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PREPARATION, CHARACTERIZATION, AND OPTIMIZATION OF MICROEMULSION FOR TOPICAL DELIVERY OF ITRACONAZOLE

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ABSTRACT

Microemulsions (ME) have been proved to increase the cutaneous absorption of both lipophilic and hydrophilic medicaments when compared to conventional vehicles (emulsions, pure oils, aqueous solutions). Hence the aim of the present investigation is to prepare, characterize and optimize microemulsion of Itraconazole (ITZ). Itraconazole is an anti fungal agent, most widely used in the ringworm infection. It is classified as class III drug as per BCS classification. It indicates lower permeability through the skin. Therefore the objective of the research is to improve permeability of Itraconazole through the skin. The microemulsion was prepared using eucalyptus oil, tween 20 and methanol as the oil phase, surfactant and co-surfactant respectively. Pseudo-ternary phase diagrams were constructed to find out the optimum ratio of oil: S_{mix} (surfactant: Co-Surfactant): water. A 3^2 full factorial design was applied to the optimization of the prepared microemulsion. The microemulsion was evaluated for globule size, zeta potential, in-vitro diffusion study etc. Results of globule size measurements and zeta potential indicated ME7 had high stability than other formulation of the microemulsion. For the optimization transdermal flux and %Q6 was selected as dependant variables. Results of optimization study also revealed ME 7 as optimized microemulsion for high permeability to the skin. Further ME7 was compared to marketed Itraconazole preparation (ITASPORE) and evaluated using similarity factor F_2. Results of F_2 value was not near to 100 indicated there is no similarity in diffusion profiles of ME7 and ITASPORE. Hence, indirectly it suggests there was increased in permeability of drug by preparing microemulsion.

Keywords: Microemulsion, Factorial Design, Eucalyptus oil, Tween 20, Desirability Analysis

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INTRODUCTION

The incidence of superficial fungal infections of skin, hair, and nails has been increased in worldwide. It has been estimated that about 40 million people have suffered from fungal infections in developing and under developed nations. The progression of fungal infections can be rapid and serious due to compromising with immune function. *Dermatophytes* are one of the most frequent causes of *tinea* and o*nychomycosis*. Candida infections are also among the most widespread superficial cutaneous fungal infections[1].

Topical treatment of fungal infections has several superiorities including, targeting the site of infection, reduction of the risk of systemic side effects, enhancement of the efficacy of treatment and, high patient compliance. The efficiency of the topical antifungal treatment depends on the penetration of drugs through the target tissue. Different type of topical effective antifungal compounds has been used in the treatment of a variety of dermatological skin infections. The main classes of topical anti-fungal are polyenes, azoles, and allylamine/benzyl amines. Currently, these antifungal drugs are commercially available in conventional dosage forms such as creams, gels, lotions, capsule, shampoo, ointment [2,3].

Development of alternative approaches for the topical treatment of fungal infections of skin encompasses new carrier systems for approved and investigational

compounds. Delivery of antifungal compounds into the skin can be enhanced with the carriers including colloidal systems (Microemulsions, Micelles, Nanoemulsion), vesicular carriers (Liposomes, Ethosomes, Niosomes, and Transfersomes), and Nanoparticles. Current dosage form available for the treatment of ringworm has some limitation & side effects like skin irritation, drug contamination. The current dosage form in the market has also poor patient compliance [4]. Microemulsion (ME) is prepared to improve penetration & Bio-availability of Itraconazole. Topical microemulsion drug delivery can be defined as the application of a drug containing formulation to the skin to directly treat the cutaneous disorder. Fast-acting & There is less risk of serious adverse effects and limited drug interactions. Topical microemulsion includes enhancing the solubility of drugs, high thermodynamic stability, and ease of preparation. ME dosage forms increase penetration enhancing ability as compared to other dosage forms [1, 5-7].

Itraconazole (ITZ) is an effective used to treat fungal infections. Conventional dosage forms of Itraconazole showed about 15-20% bioavailability. It has a biological half-life of 6 hours. Itraconazole is included in Class III as per biopharmaceutical classification system of drugs. It showed low permeability hence it showed low bioavailability in systemic circulation via the topical route of administration. Hence, it is required to formulate a newer drug delivery system with the improved permeability of Itraconazole [8-13].

The main purpose of the present research work was to develop microemulsion based drug delivery system of anti-fungal agent Itraconazole via topical route. With enhanced permeation drug targets the site. The microemulsion was optimized using 3^2 full factorial designs. For optimization % of oil and S_{mix} was selected as independent variable while transdermal flux and % Q6 was selected as the dependent variable. Statistical optimization was done using MLRA and desirability analysis.

MATERIALS AND METHODS

Materials

Itraconazole was obtained as gift sample from Astron Research Center, Ahmedabad. Eucalyptus oil, Methanol, Tween-20, Isopropyl Alcohol was procured from S. D. Fine Chemicals, Mumbai, India. All other solvents used for the analytical purpose was obtained from Sigma Aldrich, Mumbai, India. All the solvents used were of analytical HPLC grade.

Experimental Methods

Solubility study

To find out the suitable oil which can be used as the oil phase in the microemulsion, the solubility of Itraconazole in various oils, surfactants, and co-surfactants was indicated in Table 1. An excess amount of Itraconazole was added in 2.0mL of the selected oil, surfactant, and co-surfactant in stoppered vials (capacity 2.0 mL) and then preliminary mixing was carried out over magnetic stirrer for few minutes. Later on, these vials were kept in a mechanical bath shaker for 72 hours at 37±0.5C. The equilibrated samples were then centrifuged at 10,000 rpm for 10 minutes. The supernatant was separated, filtered, and after appropriate dilution with methanol, solubility was determined by UV-visible spectrophotometer [3].

Construction of pseudo-ternary phase diagrams

Microemulsion consists of three components namely Oil, Water and Surfactant & Co-surfactant. The aqueous phase and concentration of co-solvent is varied and continued addition of water in this system results in the formation of Microemulsion. Hence, the pseudo-ternary diagram can be formed.In order to find out the concentration range of components for the existing range of microemulsion pseudo-ternary phase diagram was constructed using the water titration method. Ternary plots were constructed using Eucalyptus oil, Tween-20, Methanol as oil, surfactant, and co-surfactant using different ratios of (S_{mix}) Surfactants to Co-surfactants were prepared (1:1, 1:2, 2:1, 3:1) with both the oils; respectively. For each phase diagram the ratio of oil to S_{mix} were varied as (9:1, 8:2, 7:3, 6:4, 5:5, 4:6, 3:7, 2:8, and 1:9 % w/w). The mixtures of oil and S_{mix} at certain weight ratios were diluted with water, under moderate stirring. After being equilibrated, the mixtures were assessed visually and determined as a microemulsion or coarse emulsions. The phase diagram constructed using PROSIM software [4, 14-16].

Preparation of Itraconazole Microemulsion

The microemulsion was prepared by the spontaneous emulsification method. Construction of phase diagram is a useful approach to study the complex series of interactions that can occur when different components mixed. The formulations were prepared by initially dissolving the required quantity of ITZ in oil (1% drug is incorporated in oil).Then Surfactant and Co-surfactant mixer were added and the final mixture was mixed by vortexing until a clear solution was obtained. Finally, an appropriate amount of water was added to the ITZ solution mixture drop by drop to get microemulsion. An appropriate amount of water & drug was added by stirring the mixtures at ambient temperature.

Optimization of ITZ Microemulsion

A factorial design is suitable for exploring quadratic response surface and constructing second order polynomial models. The design consists of replicated center points and the set of points lying at the midpoint of the multidimensional cube that defines the region of interest. The non-linear quadratic model generated by the design in the form:

$$Y = b_0 + b_1X_1 + b_2X_2 + b_{11} X_{12} + b_{22} X_{22} + b_{12} X_1X_2$$

Where,

Y is Response,

b_0 is intercept,

X_1 and X_2 are independent factors,

b_1 and b_2 are coefficients of independent factors.

The coefficients with second order terms (b_{11} and b_{22}) indicate the quadratic nature and b_{12} is the interaction term (combining effect of Independent factors). This study investigated the utility of a 2-factor, 3- level factorial design and optimization process for Itraconazole Microemulsion by spontaneous emulsification method. Amount of oil (%) X_1 and S_{mix} (%) X_2 were selected as the independent variables whereas transdermal flux (J_{ss}) and %Q6 (amount of drug release after 6 hrs.) selected as dependent variables. The design matrix was presented in Table 1.

Desirability Analysis

After fitting the models & residual analysis of all three responses, for optimization of these responses simultaneously optimization techniques popularize by derringer and such was used. Their procedure makes use of desirability functions the general approach is to first convert each responses y_i into an individual desirability function d_i that varies for the range $0 \leq d_i \leq 1$, where as if the responses y_i is at its goal or target then, $d_i=1$, if the response is outside acceptable region $d_i=0$. Then the design variables are chosen to maximize the overall desirability.

$$D = (d1 * d2 * \dots\dots\dots\dots dn)^{1/n}$$

Where n is a number of responses.

Statistical software package Design expert 7.0 was used to design and analyze an experiment.

Characterization of Microemulsion

Droplet size analysis

Formulations (ME1 to ME9) were analyzed for droplet size. The prepared ME was inverted twice to ensure complete dispersion of the formulation. The prepared microemulsion diluted with Water with proper dilution. After ensuring complete dispersion of the formulation the droplet size of resultant ME was determined by photon correlation spectroscopy that analyzes the fluctuation in light scattering due to the Brownian motion of the droplets as a function of time using a DLS spectrophotometer (Model DLS700, Malvern Electronics Company Ltd., Japan).The average diameter (Z-AVE) and polydispersity index (PDI) of ME were determined by using a Malvern (Model DLS700, Malvern Electronics Company Ltd., Japan) at a fixed angle of 90˚ and at 25˚C [14, 15, 17].

Zeta potential Measurement

Zeta potential of formulations (ME1 to ME9) was analyzed by using DLS spectrophotometer (Model DLS700, Malvern Electronics Company Ltd., Japan) Equipped with a 4.0 mW He-Ne red laser (633nm).

pH Determination

The pH values of prepared ME1 to ME9 were determined using digital pH meter (Digital pH meter-III, EI) standardized using pH 4 and 7 buffers before use.

Drug content

About 10 mg equivalent of the ME was weighed in a 100ml volumetric flask and dissolved in 100 ml methanol to determine drug content. It was diluted appropriately and drug content was determined by spectrophotometrically (263nm).As a blank, the ME system (in the same amount as used for making test sample) without drug was extracted in 100 ml of methanol and the same was used after proper dilution.

In-vitro skin diffusion study

The modified diffusion cell was fabricated from borosilicate glass and consisted of two compartments i.e. receptor and donor. The cell and effective receptor surface area of 3.14cm^2. The two halves of the cell were secured in place with the help of strong metallic clips. A Teflon bead of 12mm length on a magnetic stirrer stirred the receptor solution. The temperature of the cell contents was maintained at 37±1oC with the help of thermostat attached to the magnetic stirring assembly. A modified Diffusion Cell was used for evaluating drug release profiles semi-impermeable membrane (0.1mm thickness). The receptor compartment was filled with 65ml of PBS pH 7.4 containing 10% methanol stirred by the use of the Teflon coated bead on a magnetic stirrer. The whole assembly was kept tightened with the screws for the cell setup. The whole assembly was kept on the magnetic stirrer and the temperature was maintained at 37±1^{o}C with the water jacket and at the 100rpm speed of magnetic bead. The withdrawal port was covered with the glass cork which prevents air entrapment. The amount of ME was applied between to the donor compartment to receptor compartment. The amount of drug released into the receptor solution was determined by 5ml of sample withdrawal at one hr intervals for hrs. The withdrawn volume was replaced with an equal volume of fresh buffer solution. The drug was release/diffuse was determined by analyzing the samples at 263nm [1, 6, 8].

In-vitro Antifungal Activity

In vitro antifungal studies were performed against *Candida Albicans* n Sabouraud's agar medium by the agar-cup method. Suspension of *C. albicans* was inoculated in Sabouraud dextrose broth and then poured into a sterile Petri dish and allowed to solidify. Wells was done on the plate using borer of size 8 mm and1 g each of developed formulations containing 1% of ITZ was poured into the wells. These plates were kept at 4°Cfor 1 h. After 1 h plates were incubated at 37°C±1°C for24 h. The zone of inhibition of ITZ released from prepared microemulsion was calculated in centimeters [14].

Estimation of Transdermal Flux

Amount of material flowing through unit cross section barrier in unit time is called Transdermal flux. Mathematically it is expressed as

$$Jss = Qt \times A$$

Where,

J_{ss} = Transdermal flux
Q = Amount of drug transferring the membrane in time t
A = Area of the exposed membrane in cm^2

Comparison of Diffusion Profile by Similarity Factor F_2

The prepared optimized ME formulation of ITZ was compared with marketed formulation using Similarity FactorF_2. CDER, FDA, and EMEA as the "logarithmic reciprocal square root transformation of one plus the mean squared difference in percent drug dissolved between the test and the reference products" defined the similarity factor (F2). Moore and Flanner give the model independent mathematical approach for calculating a similarity factor (F_2) for comparison between dissolution profiles of a different sample. The similarity factor was calculated by formula to give below.

$$F_2 = 50 \times \log\left[\left\{1 + \frac{1}{n}\sum_{t=1}^{n}(R_t - T_t)^2\right\}^{-0.5} \times 100\right]$$

Where n= no. of time points
R_t = the reference profile at the time point t
T_t = the test profile at the same point

Table 1: Experimental Runs & Levels of independent factors as per 3^2 Factorial Design

Batch	Coded Value		Actual Value	
	X_1	X_2	Oil (%)	S_{mix} (%)
ME1	-1	-1	10	40
ME2	0	-1	20	40
ME3	+1	-1	30	40
ME4	-1	0	10	50
ME5	0	0	20	50
ME6	+1	0	30	50
ME7	-1	+1	10	60
ME8	0	+1	20	60
ME9	+1	+1	30	60

Levels Variables

Independent Variable	Low(-1)	Medium(0)	High(+1)
X_1	10	20	30
X_2	40	50	60

RESULTS AND DISCUSSION

Solubility study

The physicochemical properties of ITZ suggest that it has good potential for topical drug delivery. The important criterion for selection of materials for the microemulsion formulation development is that the components are pharmaceutically acceptable, non-irritant and non-sensitizing to the skin. The result of solubility study of ITZ in various Oils, Surfactants, Cosurfactants was shown in Table 2. Among the selected oils that were screened maximum solubility of ITZ was found in Eucalyptus oil (10.89 mg/ml). Among the surfactants, Tween 20 (0.81 mg/ml) showed reasonable solubilizing potential for ITZ. The Co-surfactant Methanol (0.72 mg/ml) shows the highest solubility of ITZ.

Construction of pseudo-ternary phase diagrams

The construction of pseudo-ternary phase diagrams was used to determine the concentration range of components in the existence range of microemulsion. The translucent microemulsion region was presented in phase diagrams with no distinct conversion from water-in-oil (W/O) to oil-in-water (O/W) Microemulsions were observed. The rest of the region on the phase diagram represents the turbid and conventional emulsions based on visual observation. Based on solubility study Eucalyptus oil selected as oil, Tween-20 selected as a surfactant, Methanol selected as co-surfactant. In Pseudo-ternary phase diagram one axis represents oil phase, other represent as aqueous phase and third represent as surfactant & co-surfactant Mixture (S_{mix}). Pseudo-ternary phase diagrams for Microemulsions with different ratio of S_{mix} were illustrated in Figure 1. The phase diagram having highest microemulsion region. Hence, it was found to be S_{mix} ratio 3:1 selected for preparation ME. Pseudo-ternary phase diagram was constructed using PROSIM software.

Table 2: Results of ITZ Solubility study in oils, surfactants, co-surfactants

Types of Excipients	Name of Excipients	Solubility (mg/ml)
	Distilled Water	0.00964
Oil	Clove oil	3.745
	Eucalyptus oil	10.89
	Peppermint oil	0.45
	IPM	1.044
	Oleic acid	5.645
	Castor oil	0.05
	Olive oil	0.028
	Cottonseed oil	0.04
Surfactant	Tween-80	0.81
	Tween-20	0.031
	Span-20	0.030
Co-surfactant	n-Butanol	0.36
	Ethanol	0.35
	Methanol	0.72
	Propylene Glycol	0.18
	PEG -400	0.30
	PEG – 600	0.27

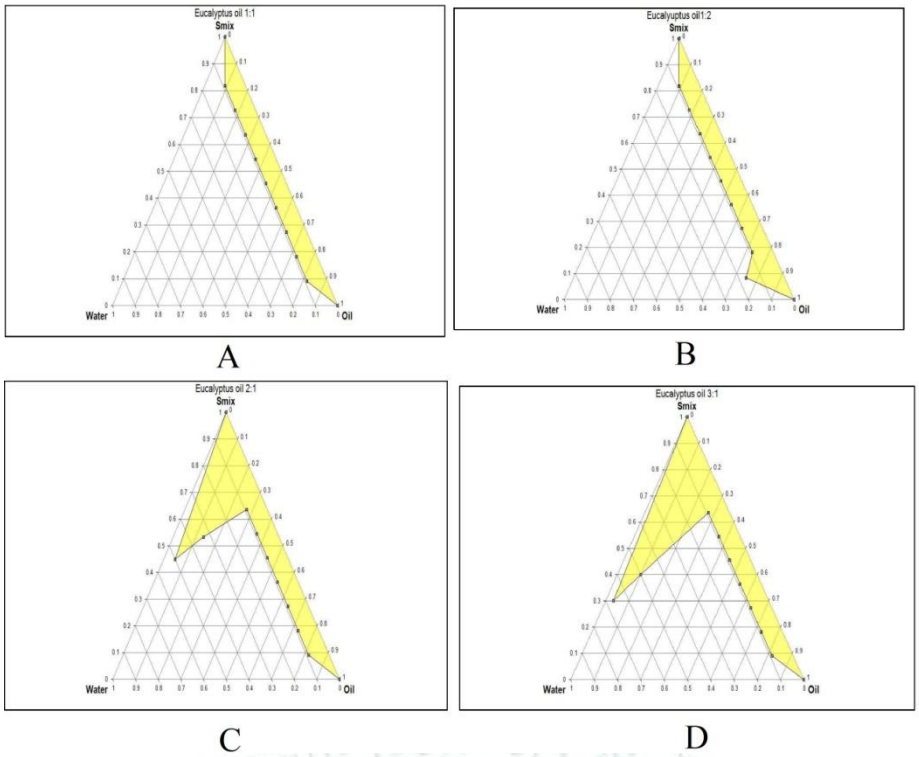

Figure 1: Psudoternary Phase diagram of Eucalyptus oil, Tween 20 & Methanol containing different S_{mix} Ratio (A) 1: 1 (B) 1: 2 (C) 2: 1 (D) 3: 1

Characterization of Microemulsion

Droplet size analysis & Zeta Potential Measurement

All 9 formulations of microemulsion were subjected to globule size and zeta potential determination using DLS. Results of globule size & zeta potential determination were presented in Table 3. Globule size of the microemulsion was ranged from 8.064nm to 687.1 nm. Results of zeta potential were found between -1.26 mv to -4.20 mv. PDI of all the formulation was in between 0.090 to 1.00. ME1, ME2, ME4, and ME5 showed lower globule size but simultaneously it had high PDI value and lower zeta potential values hence note selected as optimized formulation. ME7 may be selected as a more stable microemulsion, as it showed globule size 298.3nm and simultaneously had High Zeta potential -4.20 mV and Lower PDI value 0.090.

Viscosity Measurement

The viscosity of batch ME1 to ME9 was determined by using Brookfield viscometer. The Viscosity was measured using spindle no.64 at 62 rpm. The results of viscosity measurement were presented in Table 3. It was found between 34.5 cps to 106.1 cps.

Determination of pH

The pH values of prepared ME1 to ME9 were showed in Table 3. It indicated that all the batches showed pH Values near to the skin pH (7.01 to 7.43). Batch ME7 showed closer pH value (7.40) to the skin pH Value. Hence, ME7 was selected as best microemulsion formulation.

Table 3: Results of characterization of ME1 to ME9

Batch	Particle Size (nm)	PDI	Zeta Potential (mV)	Viscosity (cps)	pH
ME1	8.064	0.466	-2.22	34.5	7.01
ME2	9.853	0.614	-1.52	35.2	6.89
ME3	568.8	0.661	-1.28	38.3	6.25
ME4	8.857	0.361	-1.88	56.2	7.25
ME5	9.607	1.000	-1.26	59.9	6.90
ME6	687.1	0.176	-3.00	63.7	6.31
ME7	298.3	0.090	-4.20	96.5	7.40
ME8	149.5	1.000	-1.67	101.6	6.59
ME9	125.0	0.530	-2.05	106.1	6.40

In-vitro Diffusion study

The diffusion of the Itraconazole from the microemulsion was depending on the concentration of oil: S_{mix}. The release of the drug from the microemulsion formulations in high to low release order was described as below:

ME7 > ME4 > ME1 > ME8 > ME5 > ME6 > ME9 > ME2 > ME3

Where, the amounts of drug release after 6 hr were 98.74%, 90.29%, 86.56%, 85.25%, 85.01%, 82.36%, 81.36%, 80.25%, 79.12%, respectively. Amount of the drug diffused from the formulation through the diffusion membrane was completely related to the amounts of oil & S_{mix}. ME7, ME4, ME1 had high drug release compared to other Microemulsions. It indicated that if the increase in the concentration of oil, the diffusion of the drug decreases. The cumulative % drug release profile of ME1 to ME9 was illustrated in Figure 2. Among all 9 Batches, ME7 showed highest drug diffusion (98.74%) after 6 hrs. This may be due to the presence of a lower amount of oil (10%) & higher amount of S_{mix} (60%) which may be responsible for increased drug solubility and ultimately lead to higher drug diffusion.

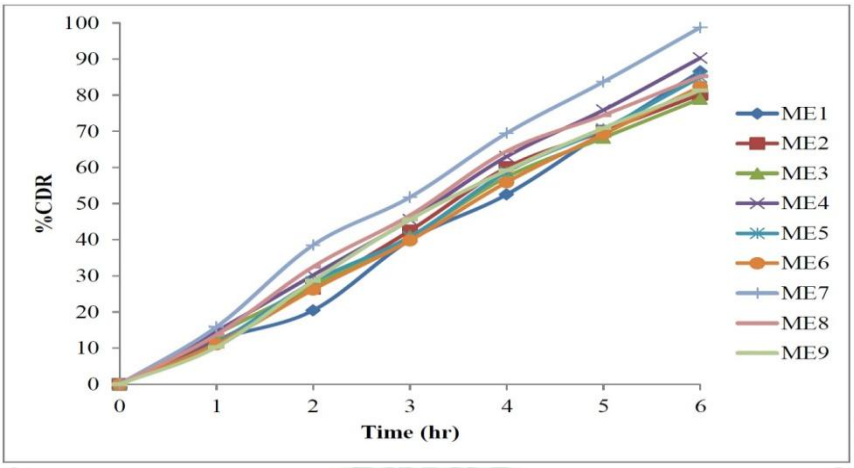

Figure 2: In-Vitro Diffusion Profile of ME1 to ME9

In-vitro Antifungal Activity

Anti-fungal activity of all 9 batches was performed to evaluate the effect of the presence of eucalyptus oil in Microemulsion. Zone of inhibition was recorded after an incubation period of 24hrs i.e. in Figure 3. All the formulations indicated the zone of inhibition similar to the marketed product. But ME7 showed a higher zone of inhibition comparatively to a marketed product which may be due to increased solubility of the drug in the microemulsion. Hence, ME7 was selected as best batch among all 9 batches and used for further evaluations.

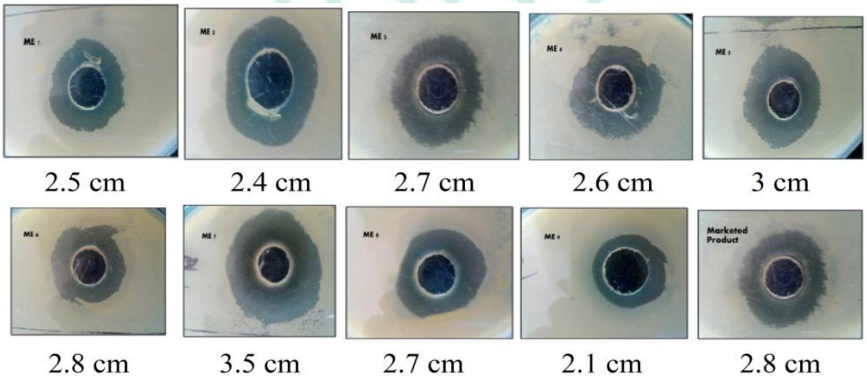

Figure 3: Results of Antifungal activity of ME1 to ME9 and Market Product

Optimization of ME using 3^2 Factorial Design

A 3^2 Full Factorial Design was implemented to optimize ITZ Microemulsion using spontaneous emulsification method. Amount of oil (X_1) and Amount of Smix (X_2) was selected as independent variables. Transdermal Flux (Jss,Y_1), % Q6 (% drug release after 6hrs, Y_2) was selected as dependent variables or responses to measure for preparation of ITZ microemulsion. Results of experimental runs as per 3^2 full factorial design and measured responses were depleted in Table 4. Responses surface curvature was examined when two variables were investigated at three levels. The design was provided the following empirical second order equation (Full model).

$$Y = b_0 + b_1X_1 + b_2X_2 + b_{11}X_{12} + b_{22}X_{22} + b_{12}X_1X_2$$

Where,

Y was represented response, b_0 was intercept, b_1 and b_2 are the coefficients of main effects, b_{12} was the coefficient for the interaction term and b_{11} and b_{22} were the coefficients for the second order quadratic terms. Nonsignificant estimated coefficients were dropped from the full model by adopting a significance test for the regression coefficient. A coefficient was significant if $P < 0.05$. Design Expert 9.0 trial software was used for statistical optimization to plot counter & 3D Response surface plots.

Table 4: Experimental Runs and Measured Responses as per 3^2 Factorial Design

Batch	Independent Variables		Responses	
	X_1	X_2	Transdermal Flux (Jss) (mg cm^2 h^{-1})	%Q6 (%)
ME1	-1	-1	4.275	86.56
ME2	0	-1	4.043	80.25
ME3	+1	-1	3.891	78.00
ME4	-1	0	4.413	90.29
ME5	0	0	4.181	89.20
ME6	+1	0	4.0716	86.79
ME7	-1	+1	4.793	98.74
ME8	0	+1	4.236	85.70
ME9	+1	+1	4.100	80.65

Transdermal Flux (Jss)

Transdermal Flux (Jss) was analyzed using Design Expert for prepared Microemulsion. A coefficient with positive sign shows a synergistic effect whereas a coefficient with negative sign shows an antagonistic effect. A coefficient of independent factor X_1 with a negative sign (-0.24) indicated that transdermal Flux (Jss) was change with change in amount of oil (batch F1, F2 and F3) similarly a coefficient of Independent factor X_2 with a positive sign (0.15) indicated that amount of S_{mix} had positive effect of transdermal Flux increase in value of S_{mix} increased value of transdermal flux & vice versa. ANOVA result of transdermal flux indicated the model F-value of 20.49 implies the model is significant. There is only a 0.21% chance that a "Model F-Value" this large could occur due to noise.

A negative value of A indicated a negative effect of variables on Microemulsion formulation. A positive value of coefficient B indicated a positive effect on S_{mix} Concentration. Increased in the value of X_1(A) range in value of microemulsion. It indicated that S_{mix} concentration increased transdermal flux was increased. Table 4 showed Results of ANOVA & Regression analysis for Transdermal Flux.

Effect of X_1& X_2 on transdermal flux was illustrated in 4.

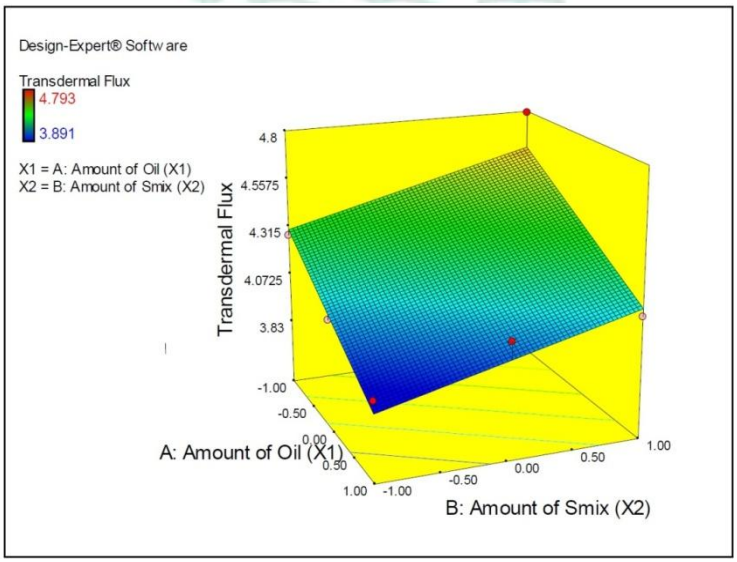

Figure 4: 3D Response Surface Plot Showing Effect of X_1 and X_2 on Transdermal flux (J_{ss})

Table 5: Results of ANOVA & Regression analysis for Transdermal Flux

Source	Sum of squares	Df	Mean square	F value	P- Value Prob >f	
Model	0.48	2	0.24	20.49	0.0021	Significant
A- Amount of oil (X1)	0.34	1	0.34	28.83	0.0017	
B- Amount of S_{mix} (X2)	0.14	1	0.14	12.16	0.0130	
Residual	0.070	6	0.012			
Cor total	0.55	8				
Std Dev		0.11	R-squared		0.8723	
Mean		4.22	Adj R-squared		0.8297	
C.V.%		2.55	Pred R-squared		0.6643	
PRESS		0.18	Adeq precision		12.525	

The "Pred R-Squared" of 0.6643 is in reasonable agreement with the "Adj R-Squared" of 0.8297. "Adeq Precision" measures the signal to noise ratio. A ratio greater than 4 is desirable. The ratio of 12.525 indicates an adequate signal. This model can be used to navigate the design space.The polynomial equation generated by using regression analysis as described below.

Final Equation in Terms of Code Factors:

Transdermal Flux = +4.22 -0.24*A +0.15*B

Final Equation in Terms of Actual Factors:

Transdermal Flux = +4.2267-0.23633*Amount of Oil (X_1) +0.15350 *Amount of Smix (X_2)

% Q6

Q6 was analyzed using Design Expert for prepared microemulsion. A coefficient with positive sign shows a synergistic effect whereas a coefficient with negative sign shows an antagonistic effect. A coefficient of independent factor X_1 with a positive sign (183.04) indicated that Q6 was decreased as the amount of oil was increased similarly a coefficient of independent factor X_2 with a negative sign (69.50) indicated that Q6 was increased as the amount of Smix was increased. ANOVA of %Q6 indicates the model F-value of 11.27 implies the model is significant. There is only a 0.93% chance that a "Model F-Value" this large could occur due to noise. Values of "Prob > F" less than 0.0500 indicate model terms are significant.In this case A, B are significant model terms. Values greater than 0.1000 indicate the model terms are not significant.In this model coefficient of X_2 (Amount of S_{mix} %) has a p-value less than 0.05 that means it was considered as a significant term in optimization formulation.

The combined effect of both of independent factors was essential to predict and achieve a targeted value of Q6. The positive sign of the interaction term indicated a favorable effect of both oil, S_{mix} on Q6. The relationship between the dependent and independent variables was presented in Figure 5. Table 5 showed ANOVA Result& Regression analysis for % Q6.

The "Pred R-Squared" of 0.5292 is in reasonable agreement with the "Adj R-Squared" of 0.7196."Adeq Precision" measures the signal to noise ratio. A ratio greater than 4 is desirable. The ratio of 9.237 indicates an adequate signal. This model can be used to navigate the design space.The polynomial equation generated by using regression analysis as described below.

Final Equation in Terms of Code Factors:

%Q6= +85.90-5.52*A+3.40*B

Final Equation in Terms of Actual Factors:

% Q6= +85.90444-5.52333*Amount of oil(X_1)+3.40333*Amount Smix(X_2)

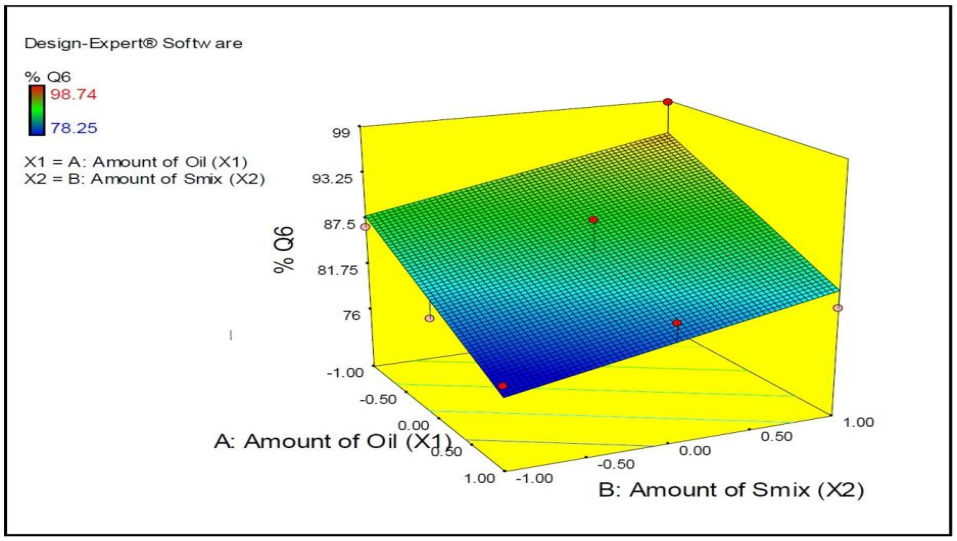

Figure 5: 3D Response Surface Plot Showing Effect of X_1 and X_2 on %Q6

Table 6: Results of ANOVA & Regression analysis for %Q6

Source	Sum of squares	Df	Mean square	F value	P- Value Prob >f	
Model	252.54	2	126.27	11.27	0.0093	Significant
A-Amount of oil (X1)	183.04	1	183.04	16.63	0.0068	
B-Amount of Smix (X2)	69.50	1	69.50	6.20	0.0472	
Residual	67.24	6	11.21			
Cor total	319.78	8				
Std Dev		3.35	R-squared		0.7897	
Mean		85.90	Adj R-squared		0.7196	
C.V.%		3.90	Pred R-squared		0.5292	
PRESS		150.56	Adeq precision		9.237	

Desirability Analysis:

The design expert software package was used to optimize the Microemulsion formulation with respect to maximum Transdermal flux and maximum %Q6. Keeping 2 independent variables within the range, which were found by experimental analysis constraints for the optimization & desirability was shown in Table 7.

We set weight for this individual desirability equal to unity. It was observed that is this analysis the medium consisting of the amount of oil and amount of S_{mix} was found to predict optimize batch with overall highest desirability 0.805 as compared to other formulation.

Table 7: Constraints for optimization and Desirability Analysis

Constraints						
Name	Goal	Lower Limit	Upper Limit	Lower Weight	Upper Weight	Importance
Amount of Oil (X1)	is in range	-1	1	1	1	3
Amount of Smix (X2)	is in range	-1	1	1	1	3
Transdermal Flux	Maximize	3.891	4.793	1	1	3
% Q6	Maximize	78.25	98.74	1	1	3

Table 8: Solutions for optimization of Batch according to Desirability analysis

Solutions						
Number	Amount of Oil (X_1)	Amount of Smix (X_2)	Transdermal Flux	%Q6	Desirability	
1	-1	1	4.6125	94.89333	0.805	Selected
2	-0.98	1	4.60858	94.7365	0.721	
3	-1	0.88	4.59453	94.4327	0.785	

It was arbitrarily decided for the selection of optimized batch, optimized batch had higher Transdermal flux and % Q6. On the basis of constraints for optimization batch, ME7 was selected as an optimized batch for ITZ Microemulsion. To validate the evolved mathematical models, two checkpoints were selected. The observed and predicted values for checkpoint analysis were presented in Table 9.

Good correlation between observed and predicted values of measured responses. Hence, it might be concluded that the evolved model might be used for theoretical prediction of responses within the factor space.

Table 9: Check Point Analysis of Microemulsion

Batch	X_1	X_2	Measured Responses			
			Transdermal Flux		%Q6 (%)	
			Observed	Predicated	Observed	Predicated
FC 1	0.5	0.25	4.21	4.22	85.71	85.713
FC 2	0.25	0.5	4.23	4.18	85.93	85.94

Batch ME7 was selected as optimized microemulsion loaded with Itraconazole and It was further used to prepare Aerosol formulation containing microemulsion.

Comparison of Diffusion Profile by Similarity Factor F_2

Optimized microemulsion batch (ME7) and marketed formulation of Itraconazole (ITASPOR) was compared using model-independent method. in vitro diffusion study data were compared in this study. Comparison of diffusion profile of ME7 and ITASPOR was showed in Figure 6. It indicated F_2 value 50.92. For similarity between in vitro diffusion of both formulations value of F_2 must be closer to the 100. In this comparison, this value was far away from 100. Hence, it was concluded that there is no similarity observed in the diffusion

profile. This suggested that there was a change in the diffusion profile of the optimized batch. Therefore, formulation of microemulsion for Itraconazole successfully increased solubility, permeation, and diffusion of the drug from the microemulsion.

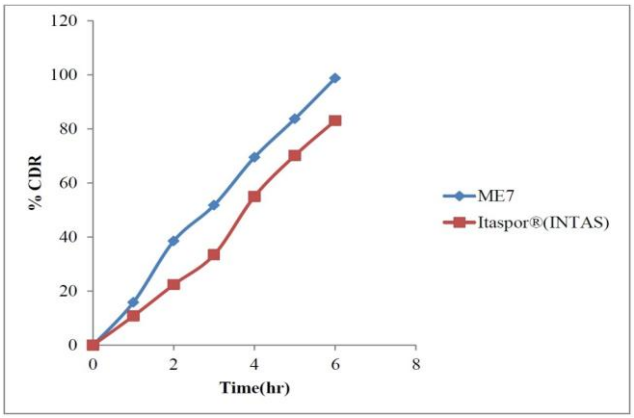

Figure 6: Comparison of In-Vitro Diffusion Profile of ME7 and ITASPOR (INTAS)

CONCLUSION

For preparation of microsimulation for itraconazole various oils, surfactants, and co-surfactants were screened via drug solubility study and a drug having maximum solubility selected for microemulsion preparation. The solubility of the drug was found higher in Eucalyptus oil (10.89mg/ml), Tween-20(0.81mg/ml), Methanol (0.72mg/ml) and Hence, selected for preparation of microemulsion. The pseudoternary phase diagrams were plotted to find out suitable microemulsion region mixture of surfactant. Surfactant: Co-surfactant (S_{mix}) was prepared in the ratio of 1:1, 1:2, 2:1 and 3:1. The various proportion of oil: S_{mix} was prepared i.e 1 to 9 and vice versa. Results of phase diagram revealed that stable microemulsion region was found oil: S_{mix} at 9:1 (S_{mix}: Oil) ratio where it contains S_{mix} ratio 3:1. The microemulsion was optimized using full factorial design. From all 9 batches of the ME7 Microemulsion was selected as an optimized batch, it showed maximum Transdermal Flux (4.739 mg $cm^{2}h^{-1}$), %Q6 (98.74%). The desirability study was also revealed that ME7 has maximum desirability i.e. 0.805. in conclusion, Microemulsion successfully increased the permeation of the Itraconazole. Batch ME7 was selected as optimized microemulsion loaded with Itraconazole.

Conflicts of Intrest

The authors of the manuscript do not have any financial conflict of interest.

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