioavailability. International Journal of Pharmaceutical & Biological Archive. 2019; 10(2): 69-77.
5. Moodahadu-Bangera LS, Martis J, Mittal R, Krishnankutty B, Kumar N, Bellary S, et al. Eberconazole-pharmacological and clinical review. Indian Journal of Dermatology, Venereology and Leprology. 2012; 78(2): 217-223.
6. Krishna MV, Idris SA, Madhavi G, Reddy BJ, Sowhardhra M, Sankar DG. Study on Dissociation Equilibria of Eberconazole Nitrate in Micellar Media by Spectrophotometry. International Journal of Pharmacy and Chemistry. 2019; 5(5): 48-51.
7. Thomas J. Eberconazole–an anti-fungal molecule revisited. International journal of scientific research. 2019; 8(3): 7-8.
8. Patil PS, Dhawale SC. Development of ritonavir loaded nanoparticles: in vitro and in vivo characterization. Development. Asian journal of Pharmaceutical and Clinical Research. 2018; 11(3): 284-288.

9. Patel J, Kevin G, Patel A, Raval M, Sheth N. Design and development of a self-nanoemulsifying drug delivery system for telmisartan for oral drug delivery. International journal of pharmaceutical investigation. 2011; 1(2): 112-118.
10. Patel J, Patel A, Raval M, Sheth N. Formulation and development of a self-nanoemulsifying drug delivery system of irbesartan. Journal of advanced pharmaceutical technology & research. 2011; 2(1): 9-16.
11. Chandra A, Sharma PK, Irchhiaya R. Microemulsion-based hydrogel formulation for transdermal delivery of dexamethasone. Asian Journal of Pharmaceutics. 2009; 3(1): 30-36.
12. Hathout RM, Woodman TJ, Mansour S, Mortada ND, Geneidi AS, Guy RH. Microemulsion formulations for the transdermal delivery of testosterone. European Journal of Pharmaceutical Sciences. 2010; 40: 188-196.
13. Dhingani A, Patel J, Garala K, Dharamsi A. Design and development of microemulsion based transdermal gel of lercanidipine hydrochloride by assimilation of rotatable central composite design and principal component analysis. Pharmaceutical Nanotechnology. 2013; 1(4): 290-305.
14. Rajaram SV, Ravindra PP, Shripal MC. Microemulsion drug delivery of imiquimod as anticancer agent for skin cancer therapy and its evaluation. Drug Designing. 2020; 9(3): 1-12.
15. Yadav V, Jadhav P, Kanase K, Bodhe A, Dombe SH. Preparation and evaluation of microemulsion containing antihypertensive drug. International Journal of Applied Pharmaceutics. 2018; 10(5): 138-146.
16. Ranade S, Londhe V, Bajaj A. Microemulsion based gels for targeting neuropathic pain. International Journal of Pharmaceutical Sciences and Research. 2014; 5(6): 2525-2534.
17. Patel MR, Patel RB, Parikh JR, Solanki AB, Patel BG. Effect of formulation components on the in vitro permeation of microemulsion drug delivery system of fluconazole. AAPS PharmSciTech. 2009; 10: 917-923.
18. Amarji B, Garg NK, Singh B, Katare OP. Microemulsions mediated effective delivery of methotrexate hydrogel: more than a tour de force in psoriasis therapeutics. Journal of drug targeting. 2016; 24(2): 147-160.
19. Yanyu X, Fang L, Qineng P, Hao C. The influence of the structure and the composition of water/AOT-Tween 85/IPM microemulsion system on transdermal delivery of 5-fluorouracil. Drug Development and Industrial Pharmacy. 2012; 38(12): 1521-1529.
20. Al Abood RM, Talegaonkar S, Tariq M, Ahmad FJ. Microemulsion as a tool for the transdermal delivery of Ondansetron for the treatment of chemotherapy induced nausea and vomiting. Colloids and Surfaces B: Biointerfaces. 2013; 101: 143-151.
21. Da Costa S, Basri M, Shamsudin N, Basri H. Stability of positively charged nanoemulsion formulation containing steroidal drug for effective transdermal application. Journal of Chemistry. 2014; 1-8.
22. Ustundag Okur N, Apaydın S, Karabay Yavasoglu NU, Yavasoglu A, Karasulu HY. Evaluation of skin permeation and anti-inflammatory and analgesic effects of new naproxen microemulsion formulations. International journal of pharmaceutics. 2011; 416: 136-144.
23. Kalam MA, Alshamsan A, Aljuffali IA, Mishra AK, Sultana Y. Delivery of gatifloxacin using microemulsion as vehicle: formulation, evaluation, transcorneal permeation and aqueous humor drug determination. Drug delivery. 2016; 23(3): 886-897.

HOW TO CITE THIS ARTICLE: Shah N, Patel K, Singhvi I. Topical Delivery of Eberconazole Nitrate Loaded Microemulsion: Formulation, Design and Evaluation. Int. J. Pharm. Sci. Drug Res. 2023;15(4):478-487. **DOI:** 10.25004/IJPSDR.2023.150412