RESEARCH ARTICLE

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Formulation and Development of W/O Type Microemulsion based Transdermal Systems for Verapamil Hydrochloride

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Abstract: The aim of the present investigation was to design and develop a novel microemulsion (ME) based transdermal drug delivery system of highly water soluble drug, verapamil HCl (VPLH) to enhance its therapeutic efficacy. Microemulsion based transdermal formulation was used to circumvent barrier properties of stratum corneum due to its lower droplet size as well as permeation enhancing effects. VPLH loaded water droplet are dispersed in oils (W/O) MEs were optimized using Box - Behnken design with amounts of oil (Capmul MCM), surfactants mixtute (Span 80 and ethanol) and water as independent variables. The critical responses; globule size, viscosity, cumulative amount of drug permeated across abdominal skin of goat in 24 h (Q_{24}) and lag time (t_L) were identified and probed as dependent variables. The optimized batch of VPLH loaded ME was further converted into microemulsion based gel (MBG) in order to increase patient compliance. The results of *in-vitro* permeation of the optimized batch of VPLH loaded MBG revealed significant increase in permeability parameters as compared to its convention gel formulation. The values of flux (J_{ss}) for optimized batch of VPLH loaded MBGs (0.5214 mg/cm^{2}h) revealed 12.08 cm^2 area requirement in order to obtain the desired input rate of VPLH within 24 h application. All these results suggested suitability of W/O type MEs as carriers for transdermal delivery of highly water soluble drug, VPLH.

INTRODUCTION

Verapamil hydrochloride (VPLH) is a calcium channel antagonists used for the treatment of hypertension and cardiac arrhythmia. VPLH endures extensive first-pass hepatic metabolism and has an absolute bioavailability of about 10-35%. VPLH is metabolized in the liver by cytochrome P450 to pharmacologically inactive metabolites. In addition to these short biological half-life (2.8-7.4 h) of VPLH demands frequent dosing to achieve its therapeutic levels. All these marked VPLH as an ideal candidate for transdermal drug delivery. [1-3] Transdermal Drug Delivery System (TDDS) is a well-accepted route for systemic delivery of drugs but only a few drugs could be effectively delivered through this route due to barrier properties of stratum corneum (SC). Microemulsions (MEs) are translucent and thermodynamically stable mixtures of oil, surfactant, cosurfactant and water with either the oil droplets are dispersed in water (O/W) or water droplet are dispersed in oils (W/O) with a droplet size typically in the range of 10-100 nm. [4-5] During the past years, MEs have received increasing attention because of the advantages including enhanced drug solubility, thermodynamic stability and increased drug permeation rate. Aqueous phase titration method had been successfully explored for the preparation of O/W type MEs of many lipophilic drugs but with respect to the hydrophilic nature of VPLH, oil phase titration method was employed for preparation of VPLH loaded W/O type MEs. [6-8] In light of these, the aim of present investigation was to design and develop W/O type ME for transdermal delivery of antihypertensive drug VPLH using QbD approaches.

MATERIALS AND METHODS

Materials

VPLH was obtained as a gift sample from Torrent Research Center, Gandhinagar, India. The materials like; Capmul MCM, Capmul PG8, Captex 355, Acconon CC-6, Capmul GMO50, Capmul PGE 860, Caprol ET and Capmul MCM C8 were generously donated by Abitec Corporation, USA. Miglyol 812 and Imwitor 742 were kindly gifted from Sasol GmbH, Witten, Germany. Capryol 90, Labrafac CC, Labrafac Lipophile WL1349, Labrafil M 2125CS, Maisine 35-1 and Paceol were gifted from Gettefosse Saint-Priest Cedex, France. Sefsol 218 was obtained as a gift sample from Nikko Chemicals, Tokyo, Japan. Cremophor RH40, Gelucire 44/14, Lauroglycol 90 and Solutol HS 15 were donated from BASF Corporation, USA. Other Chemicals like Tween 20, Tween 40, Tween 60, Tween 80, Polyethylene Glycol (PEG) 400, Propylene Glycol (PG) and Sodium alginate were purchased from Himedia Labs, Mumbai, India whereas Span 40, Span 60, Span 80, Isopropyl alcohol (IPA), n-butanol, Ethanol, White wax, Xanthan gum and Cetosteryl alcohol were procured from SD Fine Chem, Mumbai, India. Isopropyl Myristate (IPM), Olive oil, Oleic acid, Castor oil, Magnesium stearate, Titanium dioxide, Zinc oxide and Colloidal silica were procured from Loba Chem, Mumbai, India. Double distilled water was used through put the study.

Selection of Microemulsion Components

1. Selection of Oil (Solubility Studies)

The solubility of VPLH was measured in numerous oils and distilled water by shake flask method. An excess amount of drug was introduced into 2 mL of each oil and these mixtures were sealed in glass vials. Each of the samples was vortexed (GeNei, Bangalore, India) for 5 min in order to facilitate initial mixing. Further, vials were charged on an environmental shaker bath (Tempo Instruments and Equipments Pvt. Ltd., Mumbai, India) for a period of 72 h at 37°C with 300 rpm speed. After equilibrium for additional 72 h at 25°C temperature, each vial was centrifuged at 10000 rpm for 10 min using a centrifuge (Remi Laboratory Instruments, Mumbai, India). The supernatant of each sample was filtered through a membrane filter (0.45 µm) to remove any undissolved drug if present. The amount of drug in all samples was determined by their subsequent

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Table 1: Design Layout of Box-Behnken Design Batches for VPLH Loaded MEs

Batch Code	Transformed Values		
	X_1[a]	X_2[b]	X_3[c]
VPLH-ME-F1	-1	-1	0
VPLH-ME-F2	1	-1	0
VPLH-ME-F3	-1	1	0
VPLH-ME-F4	1	1	0
VPLH-ME-F5	-1	0	-1
VPLH-ME-F6	1	0	-1
VPLH-ME-F7	-1	0	1
VPLH-ME-F8	1	0	1
VPLH-ME-F9	0	-1	-1
VPLH-ME-F10	0	1	-1
VPLH-ME-F11	0	-1	1
VPLH-ME-F12	0	1	1
VPLH-ME-F13	0	0	0

Coded Values	Actual Values (mL)		
	X_1[a]	X_2[b]	X_3[c]
-1	30	30	5
0	45	45	10
1	60	60	15

[a] X_1: Amount of oil (Capmul MCM), [b] X_2: Amount of surfactant mixture [(Span 80: Ethanol (1:1)], [c] X_3: Amount of water

dilution with pH 7.4 phosphate buffer using double beam UV Visible spectrophotometer (UV-1700, Shimadzu Corporation, Tokyo, Japan) against blank. The study was repeated in triplicate and their mean values were documented. [6-8]

2. Selection of Surfactant (Emulsification Study)

For each of drug, eight lipophilic nonionic surfactants (Acconon CC-6, Capmul GMO50, Caprol ET, Lauroglycol 90, Capmul MCM C8, Span 40, Span 60 and Span 80) were screened to evaluate their propensity for emulsification of aqueous phase. For each surfactant 10 mL of 10% w/v solution was prepared in preselected oil phase and distilled water was subsequently added to each of these solutions with an increment of 10 µL alongwith vortexing until the system becomes cloudy. The study was performed in triplicates. [9-10]

3. Selection of Cosurfactant (Emulsification study)

The optimized blend of surfactant was combined with five different cosurfactants, namely, PEG 400, PG, IPA, n-butanol and Ethanol at a fixed surfactant to cosurfactant ratio (S_{mix}) of 1:1. Different combinations of water and S_{mix} in weight ratios (1:9, 1:8, 1:7, 1:6, 1:5, 1:4, 1:3, 1:2, 1:1, 2:3, 3:7, 3:2, 7:3, 4:1 and 9:1) were titrated by optimized oil phase in order to delineate all the boundaries of phases precisely formed. At the end of titration, the concentrations of each components were calculated and pseudo-ternary phase diagrams were constructed with apex representing oil, S_{mix} and water using Sigma Plot® software (Stat-Ease. Inc. Minneapolis, USA). The microemulsifying phase was identified as the region in phase diagram where clear and transparent formulations were obtained by visual observation. The cosurfactant with highest microemulsification area in pseudo-ternary phase diagram was optimized for all further trials. [9-10]

Selection of Surfactant and Cosurfactant Ratio (K_m)

The K_m ratio was optimized by constructing pseudo-ternary phase diagrams for various ratios (1:3, 1:2, 1:1, 2:1, 3:1) by oil titration method as mentioned earlier for cosurfactant selection. The pseudo-ternary phase diagram with highest microemulsion area was selected as optimized ratio of K_m for all further trials. [9-10]

Preparation of VPLH Loaded W/O Type MEs

VPLH loaded MEs were prepared by dissolving fixed amount of VPLH (4% w/w) in distilled water with subsequent addition of optimized surfactant and cosurfactant (S_{mix}). The resultant mixtures were continuous stirred for a period of 2 min on vortex mixer. The optimized amount of oil phase was further added slowly with continuous stirring using a high speed homogenizer (Remi Laboratory Instruments, Mumbai, India). All formulations were vortexed for 10 min on vortexer and sonicated for 5 min in an ultrasonicator bath (Frontline FS-4, Mumbai, India). The resultant MEs were sealed in a glass vial and stored at room temperature before further evaluations. [6-8]

Optimization of VPLH Loaded MEs

On the basis of pseudo-ternary phase diagrams the levels of oil, surfactant cosurfactant and water were decided in terms of their maximum possibility of microemulsification. Box - Behnken design (BBD) was implemented with three independent variables (factors) as; amount of oil (X_1), amount of surfactant mixture (surfactant and cosurfactant - X_2) and amount of water (X_3). In this design, three factors with three levels were probed to investigate the main and interaction effects on previously selected critical responses. The design consisted total 13 runs (VPLH-ME-F1 to VPLH-ME-F13) (Table 1) and each of them was formulated in triplicates in order to estimate reproducibility of the model applied. [11-12] A second order quadratic model

incorporating interactive and polynomial terms was exercised to evaluate the responses.

$$Yi = b_0 + b_1X_1 + b_2X_2 + b_3X_3 + b_{12}X_{12} + b_{13}X_{13} + b_{23}X_{23} + b_{11}X_1^2 + b_{22}X_2^2 + b_{33}X_3^2$$

Eq. ---------- 1

Where, Yi was dependent variable, b_0 was arithmetic mean response of 13 runs and b_i was the expected coefficient for factor X_i. The main effects (X_1, X_2 and X_3) highlighted average result of altering one factor at a time from its lowest value to highest value. The interaction terms (X_{12}, X_{13} and X_{23}) depicted change in responses when two factors were simultaneously altered. The polynomial terms (X_1^2, X_2^2 and X_3^2) were incorporated to investigate nonlinearity (Table 1).

Data were further analyzed by Microsoft Excel® version 2010 (Microsoft corporation, Washington, USA) for regression analysis. Contour plots were generated to study response variations against independent variables using Sigma Plot® and Design Expert® softwares (Stat-Ease. Inc. Minneapolis, USA). Additionally the composition of optimized (check point) batch was derived by constructing overlay plots. The percentage relative error of each response was calculated using following equation in order to judge validity of the model. [13-14]

$$\% \text{ Relative Error} = \frac{(\text{Predicted value} - \text{Experimental value})}{\text{Predicted value}} \times 100$$

Eq. ---------- 2

Evaluation Parameters of VPLH Loaded W/O Type MEs

1. Globule Size and Size Distribution

All the batches of VPLH loaded MEs were subjected to measurement of globule size and size distribution immediately after preparation. The sample was subjected to a brief period of sonication (15–30 sec) in order to minimize any aggregation if present. The samples were analyzed by particle size analyzer (Zetatrac, U2552, New York, USA) at 25°C with an angle of 90°. The study was repeated in triplicates for confirmation of reproducibility. [6-8]

2. Zeta Potential (ζ)

The zeta potential (ζ) of all the batches of VPLH loaded MEs was determined by the particle size analyzer. The analysis was performed with purified water adjusted to a standardized conductivity of 50 µS/cm with sodium chloride solution in order to avoid changes in ζ values due to day-to-day variations occurring in the conductivity of water. The mean values of ζ for three independent samples were documented. [6-8]

3. Refractive Index (RI)

The RI values of all the batches of VPLH loaded MEs were determined using refractometer (Bausch and Lomb Optical Company, Rochester, NY, USA). One drop of the sample was placed on the sample holder and the values of RI were recorded in triplicates against distilled water as blank. [6-8]

4. Percentage Transmittance (%T)

The percent transmittance of all the batches of VPLH loaded MEs was measured by subjecting each sample to UV spectrophotometer at 650 nm using distilled water as a blank. [6-8]

5. Percentage Drug Content (% DC)

All the experimental design batches of VPLH loaded MEs were subjected to assay analysis in order to determine their percentage drug content. Accurately weighed samples were dissolved individually in 10 mL of methanol and stirred by vortex mixer for a period of 10 min. Each of the solutions was filtered, using membrane filter (0.45 µm) and the drug content of each filtrate was estimated spectrophotometrically against blank. [6-8]

6. Viscosity

The viscosity of all the batches of VPLH loaded MEs was determined by using rheometer (Brookfield Engineering Laboratories, Inc., Middleboro, MA) with S62 spindle and 25°C temperature in triplicates. [6-8]

7. *In-vitro* Permeation Study

Preparation of skin: The *in vitro* skin permeation study was carried out under the guideline compiled by Committee for the Purpose of Control and Supervision of Experiments on Animal (CPCSEA, Ministry of Culture, Government of India) and all the study protocols were approved by the Local Institutional Animal Ethics Committee of Atmiya Institute of Pharmacy, Rajkot, Gujarat, India (CPCSEA No.: 1004/9O/Q/06//CPCSEA/PG-1005, Dated: 10/01/2012). The abdominal skin of rat was obtained from local slaughter house within 1 h of scarification in order to analyze *in vitro* permeation of developed MEs. After hair was shaved carefully with an electric clipper, the skin was subjected for removal of subcutaneous fat and other extraneous tissues without damaging the epidermal surface. The excised skins were washed and examined for integrity, and then stored at 4°C for 24 h in phosphate buffer pH 7.4 and then used for the permeation experiments. [15-18]

8. Permeation Study

Goat abdominal skin was mounted with the SC facing opposite to the receptor compartment on the Franz diffusion cell (Orchid scientific, Nasik, India) containing a diffusion area of 1.77 cm^2. The receptor compartment was filled with 16 mL of pH 7.4 phosphate buffer and the content was magnetically stirred at 300 rpm to prevent stagnant layer formation. The temperature of the system was maintained at 32°C. The donor compartment was filled with 1 mL of VPLH loaded MEs to achieve desired drug concentration at the site. Aliquots of 0.5 mL was withdrawn at predetermined intervals (0.5, 1, 1.5, 2, 3, 4, 5, 6, 8, 10, 12 and 24 h) from receptor medium and replaced immediately with an equal volume of receptor solution to maintain the volume constant. The amount of drug permeated across abdominal skin was measured after suitable dilution using UV visible spectrophotometer against blank. In order to

estimate extent of enhancement by ME formulations, an aqueous solution of VPLH with same concentration was also subjected to *in vitro* permeation under the same circumstances. The study was repeated in triplicates and the average values were used for the calculation.

9. Data Analysis

Average cumulative amount of drug permeated per unit surface area of the skin was plotted against time. The steady state flux (J_{ss}) was calculated from the slope of the linear portion of the plot and expressed as:

$$J_{ss} = \frac{dM}{dt}$$

Eq. ---------- 3

where, M was the cumulative amount of VPLH permeated (mg) through skin per unit area (cm^2) within experimental time t (h). Other parameters such as, permeability coefficient (K_p) and enhancement ratio (ER) were also calculated according to the following equations.

$$K_p = \frac{J_{ss}}{C_{donor}}$$

Eq. ---------- 4

$$ER = \frac{\text{Flux from microemulsion formulation}}{\text{Flux from drug solution}}$$

Eq. ---------- 5

where, J_{ss} was the flux, C_{donor} was the total amount of VPLH in donor compartment, h was the thickness of skin sample and t_L was the lag time. Further, in order to quantify the drug concentration in the skin (drug retained in skin) after permeation study, donor solutions were removed and the skin was washed twice with distilled water before unclamping from diffusion cells. The skin pieces were transferred in 10 mL of ethanol (95% v/v) and subsequently mixed with a mechanical stirrer. After 2 h stirring, the contents were centrifuged at 5000 rpm for 15 min in a centrifuge. The supernatants were analyzed for amount of drug present after adequate dilution with methanol using UV visible spectrophotometer against blank.

Preparation of VPLH Loaded Microemulsion Based Gels (MBGs)

An amount of drug representing 4% w/w for VPLH loaded MEs was added to distilled water, consisting of the optimized quantities of surfactant and cosurfactant. This aqueous part of optimized ME was vortexed for 10 min on vortexer and sonicated in an ultrasonicator bath until the drug was completely dissolved. Pre-optimized amount of thickening agent was dispersed in optimized amounts of selected oil phase by using high speed homogenizer at 1,000 rpm. This dispersion was kept in dark for 24 h for complete swelling of selected thickening agent. The previously prepared aqueous part loaded with VPLH was added slowly to this nonaqueous dispersion under magnetic stirring. All the batches of MBGs were allowed to stand for 24 h for complete gelation with subsequently sealing in glass vials and storage at room temperature until further evaluations. [19-20]

Selection of Thickening Agent

Various thickening agents, namely, white wax, cetostearyl alcohol, magnesium stearate, titanium dioxide, zinc oxide and colloidal silica were evaluated for their ability to thicken VPLH loaded MEs. The optimized thickening agent was further evaluated for the effect of its concentrations on the optimized batches of VPLH loaded MEs. [21-22]

Evaluation Parameters of VPLH Loaded W/O Type MBGs

1. Appearance

All the batches of VPLH loaded MBGs were evaluated visually for their color, homogeneity, consistency and phase separation. [21-22]

2. Globule Size and Size Distribution

The optimized batches of VPLH loaded MBGs were diluted (100 times) in respective optimized oil phase and endangered to measurement of globule size and size distribution immediately after preparation. The sample was subjected to a brief period of sonication (15–30 sec) in order to minimize any aggregation if present. The samples were analyzed by particle size analyzer at 25°C with an angle of 90°. The study was repeated in triplicates for confirmation of reproducibility. [21-22]

3. Zeta Potential (ζ)

The zeta potential (ζ) of optimized batch of VPLH loaded MBGs were determined by the particle size analyzer after their dilution (100 times) with optimized oil phase. The analysis was performed similar to as with MEs. The mean values of ζ for three independent samples were documented. [21, 22]

4. pH

The pH of all the batches of VPLH loaded MBGs was measured by digital pH meter. Each of samples was subjected to 10 times dilution by optimized oil phase before analysis. The study was repeated in triplicates and the average values were summarized. [21-22]

5. Viscosity

The viscosity of all the batches of VPLH loaded MBGs was determined by using rheometer with S61 spindle and 25°C temperature in triplicates. [21-22]

6. Percentage Drug Content (% DC)

Accurately weighed amount (1 gm) of the optimized batch of VPLH loaded MBG was transferred in a 100 mL volumetric flask and the volume was made up to the mark with methanol. The formulations were vortexed for 10 min on vortexer and sonicated in an ultrasonicator bath until the drug was completely dissolved. Each of the solutions was filtered, using membrane filter (0.45 µm) and the drug content of each filtrate was estimated

Table 2: Solubility of VPLH in Various Oils, Surfactants, Cosurfactants and Distilled Water

Oils	Solubility (mg/mL)	Surfactants	Solubility (mg/mL)
Capmul MCM	130.34 ± 10.61	Acconon CC-6	30.54 ± 4.89
Capmul PG8	105.31± 7.56	Capmul GMO50	324.57 ± 20.60
Captex 355	75.29 ± 6.48	Caprol ET	18.35 ± 2.16
Capryol 90	70.85 ± 5.89	Lauroglycol 90	47.34 ± 5.11
Labrafac CC	70.77 ± 7.32	Capmul MCM C8	234.54 ± 13.54
Labrafac Lipophile WL 1349	89.49 ± 8.57	Span 40	176.52 ± 13.46
Labrafil M 2125 CS	39.29 ± 3.95	Span 60	155.07 ±11.53
Maisine 35-1	37.22 ± 3.58	Span 80	160.46 ± 10.67
Imwitor 742	30.62 ± 2.93	**Cosurfactants**	**Solubility (mg/mL)**
Miglyol 812	25.01 ± 2.85	Polyethylene Glycol (PEG) 400	198.14 ± 13.02
Isopropyl Myristate	74.77 ± 8.60	Propylene Glycol (PG)	146.22 ± 10.33
Paceol	102.52 ± 7.57	Isopropyl Alcohol (IPA)	245.24 ± 14.72
Sefsol 218	116.27 ± 11.21	n-Butanol	188.44 ± 13.33
Oleic Acid	60.21 ± 3.45	Ethanol	402.24 ± 25.46
Olive Oil	12.8 ± 3.24		
Castor Oil	19.17 ± 2.16		
Distilled Water	150.18 ± 10.57		

The results are of mean ± SD (n=3)

spectrophotometrically against blank. The study was repeated for three independent samples in order to confirm reproducibility of the results. [21-22]

7. *In-vitro* Permeation Study

The *in vitro* skin permeation study of VPLH loaded MBGs was carried out under the guideline compiled by CPCSEA, Ministry of Culture, Government of India and all the study protocols were approved by the Local Institutional Animal Ethics Committee of Atmiya Institute of Pharmacy, Rajkot, Gujarat, India (CPCSEA No.:1004/9O/Q/06//CPCSEA/PG-1005, Dated: 10/01/2012). Goat abdominal skin was mounted with the SC facing opposite to the receptor compartment on the Franz diffusion cell containing a diffusion area of 1.77 cm^2. The receptor compartment was filled with 16 mL of pH 7.4 phosphate buffer and the content was magnetically stirred at 300 rpm to prevent stagnant layer formation. The temperature of the system was maintained at 32°C. The donor compartment was filled with 1 gm of optimized batch of VPLH loaded MBGs to achieve desired drug concentration at the site. An aliquots of 0.5 mL was withdrawn at predetermined intervals from receptor medium and replaced immediately with an equal volume of receptor solution to maintain the volume constant. The amount of VPLH permeated across goat abdominal skin was measured after suitable dilution using UV visible spectrophotometer against blank. In order to estimate extent of enhancement by MBG formulations, a conventional gel of VPLH with same concentration was subjected to *in-vitro* permeation under the same circumstances. The study was repeated in triplicates and the average value were used for the calculation The diffusion profile of optimized batch of drug loaded MBG was further subjected to estimation of release kinetic models by using in house developed software. [21-22]

RESULTS AND DISCUSSION

Selection of Microemulsion Components

1. Screening of Oil

The solubility of VPLH in different oils as well as in distilled water were determined and it was found to be highest in Capmul MCM (130.34 ± 10.61 mg/mL) as compared to other oils (Table 2). The data illustrated high solubilization capacity of novel synthetic oils as compared to edible oils. Therefore Capmul MCM was selected as the optimized oil phase for the development of W/O type ME formulation of VPLH. The solubility of VPLH in distilled water was found to be 150.18 mg/mL which was higher compared to oil phase. To observe the part of surfactants and cosurfactant in drug solubilization the solubility studies were accompanied in different surfactants and cosurfactants for VPLH. Highest solubility for VPLH was observed with Capmul GMO50 (324.57 mg/mL) from surfactant category whereas from numerous cosurfactants selected, ethanol exhibited highest solubility (402.24 mg/mL) for VPLH (Table 2). [23-24]

2. Screening of Surfactant

The surfactant selected must be able to lower the interfacial tension to a very small value and should be of the appropriate lipophilic character to provide the correct curvature at the interfacial region of W/O type MEs. [4-5] The results of emulsification study optimized oil phase (Capmul MCM) by lipophilic surfactant depicted highest emulsification potential of Span 80 than all other lipophilic surfactants (Table 2). Thus, Span 80 was selected as surfactants for further optimization of VPLH loaded MEs.

3. Screening of Cosurfactant

Cosurfactants provide a flexible film that can readily deform around droplets of MEs and be of the appropriate characteristic to provide the correct curvature at the interfacial region. It also penetrates into surfactant monolayer providing additional fluidity to interfacial film and thus disrupting the liquid crystalline phases. [4-5] Pseudo-ternary phase diagrams of different cosurfactants

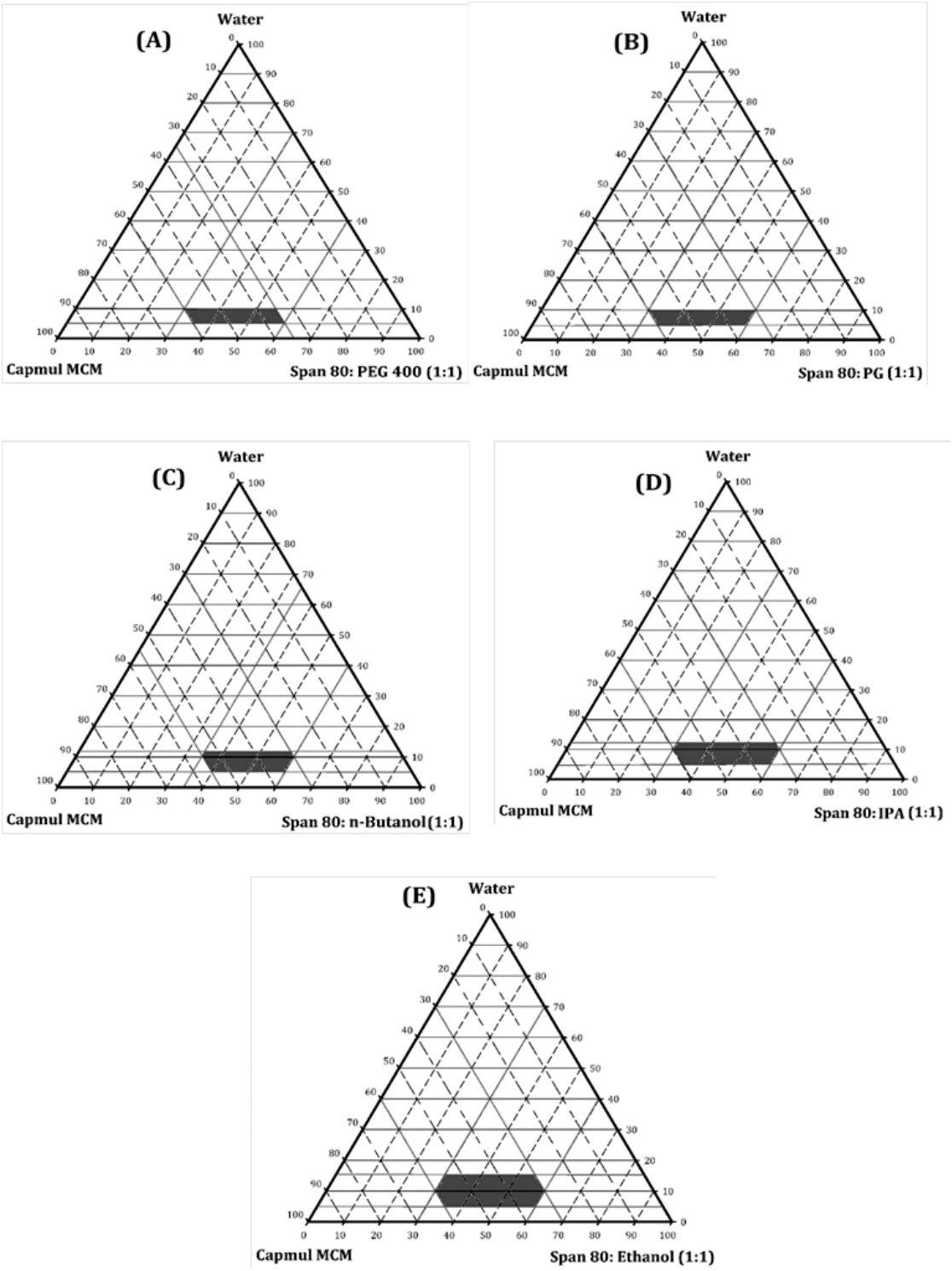

Figure 1: Pseudo-ternary phase diagrams with different cosurfactant (A) PEG 400, (B) PG, (C) n-Butanol, (D) IPA (E) ethanol for VPLH loaded MEs.

are illustrated in Figure 1. The results depicted highest microemulsification region with ethanol as cosurfactant.

Optimization of Surfactant and Cosurfactant Ratio (K_m)

The surfactant to cosurfactant ratio plays a crucial role in the characteristics of MEs and hence it was optimized by constructing pseudo-ternary phase diagrams. Pseudo-ternary phase diagrams of optimized surfactant blend (Span 80) and cosurfactant (ethanol) with different ratios (K_m) (3:1, 2:1, 1:1, 1:2, 1:3) are shown in Figure 2. Ethanol at higher concentration is reported to have skin irritation effect and hence 1:1 K_m ratio was selected as optimized for all further trials.

Optimization of VPLH Loaded Microemulsion

The present study was persisted with Box-Behnken design for VPLH loaded MEs using amount of oil, surfactant mix (S_{mix}) and water as three critical factors. Based on

Table 3(a): Results of Box Behken Design Batches of VPLH Loaded MEs

Batch Code	Globule Size (nm)	PI[a]	ζ [b] (mV)	RI [c]	%T [d]	%DC [e]	η [f] (cps)
VPLH-ME-F1	51.36 ± 4.24	0.17 ± 0.02	33.40 ± 1.45	1.46 ± 0.05	100.30 ± 0.36	98.45 ± 0.35	17.46 ± 1.34
VPLH-ME-F2	49.04 ± 2.46	0.18 ± 0.03	39.25 ± 1.56	1.50 ± 0.07	99.67 ± 0.30	99.10 ± 0.66	31.25 ± 2.14
VPLH-ME-F3	43.13 ± 4.34	0.14 ± 0.02	40.15 ± 1.73	1.51 ± 0.04	98.45 ± 0.98	100.65 ± 0.17	21.26 ± 1.65
VPLH-ME-F4	40.14 ± 3.10	0.16 ± 0.04	34.60 ± 2.76	1.40 ± 0.06	100.70 ± 0.26	98.54 ± 0.17	44.25 ± 3.60
VPLH-ME-F5	65.09 ± 5.65	0.18 ± 0.02	32.56 ± 2.15	1.55 ± 0.06	99.45 ± 0.70	100.64 ± 0.20	19.50 ± 1.04
VPLH-ME-F6	61.12 ± 3.76	0.15 ± 0.03	35.67 ± 1.09	1.54 ± 0.06	98.45 ± 0.46	100.65 ± 0.15	35.27 ± 2.54
VPLH-ME-F7	35.76 ± 2.56	0.17 ± 0.01	42.78 ± 2.60	1.57 ± 0.06	100.00 ± 0.57	99.00 ± 0.12	18.46 ± 1.11
VPLH-ME-F8	33.00 ± 3.43	0.16 ± 0.04	48.00 ± 3.57	1.44 ± 0.04	101.46 ± 0.23	101.65 ± 1.06	41.26 ± 4.65
VPLH-ME-F9	67.26 ± 4.09	0.21 ± 0.03	43.16 ± 4.76	1.49 ± 0.06	99.67 ± 0.64	99.15 ± 1.05	23.23 ± 2.20
VPLH-ME-F10	55.10 ± 3.09	0.12 ± 0.02	40.15 ± 1.05	1.50 ± 0.07	99.40 ± 0.64	101.00 ± 1.09	33.14 ± 2.32
VPLH-ME-F11	37.57 ± 2.20	0.23 ± 0.01	35.65 ± 1.11	1.52 ± 0.06	99.32 ± 0.16	100.13 ± 0.40	27.54 ± 1.98
VPLH-ME-F12	30.12 ± 2.35	0.14 ± 0.04	37.40 ± 1.45	1.55 ± 0.05	100.00 ± 0.70	99.43 ± 0.57	35.32 ± 3.07
VPLH-ME-F13	45.56 ± 3.64	0.17 ± 0.04	39.16 ± 1.40	1.53 ± 0.07	100.04 ± 0.14	98.74 ± 0.20	26.36 ± 2.40

The results are of mean ± SD (n=3), [a] PI: Polydispersibility index, [b] ζ: Zeta potential, [c] RI: Refractive index, [d] %T: Percentage transmittance, [e] %DC: Percentage drug content, [f] η: Viscosity

Table 3(b): Results of Box Behken Design Batches of VPLH Loaded MEs

Batch Code	Q_{24} [a] (mg/cm²)	J_{ss} [b] (mg/cm²h)	t_L [c] (h)	ER [d]	K_p [e] X 10^{-2}	%DRS [f]
VPLH-ME-F1	10.55 ± 0.18	0.5502 ± 0.0934	0.50 ± 0.02	3.5729 ± 0.1458	2.4346 ± 0.0755	150.45 ± 10.50
VPLH-ME-F2	10.70 ± 0.12	0.5005 ± 0.0635	0.42 ± 0.03	3.2503 ± 0.7687	2.2148 ± 0.1236	223.35 ± 13.56
VPLH-ME-F3	12.58 ± 0.20	0.5134 ± 0.0737	0.34 ± 0.04	3.3338 ± 0.1365	2.2717 ± 0.1447	210.25 ± 12.45
VPLH-ME-F4	12.68 ± 0.21	0.5141 ± 0.0476	0.25 ± 0.02	3.3386 ± 0.4580	2.2750 ± 0.1414	223.54 ± 16.65
VPLH-ME-F5	11.23 ± 0.13	0.4650 ± 0.0825	0.42 ± 0.05	3.0195 ± 0.3424	2.0575 ± 0.0768	250.15 ± 16.63
VPLH-ME-F6	11.71 ± 0.16	0.4696 ± 0.0634	0.42 ± 0.06	3.0491 ± 0.2768	2.0777 ± 0.3025	167.43 ± 12.15
VPLH-ME-F7	12.08 ± 0.21	0.5251 ± 0.0468	0.42 ± 0.02	3.4103 ± 0.4815	2.3238 ± 0.2547	189.9 ± 10.11
VPLH-ME-F8	12.75 ± 0.15	0.5281 ± 0.0634	0.34 ± 0.05	3.4295 ± 0.5057	2.3369 ± 0.4387	178.92 ± 9.50
VPLH-ME-F9	10.29 ± 0.17	0.4560 ± 0.0947	0.50 ± 0.03	2.9610 ± 0.3325	2.0177 ± 0.1325	200.34 ± 15.35
VPLH-ME-F10	12.70 ± 0.23	0.5211 ± 0.0447	0.42 ± 0.05	3.3838 ± 0.6787	2.3058 ± 0.1315	234.25 ± 10.33
VPLH-ME-F11	11.21 ± 0.17	0.5273 ± 0.0709	0.50 ± 0.07	3.4237 ± 0.8062	2.333 ± 0.1576	246.47 ± 16.35
VPLH-ME-F12	13.10 ± 0.23	0.5371 ± 0.0625	0.25 ± 0.03	3.4879 ± 0.3413	2.3767 ± 0.1612	234.15 ± 12.14
VPLH-ME-F13	12.17 ± 0.31	0.5163 ± 0.0725	0.42 ± 0.06	3.3530 ± 0.4514	2.2848 ± 0.1960	210.36 ± 14.34

The results are of mean ± SD (n=3), [a] Q_{24}: Cumulative amount of drug permeated, [b] J_{ss}: Flux, [c] t_L: Lag time, [d] ER: Enhancement ratio, [e] K_p: Permeability coefficient, [f] DRS: Drug retained in skin

preliminary trials three independent variables (factors) were determined as X_1 - amount of oil (Capmul MCM), X_2 - amount of surfactant mixture [Span 80: ethanol (1:1)] and X_3 - amount of water. The actual values of each of the selected factor has been summarized against their respective coded values in Table 1. The results of responses like globule size, polydispersibility index (PI), zeta potential (ζ), refractive index (RI), percentage transmittance (%T), percentage drug content (%DC), viscosity (η), cumulative amount of drug permeated after 24 h (Q_{24}), flux (J_{ss}), lag time (t_L), enhancement ratio (ER), permeability coefficient (K_p) and drug retained in skin (DRS) for experimental design batches of VPLH loaded MEs have been summarized in Table 3(a) and 3(b). For all 13 batches four dependent variables; globule size (Y_1), viscosity (Y_2), cumulative amount of drug permeated in 24 h (Q_{24} – Y_3) and lag time (t_L – Y_4) exhibited wide variations from 30.12 to 67.12 nm, 17.46 to 44.25 cps, 10.29 to 13.10 mg/cm² and 0.25 to 0.5 h, respectively (Table 3). The data clearly indicated strong influence of selected factors (X_1, X_2 and X_3) on all four responses (Y_1, Y_2, Y_3 and Y_4). A stepwise multivariate regression was performed to evaluate the observations.

The fitted polynomial equations (full and reduced model) relating the responses to the transformed factors are shown in Table 4.

The polynomial equations could be used to draw conclusions after considering the magnitude of coefficients and their mathematical sign. For globule size (Y_1), coefficients b_{12}, b_{13}, b_{23}, b_{11} and b_{22}; for viscosity (Y_2), coefficients b_{23} and b_{11}; for Q_{24} (Y_3) coefficients b_{12}, b_{13}, b_{23} and b_{33} whereas for lag time (Y_4) coefficients b_{12}, b_{13}, b_{11}, b_{22} and b_{33} were found to be insignificant ($P > 0.05$) and hence, they were separated from full model to develop a reduced model. [13-14]

The high value of correlation coefficients for globule size (Y_1), viscosity (Y_2), Q_{24} (Y_3) and lag time (Y_4) illustrated goodness of fit. The critical values of F for Y_1, Y_2, Y_3 and Y_4 were found to be 9.01 (df = 5, 3), 9.55 (df = 2, 3), 9.12 (df = 4, 3) and 9.12 (df = 4, 3) respectively. For all four responses, calculated F values [1.76 (Y_1), 3.28 (Y_2), 2.59 (Y_3) and 1.6 (Y_4)] were less than their respective critical values which supported nonsignificant difference between full and reduced models. The data of all the 13 batches of experimental design were used to generate interpolated values with the assistance of contour plots.

Table 4: Regression Analysis of Box-Behnken Design Batches of VPLH Loaded MEs

Coefficients	Globule Size (Y_1)		Viscosity(Y_2)	
	FM[a]	RM[b]	FM[a]	RM[b]
b_0	45.5600	45.8460	26.3600	26.9471
b_1	-1.5050	-1.5050	9.4100	9.4100
b_2	-4.5925	-4.5925	4.3112	4.3112
b_3	-14.0150	-14.015	1.4212	1.4212
b_{12}[c]	-0.1675	----	2.3000	2.3000
b_{13}[c]	0.3025	----	1.7750	1.7750
b_{23}[c, d]	1.1775	----	-0.5325	----
b_{11}[c, d]	0.7937	----	0.5137	----
b_{22}[c]	-0.4362	----	1.6812	1.4610
b_{33}	2.3887	2.2815	1.7662	1.5460

Coefficients	Q_{24} (Y_3)		t_L (Y_4)	
	FM[a]	RM[b]	FM[a]	RM[b]
b_0	12.1732	12.0829	0.4200	0.4000
b_1	0.1123	0.1123	-0.0312	-0.0312
b_2	1.0371	1.0371	-0.0825	-0.0825
b_3	0.3383	0.3383	-0.0312	-0.0312
b_{12}[e, f]	-0.0143	----	-0.0025	----
b_{13}[e, f]	-0.0764	----	-0.0200	----
b_{23}[e]	-0.1306	----	-0.0425	-0.0425
b_{11}[f]	-0.2775	-0.2437	-0.0300	----
b_{22}[f]	-0.2687	-0.2348	-0.0125	----
b_{33}[e, f]	-0.079	----	0.0100	----

[a] FM: Full model, [b] RM: Reduced model, [c] Nonsignificant (P>0.05) coefficients for Y_1, [d] Nonsignificant (P>0.05) coefficients for Y_2, [e] Nonsignificant (P>0.05) coefficients for Y_3, [f] Nonsignificant (P>0.05) coefficients for Y_4

1. Influence of Formulation Factors on Globule Size (Y_1)

A lowest value of globule size (30.12 nm) was observed with batch VPLH-ME-F12 (Table 3). Moreover, contour plots (Figure 3) for Y_1 also illustrated strong influence of all three factors (oil, S_{mix} and water) studied. The data of regression analysis revealed negative values for coefficients b_1, b_2 and b_3 which indicated that globule size was decreased with increasing oil, S_{mix} and/or water concentration. This might be attributed solubilization potential of selected oil, surfactant, cosurfactant and water for VPLH.

2. Influence of Formulation Factors on Viscosity (Y_2)

A highest value of viscosity (44.25 cps) was observed with batch VPLH-ME-F4 (Table 3). Moreover, contour plots (Figure 3) for Y_2 also illustrated strong influence of all three factors (oil, S_{mix} and water) studied. The data of regression analysis revealed positive value for coefficients b_1, b_2 and b_3 which indicated that viscosity was increased with increasing oil, S_{mix} and/or water concentration. This might be attributed to viscous nature of these agents.

3. Influence of Formulation Composition Factors on Q_{24} (Y_3)

A highest value of Q_{24} (13.10 mg/cm^2) was observed with batch VPLH-ME-F12 (Table 3). Moreover, contour plots (Figure 3) and for Y_3 also illustrated strong influence of all three factors (oil, S_{mix} and water) studied. The data of regression analysis revealed positive value for coefficients b_1, b_2 and b_3 which indicated that Q_{24} was increased with increasing oil, S_{mix} and/or water concentration. This might be attributed to reduction in globule size of MEs with increase in amount of water, membrane disturbing potential of surfactant (Span 80) and cosurfactant (ethanol) and enhanced lipophilicity of the water soluble drug by lipid phase.

4. Influence of Formulation Factors on t_L (Y_4)

Lag time was highly influenced by all three independent variables studied with lowest t_L value of 0.5 h for batch VPLH-ME-F1 (Table 3). Moreover, contour plots (Figure 3) for Y_4 also illustrated strong influence of three factors (oil, S_{mix} and water) analyzed. The data of regression analysis revealed negative value of b_1, b_2 and b_3 coefficients which indicated that t_L was decreased with increasing amount of oil, S_{mix} and/or water. This might be attributed to reduction in globule size with increase in water amount, alteration of diffusivity with increase in surfactant and cosurfactant amounts and enhanced lipophilicity by oil phase.

5. Model Validation and Selection of Optimized Batch

The criterias for selection of optimum formulation of VPLH loaded ME was arbitrarily selected as lowest values of globule size, viscosity and lag time with maximum value of Q_{24}. On the basis of these criteria the check point/ optimized batch of VPLH loaded ME was constructed practically according to the levels of factors illustrated in Table 5. The predicted as well as observed values of all four responses for optimized batch of VPLH loaded ME have been summarized in Table 5. The results depicted nonsignificant (P>0.05) difference and lower % relative error between experimentally obtained and theoretically computed data of all four responses (globule size, viscosity, Q_{24} and t_L) which suggested suitability of design applied. [25-26]

Table 5: Formulation Composition and Results of Check Point Batch for VPLH Loaded MEs

Type of Component		Name of Component	Amount (mL)	Concentration (%w/w)
Oil (X_1)		Capmul MCM	40.80	38.44
Surfactant Mixture (X_2)	Surfactant	Span 80	29.00	27.32
	Cosurfactant	Ethanol	29.00	27.32
Water (X_3)		Distilled water	7.34	6.92

Responses	Predicted Value	Experimental Value [a]	% Relative Error
Globule Size (Y_1)	49.43 nm	52.00 ± 3.33 nm	5.19
Viscosity (Y_2)	28.47 cps	29.14 ± 1.67 cps	2.35
Q_{24} (Y_3)	12.67 mg/cm^2	12.16 ± 0.97 mg/cm^2	4.02
Lag time (Y_4)	0.3822 h	0.3922 ± 0.07 h	2.61

[a] The results are of mean ± SD (n=3)

Table 6: Comparison of Diffusion Parameters of Optimized Batches of Drug Loaded ME and MBG with Control Formulations

Parameters	ME	Pure Drug	MBG	Conventional Gel
Q_{24}[a] (mg/cm^2)	12.16 ± 1.58	3.29 ± 0.77	11.78 ± 1.45	2.68 ± 0.45
J_{ss}[b] (mg/cm^{2}h)	0.5497 ± 0.0361	0.1246 ± 0.0143	0.5214 ± 0.0256	0.1036 ± 0.0127
t_L[c] (h)	0.39 ± 0.09	1.00 ± 0.12	0.5 ± 0.07	1.5 ± 0.23
K_p[d] x 10^{-2} (cm/h)	2.74 ± 0.67	0.62 ± 0.07	2.60 ± 0.52	0.51 ± 0.11
ER[e]	4.41 ± 0.96	-	3.25 ± 0.12	-
%DRS[f] (µg/cm^2)	200.34 ± 18.48	35.79 ± 3.68	178.30 ± 16.58	30.25 ± 3.03
f_1[g]	76.84	-	67.37	-
f_2[h]	0.89	-	2.37	-

The results are mean ± SD (n=3), [a] Q_{24}: Cumulative amount of drug permeated at the end of 24 h, [b] J_{ss} : Flux (h), [c] t_L: Lag time, [d] K_p: Permeability coefficient, [e] ER: Enhancement ratio, [f] DRS: Drug retained in skin, [g] f_1: Difference factor, [h] f_2: Similarity factor

Evaluation Parameters VPLH Loaded W/O Type MEs

1. Globule Size and Size Distribution

The globule size of the optimized batch of VPLH loaded MEs was found to be 52 nm which confirmed nanometer size of developed formulation. The PI of the optimized batch of VPLH loaded MEs was found to be 0.16 which illustrated narrow size distribution of developed formulations.

2. Zeta Potential (ζ)

The ζ value of the optimized batch of VPLH loaded MEs was found to be - 34.57 mV. All the values of zeta potential were higher than |30 mV| which supported stability of dispersed systems.

3. Refractive Index (RI)

The RI values of all formulations were in the range of 1.4 – 1.6. The RI value of the optimized batch of VPLH loaded MEs was found to be 1.41.

4. Percentage Transmittance (%T)

The %T value of the optimized batch of VPLH loaded MEs was found to be 100.07.

5. Percentage Drug Content

The value of % drug content for the optimized batch of VPLH loaded MEs was found to be 99.98.

6. Viscosity

The viscosity of the optimized batch of VPLH loaded MEs was found to be 29.14 cps at 25°C.

7. *In vitro* Permeation

Permeation profile of all batches exhibited significant enhancement in *in vitro* permeation by MEs compared to their aqueous solution (control) ($P<0.05$). All permeability parameters like (Q_{24}, J_{ss}, K_p and ER) were significantly increased with MEs as compared to their aqueous solution ($P<0.05$). These could be due to the difference in mean size of internal phase droplets of MEs as compared to their respective aqueous formulations. Moreover, different mechanisms have been proposed to explain the enhanced transdermal delivery of drugs using MEs which includes; increased thermodynamic activity of the drug due to increase in its solubilization, through the action of MEs ingredients (surfactants and cosurfactants) as permeation enhancers and increased skin lipophilicity by presence of oil. All these possibilities were supported in our results as values of Q_{24} and J_{ss} were increased with increasing oil, surfactant, cosurfactant and water contents. [15-18] The optimized batch of VPLH loaded MEs also exhibited significant enhancement ($P<0.05$) in all studied permeability parameters as compared to aqueous solution of drug (control) and their results are documented in Table 6 and Figure 4(A). The diffusion pattern of the optimized batch of VPLH loaded MEs depicted 1.277 mg/cm^2 of VPLH permeated with respect to only 0.045 mg/cm^2 for pure VPLH within 1 h. Moreover, parameters like difference factor (f_1) and similarity factor (f_2) confirmed nonsimilarity of diffusion profiles of VPLH loaded MEs and their respective pure drug as f_1 values were higher than 15 and f_2 values were lower than 50 for both drugs (Table 6). The increase in diffusion velocity of drug loaded MEs could be

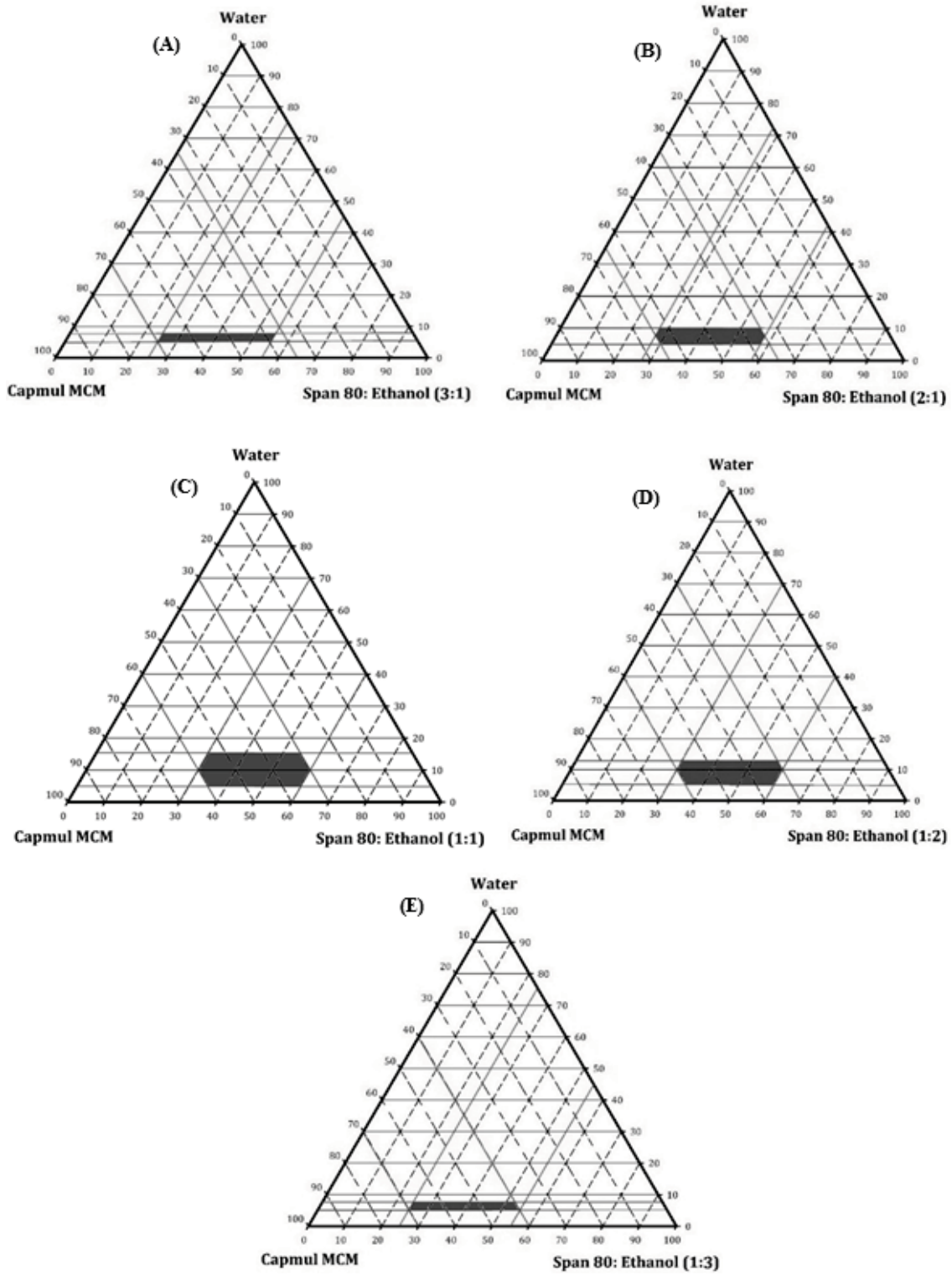

Figure 2: Pseudo-ternary phase diagrams with different ratios of S_{mix} (A) 3:1, (B) 2:1, (C) 1:1, (D) 1:2, (E) 1:3 for VPLH loaded MEs

attributed to reduction in globule size and decrease in diffusion distance. [6-8]

Table 6 shows comparison of diffusion parameters of optimized batches of drug loaded ME and MBG with control formulations.

Formulation and Development of W/O Type Microemulsion Based Gels

1. Selection of Thickening Agent

Various gelling agents such as white wax, cetostearyl alcohol, magnesium stearate, titanium dioxide, zinc oxide and colloidal silica were evaluated for the gelling of optimized batch of drug loaded MEs. [20-21] It was observed that white wax and cetosteryl alcohol affected the structure of the ME and resulted in macroemulsion formation. Titanium dioxide, zinc oxide and magnesium stearate were unable to yield desirable viscosity for the gel formulation and had resulted into sedimentation. Only colloidal silica

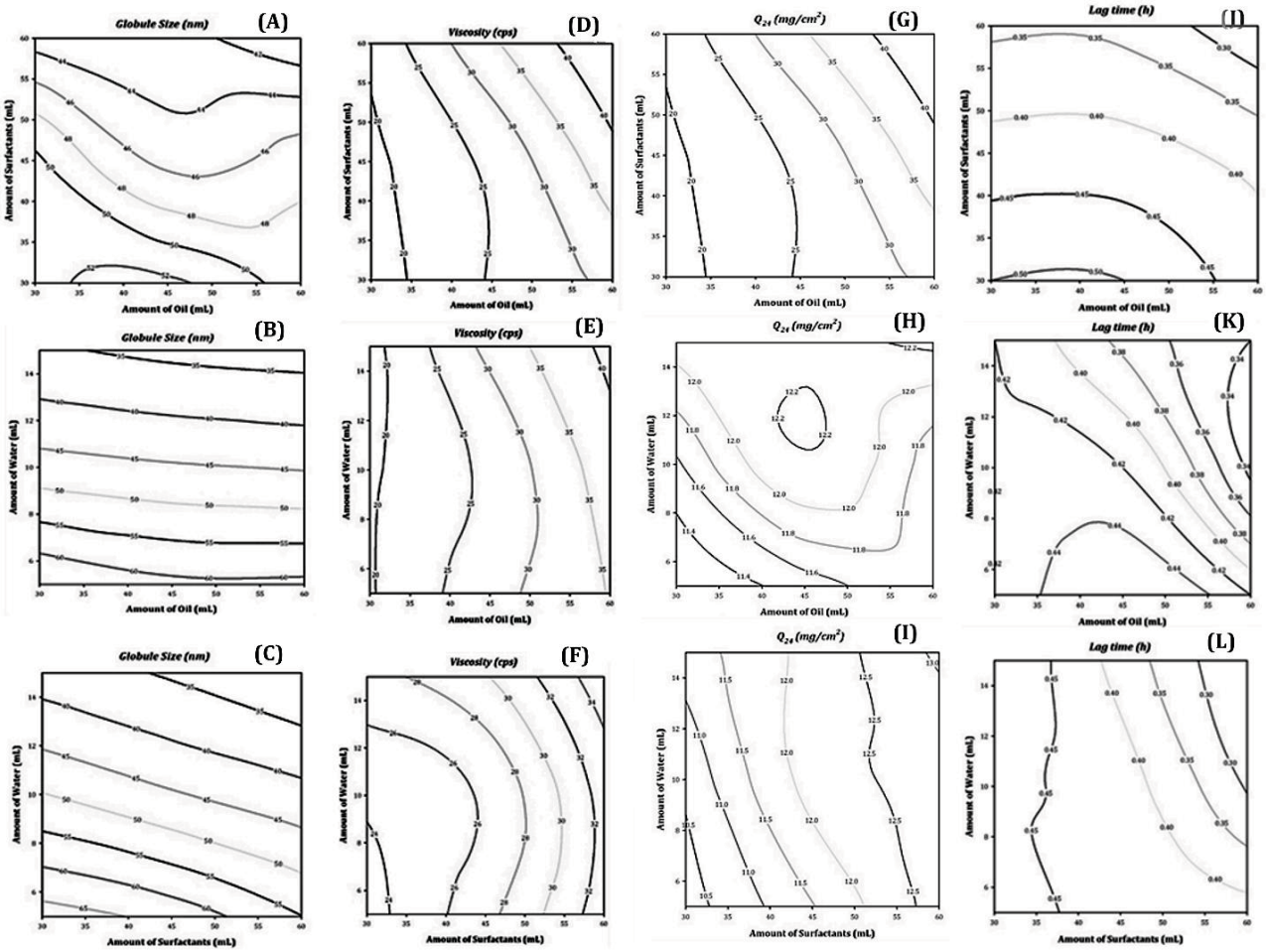

Figure 3: Contour plots for influence of formulation factors on globule size (Y_1) (A-C); viscosity (Y_2) (D-F); Q_{24} (Y_3) (G-I); Lag time (Y_4) (J-L) of VPLH loaded MEs

could yield gel without disturbing the microstructure of the optimized batch of ME. The selected thickening agent was further evaluated for the effect of its concentration on optimized batches of drug loaded MEs. The results revealed that only 5% w/v of colloidal silica concentration provided suitable viscosity to VPLH loaded MBG formulations.

Evaluation Parameters of VPLH Loaded W/O Type MBGs

1. Appearance

The optimized batch of VPLH loaded MBG was almost transparent, homogeneous, consistent alongwith no signs of phase separation for 24 h. [20, 21]

2. Globule Size and Size Distribution

The results of globule size and its distribution of the optimized batch of VPLH loaded MBG illustrated no significant difference ($P<0.05$) in the values of globule size and PI which conformed that inspite of conversation of liquid formulation (ME) into a semisolid form (MBG) the globule size and its distribution remains same. [20, 21]

3. Zeta Potential (ζ)

The results of zeta potential of the optimized batch of drug loaded MBG illustrated no significant difference ($P<0.05$) in the values of ζ which conformed stability of final formulation inspite of conversation of liquid formulation (ME) into a semisolid form (MBG). [20-21]

4. pH

The value of pH for the optimized batch was found to be 6.58 for VPLH loaded MBG, which was in the acceptable range for a transdermal formulations. [20-21]

5. Viscosity

The optimized batch of VPLH loaded MBG showed enhancement in the values of viscosity (193.58 cps) as compared to its ME. This enhancement in viscosity was attributed to the presence of colloidal silica as thickening agent. This enhancement might be predicted for the convenience in application of final formulations. [20-21]

6. Percentage Drug Content (% DC)

The percentage drug contents of the optimized batch of VPLH loaded MBG was found to be 97.68. The values of % drug content were almost 100% alongwith very low standard deviations, suggested uniform dispersion of drug in developed formulations. [20-21]

7. *In-vitro* Permeation

The optimized batch of VPLH loaded MBG exhibited significant enhancement ($P<0.05$) in all studied

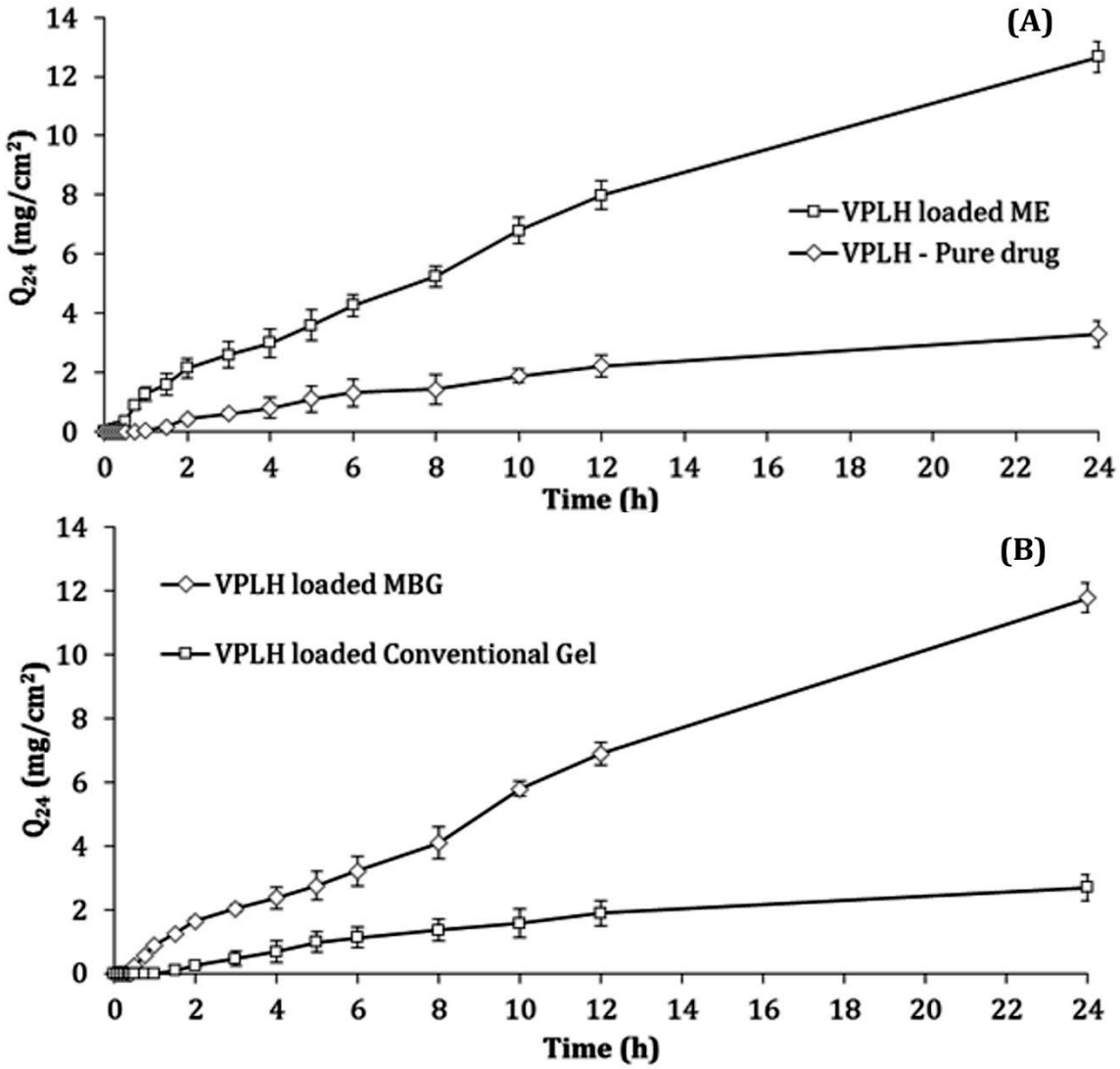

Figure 4: Comparison of *in vitro* drug permeation profiles of (A) optimized batch of VPLH loaded ME against their respective pure drug and (B) optimized batch of VPLH loaded MBG against their respective conventional gel, Error bar represents SD (n=3)

permeability parameters as compared to its conventional gel [Figure 4(B)]. The diffusion pattern of the optimized batch of VPLH loaded MBG depicted 1.64 mg/cm^2 of VPLH release compared to 0.24 mg/cm^2 for conventional gel within 2 h. The significantly higher values of Q_{24}, J_{ss}, K_p and lower values of t_L for the optimized batch of drug loaded MBG as compared to their respective conventional gel suggested marked improvement in diffusion rate (Table 6). The desired input rate of VPLH was decided by calculating drug concentrations required to elicit the pharmacological effect as per following equation:

$$\text{Desired Input Rate} = C_{ss} \times C_L \times \text{B. W.} \quad \text{Eq. ---------- 6}$$

where, C_{ss} was drug concentration at therapeutic level; BW was the standard human body weight (70 kg) and C_L was the total body clearance of the drug. The desired values of drug input rate was found to be 6300 µg/h whereas the practical value of J_{ss} for optimized batch of VPLH loaded MBG was found to be 0.5214 mg/cm^{2}h which revealed an application area of 12.08 cm^2 in order to match the desired input rate. Since this area was highly manageable for a transdermal formulation it fulfills the criterias for patient compliance.

CONCLUSIONS

The present investigation revealed that both rate and extend of VPLH transport across animal skin were highly dependent on the amounts of oil, surfactant, cosurfactant and water of developed formulations. The VPLH loaded ME; composed with 38.44% w/w of Capmul MCM as oil phase, 27.32% w/w of Span 80 as surfactant phase, 27.32% w/w of ethanol as cosurfactant phase and 6.92% w/w of water as aqueous phase was found to be optimum with a mean globule size of 52 nm. The drug loaded ME was successfully converted into MBG by using colloidal silica as thickening agent. The optimized batch of VPLH loaded MBG delivered VPLH with a flux value of 0.5214 mg/cm^{2}h in the *in vitro* permeation study. Thus, it could be concluded from the present investigation that a W/O type microemulsion could be an excellent approach for successful transdermal

delivery of highly water soluble drugs like, VPLH. However further, *in vivo* investigations are required to confirm improved antihypertensive efficacy of VPLH.

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