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Formulation, Design and Evaluation of Microemulsion and Micro-Emulgel of Itraconazole for Topical Application

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ABSTRACT

The aim of the present investigation was to develop and evaluate itraconazole-loaded microemulsion based microemulgel for a treatment of fungal infection. Before making the formulation of itraconazole microemulsion carried out the preformulation study. Find out maximum solubility of ITZ in oils, surfactants and co-surfactants were evaluated to identify potential of excipients. The microemulsion area was selected through the construction of the pseudo ternary phase diagrams by phase titration method. A 3^2 full factorial design was applied to examine the combined effect of two formulation variables, each at 3 levels and the possible 9 combinations of ITZ ME were prepared. Optimized ME was prepared and incorporated into carbopol 934 added as gel matrix to convert microemulsion into microemulgel.Microemulsion and microemulgel evaluated by %transmittance, viscosity, pH, Density, conductivity, TEM, particle size, Zeta potential, surface tension, refractive index, In-vitro diffusion study, Physical appearance, viscosity, spreadability, extrudability measurement, syneresis measurement, comparison with marketed product having antifungal activity and accelerated stability studies. From FTIR and DSC study found that there is no interaction between drug and excipients. On the basis of pseudo ternary phase that the system consisting of capryol 90, tween 80 and PEG 400 showed the good emulsifying property at Mix ratio 4:1. skin irritation study was performed and it shows no irritation on rat skin after 48 hr of application. Microbiological assay of ITZ was performed where it shows the better zone of inhibition than marketed formulation.

INTRODUCTION

The frequency of obtaining bacterial, viral or fungal infections diseases increases each year due to the ease of transmission from person to person.[1]Swift and effective treatment options are a necessity to avoid spreading the disease to peripheral organs and potential death.[2,3]Also Fungal infection is a common infection which affects two-thirds of population among the world. Recently, there have been an increasing number of fungal infections caused by fungi, such as those belonging to the genus *Candida*, *Aspergillus*, and *Cryptococcus*. Fungal infections are varied and range from superficial infection with candida species to life-threatening infections of immunosuppressed individuals with *Aspergillus* species.

Fungal infections of the skin are also known as 'mycoses'. Fungal infections are common and a variety of environmental and physiological conditions can contribute to the development of fungal diseases. Candida skin infections can occur on almost any area of the body, but they are more commonly found in intertriginous regions, where two skin areas may touch or rub together.[4,5]

Many antifungal drugs are available for treatment of fungal infection like Itraconazole, Miconazole, Ketoconazole etc. Itraconazole is a triazole antifungal agent with the broad spectrum of activity. It belongs to BCS class-II drugs i.e. lower solubility with 55% oral bioavailability. The log partition coefficient of ITZ is 5.66 in a system of n-octanol and aqueous buffer solution at pH 8.1 which indicates hydrophobicity of drug. ITZ is a weak base with a pKa 3.7 and relatively insoluble in water (S<1 µg/ml). It has an extremely low aqueous solubility and poor dissolution rate in gastrointestinal tract so its oral administration is faced with large inter-individual variations in bioavailability. It is having certain oral side effects like nausea, vomiting, dizziness, stomach upset, trouble breathing, swelling feet, hair loss, irregular heartbeat, hearing loss etc. upon recurrent prolonged use. Log P is high which indicates high permeability through the membrane and it is beneficial for topical delivery.[6]It has been used successfully in the treatment and prevention of *Aspergillus* infections with a lower toxicity than amphotericin B, indicating a better therapeutic index. However, the bioavailability of ITZ from the existing market formulation like the pellet capsule form is very low in neutropenic patients and inadequate plasma concentrations are often found in patients receiving antineoplastic therapy.

Topical drug delivery opens up a number of opportunities with regard to efficient drug therapy for fungal infection and would be more effective in these individuals. A topical application may be helpful for many neutropenic and other immunocompromised patients who have difficulty swallowing the oral capsule formulation.[7]

Probable advantages of topical administration route include site-directed delivery, which can prevent the need for oral and other systemic treatment and can reduce the total drug dose, dose frequency and improve patient compliance, thereby reducing non-target site toxicities. A useful case in point is the treatment of cutaneous fungal infections where many useful agents must be administered orally to achieve clinically respectful cure rates.[8]For topical delivery semisolid preparations are widely accepted over solid and liquid dosage forms.

One of the most promising techniques for enhancement of transdermal permeation of drugs is by using microemulsion technique. Microemulsion is defined as thermodynamically stable transparent and translucent dispersions of oil and water stabilized by an interfacial film of surfactant and co-surfactant molecules having the droplet size less than 200 nm.It has been studied as drug delivery systems because of their capacity to solubilize poorly water-soluble drugs as well as their enhancement of topical and systemic availability. It helps to solubilize the lipophilic drug moiety and it shows rapid and efficient penetration to the skin. So it is beneficial for the topical drug.[9]

Many studies proved that microemulsion formulations possess improved transdermal and dermal delivery properties both *in Vitro* and *in vivo* over emulsions and gels. In principle, microemulsion can be used to deliver drugs to the patients via several routes, but the topical application of microemulsion has gained increasing interest. The three main factors determining the transdermal permeation of drugs are the mobility of drug in the vehicle, release of drug from the vehicle and permeation of drug into the skin. The concentration of surfactant must be high enough to provide the number of surfactant molecules needed to stabilize the microdroplets to be produced by an ultra-low interfacial tension.[10,11]This paper highlights the perspective of the microemulsion system transdermal delivery of itraconazole consisting of Capryol-90 as the oil phase, Tween 80 as surfactant and PEG 400 as co-surfactant. Further, the optimized formulations were incorporated into carbopol-934gel and further other studies were performed.

MATERIALS AND METHODS

Materials:

Itraconazole(ITZ) was gifted by Emcure Pharmaceutical (Gandhinagar), capryol 90 and labrasol were procured from Gattefosse (Mumbai), captex 200 and capmulMCM from Abiteccorp. USA, Tween 80, tween 20, PEG 400, PG, triethanolamine from Sulab (pioneer sales, Baroda),carbopole 934P from Balaji drug (Surat).All other chemicals and solvents used were of analytical grade.

Experimental:

- **Calibration curve of ITZ:**

Sample Preparation of stock and standard solutions for Itraconazole in Phosphate buffer pH 7.4.

Briefly, the stock solution was prepared by accurately weighing the quantity of 100 mg Itraconazole drug, and transferred into the 100 ml volumetric flask and dissolve and dilute up to the mark with methanol to give a stock solution having strength 1000µg/ml. 10ml of this solution was pipetted out in the separate 100ml volumetric flask and diluted with phosphate buffer pH 7.4 with 1% SLS for prepare working standard solution having strength 100µg/ml.The stock solution was serially diluted with Phosphate buffer pH 7.4 with 1%SLS to get solution range of 10 to 70µg/ml.

- **Screening of oils, surfactants, and co-surfactants for microemulsion:**

The solubility of itraconazole was determined in different oils, surfactants and cosurfactants were measured using shake flask method and the excess amount of drug was introduced into each 1 ml of selected vehicles.1ml of selected vehicles i.e. different oils, surfactants, and cosurfactants. Followed by sealing in vials. A vortex mixture was used to facilitate solubilization for 10 minutes. Sealed vials were stirred in water bath shaker for 48 hr and maintain at RT.Each vial was centrifuged at 1500 RPM for 10 min using centrifuge followed by removal of undissolved drug by filtering with membrane filter (0.45µm).Samples were suitably diluted with

methanol and drug concentration was obtained via a validated UV method at to 261 nm using methanol as a blank.[12]

- **Selection of Surfactants**

Surfactants selection was done on the basis of percentage of transparency(% Transparency) and Ease of emulsification.Briefly, the 0.3 ml of each surfactant was added to the selected 0.3 ml oil phase.The mixture was gentelyheayed at 50ºC homogenization of the components.Each 0.05 ml of mixture diluted with distilled water in stoppered conical flask.Ease of emulsification was judged by the number of flask inversion. Required to yield the homogeneous emulsion. Emulsion was allowed for stand for 2hrs and they %transperancy was evaluated by double beam UV spectrophotometer using distilled water as a blank at 638.2nm.

- **Selection of Co- Surfactants.**

The screening of co-surfactants was conducted on the basis of % transparency and ease of emulsification.0.1 ml of each co-surfactants mixed with 0.2 ml of selected surfactants and the 0.3ml of selected oil phase wad added and evaluated in a similar fashion as described in above section of surfactants.

➢ **Construction of pseudo ternary phase diagrams and formulation of ITZ microemulsion:**

Construction of pseudo ternary phase diagrams:

A pseudo ternary phase diagram was constructed using a water titration method at ambient temperature (25°C) with the help of Chemix ternary diagram software. The surfactant and co-surfactant, Smix (km)was mixed in the differents weight ratios 1:1,2:1,3:1,4:1,1:2.For each phase diagram the ratio of oil to the Smix was mixed as 1:9,2:8.3:7,4:6,5:5,6:4,7:3,8:2,9:1 (%w/w) respectively. After Water added dropwise to each oil-Smix mixture under vigorous stirring to identify microemulsion region until the mixture became clear at certain point. The concentration of components was recorded in order to construct the pseudo ternary phase diagram, and then the contents of oil, surfactant, co-surfactant and water at appropriate weight ratios were selected

based on these results. After phase diagram was plotted based on readings. The resulting phase diagram permits identifying the coarse emulsion and microemulsion regions.[13]

Formulation of final batches (preliminary trial batches)of ITZ loaded ME:

According to the microemulsion area in the phase diagram, the ITZ loaded ME for formulations were selected at mixing. Itraconazole loaded in accurately weight amount of oil and melted in water bath at 37ºC oil, The surfactant and co-surfactant were added to be oily phase using positive displacement pipette and After required quantity of water was added dropwise and stirred with magnetic stirrer for 15 min. Allow the solution to form clear and transparent liquid, which was o/w microemulsion. After that these batches were evaluated. On basis of result of evaluation, concentration of oil, Smix and water was selected and the preliminary batches were taken after screening to optimize various types and levels of variables for DoE study.

Formulation optimization:

The component proportion of microemulsion was optimized by using Design expert software version 10.0. In this research, from preliminary results, a 3^2 full factorial design was utilized in which two factors were evaluated, separately at three levels and possible nine combinations were formulated. Three level factorial studies were carried out using two different variables. In factorial design, amount of oil concentration (X1) and Smix concentration (X2) were taken as independent variables while %Transmittance (Y1), Viscosity (Y2) and %CDR (Y3) were selected as dependent variables for factorial design. Various batches of Itraconazole microemulsion by using DoE approach was prepared according to 3^2 factorial designs which are shown in Table 7,8,9

Evaluation of Microemulsion systems: [12,13,14,15]

1. **Viscosity measurement**

The viscosity was measured to determine the rheological properties of formulation Brookfield rotational r type viscometer was used to measure the viscosity at different rpm.

2. **Transmittance measurement:**

The percent transmittance of formulation was measured at λmax 638 using UVvisible spectrophotometer

3. **Zeta potential and globule size analysis**

Zeta potential of microemulsion was determined by using dynamic light scattering technique by malven zeta sizer**.**

4. **pH measurement**

The pH was done by digital pH meter.

5. **Measurement of Density**

The density was determined by using a pycnometer. Take empty weight of the pycnometer. Microemulsion was taken into the neck of the pycnometer and the weight was determined by using electronic balance. Now the difference between the total weights and empty pycnometer weight would give the weight of formulation. The density of formulation was calculated by the formula,

$$\text{Density [g/mL]} = \frac{\text{Weight[gm]}}{\text{Volume[mL]}}$$

6. **Conductivity test**

The measurement of electrical conductivity gives the quantitative idea of the solubilization of water phase in the selected mixture containing oil phase, surfactant and co-surfactant. The conductivity was measured by digital conductometer**.**

7. **Dilution test:**

If the continuous phase is added in microemulsion, it will not crack or separate into phase. It is confirmatory test of microemulsion to know which type of microemulsion was formed.

8. **Refractive Index**

The refractive index of microemulsion was measured by Abbe's refractometer

Surface tension

Surface tension of microemulsion was measured by using stalagmometer

9. **Transmission electron microscopy (TEM) study.**

Morphology and structure of the microemulsion were observed by using transmission electron microscopy(TEM).

10. **Determination of Drug content of Itraconazole Microemulsions**

For determination of drug content about 1 gm of each Microemulsion was weighed into a 10 ml volumetric flask and dissolved in methanol and diluted appropriately.Methanol was taken as blank and analyzed spectrophotometrically at 262nm.

11. ***In-vitro*** **diffusion study of Itraconazole microemulsion**

In-vitro study was carried out using cellophane membrane. The cellophane membrane was activated in glycerine for 24 hours. The cellophane membrane was placed in the receiver chamber and the donor chamber was clamped in place. The receiver chamber was filled with Phosphate buffer pH 7.4 as diffusion medium. The whole assembly was put on a magnetic stirrer. 1 ml of microemulsion was withdrawn from the receiver solution at different time intervals, and the cell was replenished to their marked volumes with fresh buffer solution. Addition of the solution to receiver compartment was done with great care to escape air trapping. The samples were filtered and %drug release was calculated by taking absorbance at λmax 262nm.

12. **Thermodynamic stability studies**

Microemulsion was subjected to six refrigerator cycles between temperatures of4ºC to 45 ºC with storage not less than 48 hrs in a heating cooling incubator. Formulations stable at these temperatures will be further subjected to centrifugation test at3,500 rpm for 30 min using microcentrifuge. Formulations which did not show any phase separation upon centrifugation was taken for freeze thaw stress test by three cycles between −21ºC and 25ºC temperature for48 hr. Based on the thermodynamic stability studies the microemulsion formulations was selected.

Preparation of microemulgel:[16]

1. Preparation of gel base by using gelling agent(1 % carbopol 934) and water by constant stirring and pH was adjusted by using triethanolamine.

2. Prepare API loaded o/w Micro Emulsion.

3. Incorporation of microemulsion into gel base.

Chracterizationof microemulgel:[17,18,19,20]

1. **Physical appearance**

The prepared gel formulations were inspected visually for their color, odor, feel on application, Texture, consistency.

2. **Homogeneity**

All developed emulgels were tested for homogeneity by visual inspection after the emulgel have been set in the container. They were testing for their appearance and presence of any aggregates.

3. **Grittiness**

All the formulations were observed under light microscope for the presence of any appreciable particulate matter.

4. **Measurement of pH:**

The pH of various emulgel formulations was determined by using digital pH meter.

5. **Measurement of Conductivity:**

The electric conductivity of Emulgel was measured by conductivity meter.

6. **Measurement of viscosity:**

The viscosity was measured to determine the rheological properties of formulation. Brookfield rotational viscometer was used to measure the viscosity at different rpm.

7. **Spreadability:**

Spreadability was determined by apparatus which is suitably modified in the laboratory and used or the study. It consists of a wooden block, which is provided by a lifter at one end. By this method, Spreadability was measured on the basis of 'Slip' and 'Drag' characteristics of emulgels.

Spreadability was measured by following formulation:

$$S=\frac{M\times L}{T}$$

Where,

S = Spreadability.

M =Weight tied to upper slide.

L = Length of glass slides.

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

8. **Extrudability Study of Topical microemulgel**

Here, in this method take quantity in percentage of microemulgel and microemulgel was extruded from lacquered aluminum collapsible tube on application of weight in grams required to extrude at least 0.5 cm ribbon of microemulgel in 10 seconds. More quantity extruded better is extrudability. The measurement of extrudability of each formulation is in triplicate and the average values are presented. The extrudability was calculated by using the following formula:

Extrudability = Applied weight to extrude emulgel from tube (gm) /Area (cm^2)**.**

9. **Syneresis measurement test**

In this test, microemulgel was put in a cylindrical plastic tube with a perforated bottom which was covered with filter paper (Whatman No. 41). These tubes were placed in centrifuge tubes and centrifuged for 15 min. The cylindrical plastic tube and liquid which separated from microemulgel were weighed. The percentage of Syneresis was calculated as

$$\% \text{ of Syneresis} = \frac{\text{weight of liquid separated from emulgel}}{\text{total weight of microemulgel before centrifugation}} \times 100$$

10. **Drug content determination:**

Weigh up 1gm of microemulgel and mix it in suitable solvent, filter it to obtain clear solution. Find out its absorbance by using UV spectrophotometer. Standard plot of drug was prepared in same solvent. Drug content was measured.

11. **Comparison study of optimized formulation of ItraconazoleMicroemulgel with marketed formulation.**

Optimized itraconazole microemulgel was compared with marketed itraconazole formulation.

12. **Drug Release Kinetic Study**

In order to discover the mechanism of drug release from microemulgel. The release data obtained from *in-vitro* diffusion studies was fitted into various kinetic equations. The kinetics models was used such as Zero order kinetic model (*C = K0t)*

Where,K0 = zero-order rate constant expressed in units of concentration(%cpr) time ,

t =time in hrs. First order kinetic model (Log C = Log C0 - Kt / 2.303**)** Where, C0 = initial concentration of drug, K = first order rate constant, and t = time. Hixson-Crowell kinetic model($W_0^{1/3} - W_t^{1/3} = \kappa t$) Where, W0 = initial amount of drug in the pharmaceutical dosage form, Higuchi's equation($Q = K_H \times t^{1/2}$)Where,

Q = percentage of drug release at time t, KH = Higuchi diffusion rate constant. Korsmeyer-Peppas Model ($M_t / M_\infty = k t^n$) Where, M_t / M_∞ = fraction of drug released at time t, k = release rate constant and n = release exponent.

Table 1: Release Kinetic Mechanism

Release Exponent 'n'	**Drug Transport Mechanism**	**Rate as a function of Time**
0.5	Higuchi Matrix	tn-0.5
0.5<n<1.0	Non- Fickian Diffusion	tn-1
1.0	Zero Order Release special Case–II Transport	Zero Order Release
Higher release (n>1)	Super Case–II Transport	tn-1

13. ***Ex-vivo*** **permeability study of microemulsion based emulgel.**

The *ex-vivo* study was carried out by using rat skin that was mounted on Franz diffusion cell using fevi-quick glue at the edge of donor compartment to escape leakage of test sample. Rat skin was placed in the receiver chamber and the donor chamber was clamped in place. The receiver chamber was filled with Phosphate buffer pH 7.4 as diffusion medium. Whole assembly was put on magnetic stirrer. 1 gm of microemulsion based emulgel was put on the rat skin and stirring was started with note down of time. Samples were withdrawn from the receiver solution at predetermined time intervals, and the cell was replenished to their marked volumes with fresh buffer solution. Addition of the solution to receiver compartment was done with great care to escape air trapping. The samples were filtered and % drug release was calculated by taking absorbance at λmax 262 nm.

14. **Flux and permeability coefficient**

The flux (mg/cm^2/hr) of Itraconazole was been calculate from the slope of the plot of the cumulative amount of Itraconazole permeated per cm^2 of skin at steady state against the time using linear regression analysis. The steady state permeability coefficient (Kp) of the drug through rat epidermis was calculated by using the following equation.

Kp = J/ CWhere,*J* = the flux,C =the concentration of Itraconazole in the gel.

15. **Skin Irritation Test**

The preparation was applied on the appropriately shaven skin of rats and its adverse like change in color, change in skin morphology should be checked up to 24 hours. The total set of 8 rats can be used of the study. If found no irritation than test will pass. If the skin irritation symptom occurs in more than 2 rats the study will be repeated.

16. ***In-Vitro* Antifungal activity**

Antifungal activity of formulation was checked by cup plate method. Certain volume of *Candida albicans* suspension was poured into sterilized dextrose agar media (cooled at 40 ºC) and mixed systematically. About 20 ml of this suspension was poured aseptically in petri dish and kept till the solidification. The surface of agar plates was pierced by using a sterile cork borer. The prepare wells were filled with an equal volume of optimized batch of microemulsion based gel and marketed itraconazole formulation after that it was incubated at 18-24 ºC, for 72 hrs. Fungal growth was found and the zone of inhibitions was measured using antibiotic zone reader.

17. **Accelerated Stability studies**

Drug or dosage form quality may affect under impact of by varying temperature, humidity and light with time which could found out by stability testing. It was carried out at 25°C ± 2°C/ 60% RH ± 5% RH and 40°C ± 2°C/ 75% RH ± 5% RH for the selected formulation for three months. Samples were withdrawn on 0th, 30th, 60th and 90th day and were analyzed for physical appearance and drug content.

RESULTS AND DISCUSSION

- **Calibration curve of ITZ:**

The calibration curve for Itraconazole was obtained by using the 100 µg/mL solution of Itraconazole in Phosphate Buffer pH 7.4. The calibration curve shows regression equation Y=0.011x and R^2value for PB pH 7.4 was 0.998. The result revealed that drug concentration between 0 – 70 µg/mL follows Beer Lambert's law as the regression coefficient was 0.998.

➢ **Screening of oils, surfactants and co-surfactants for microemulsion:**

➢ The physicochemical properties of ITZ suggest that it has good potential for topical drug delivery. The important criterion for selection of the excipients is that all the components are pharmaceutically acceptable for topical administration and fall under GRAS (Generally regarded as safe) category. Among the selected oils that were screened [Table 1], maximum solubility of ITZ was found in Capryol 90, so it was selected as an oil. Among the surfactants [Table 1], Tween 80 showed reasonable solubilizing potential for ITZ and it has high HLB value >15 which is suitable for o/w microemulsion as well as give 99.3% transmittance which shows excellent emulsifying ability for capryol 90. Therefore, tween 80 was selected as the surfactant for Itraconazolemicroemulsion formulation.

➢ Furthermore, PEG400 show 98.5% transmittance which is indicative of good emulsifying ability for capryol 90 and tween 80. Therefore, PEG 400 was selected as the co-surfactant for microemulsion formulation of Itraconazole. The suitable combination of surfactant and co-surfactant gives better stability of microemulsion.The value of solubility study shown in table 2 and result of selection of surfactant and co-surfactant result shown in table 3.

Table 2: Solubility of Itraconazole in various oils, surfactants and co-surfactants

Excipients	Solubility (mg/ml) (n=3)	SD
Oils		
Isopropyl myristate	0.204	0.55
Captex 200	0.986	0.86
Capryol 90	28.152	1.59
Capmul MCM	2.12	1.57
Castor Oil	1.35	1.43
Sunflower oil	1.73	1.52
Surfactants		
Labrasol	6.136	1.65
Tween 80	16.78	1.15
Tween 20	1.25	1.04
Co-surfactants		
PEG 400	5.43	0.55
Propylene Glycol	1.184	1.02

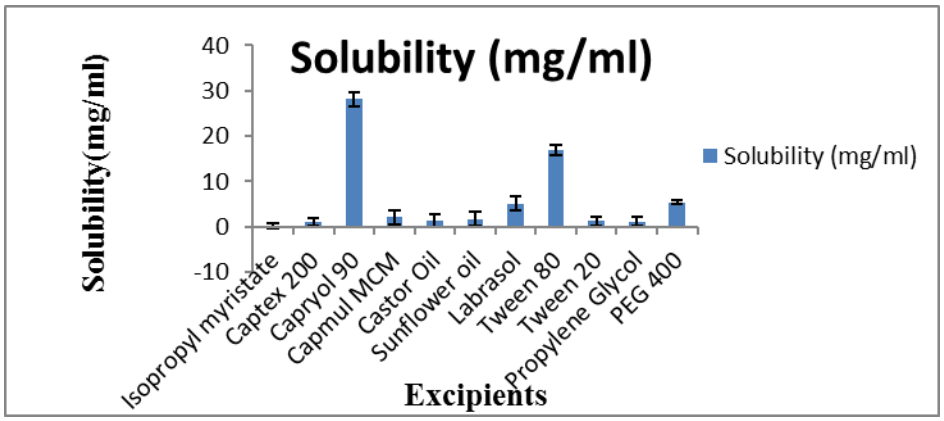


Figure 1: Solubility of Itraconazole in various oils, surfactants and co-surfactants

- **Selection of Surfactants and co-surfactants.**

Table 3: Selection of surfactants and co-surfactants

Sr. no.	Excipients	%Transmitance	No. of flask inversion
1	Tween 80	99.3	5
2	Tween 20	98	18
3	Labrasol	89	20
4	PEG 400	98.5	9
5	PG	94.2	15

- **Construction of pseudoternary phase diagrams and formulation of ITZ microemulsion:**

➢ **Construction of pseudoternary phase diagrams:**

Phase diagrams were constructed using Chemix school software ver.3.60.The rest of region represent turbid and conventional emulsions based on visual inspection, from the pseudoternary diagram it has been found that the system consisting of capryol 90, tween 80, PEG400 formation of maximum microemulsion (colored area) existence region at Smix 4:1ratio.Pseudoternary Diagram reading of maximum region for microemulsion shown in table 4**.**

Table 4: Pseudoternary Diagram reading of maximum region for microemulsion

Surfactant:Cosurfactant	Oil (%w/w)	Smix (%w/w)	Water (%w/w)	%Transmittance
4:1	6.33	56.96	36.71	98.2%
	12.90	51.61	35.49	97.6%
	20	46.67	33.33	95%
	26.85	40.27	32.88	92%
	34.97	34.97	30.06	89.7%
	42.86	28.57	28.57	90.1%
	51.09	21.90	27.01	80.7%
	60.15	15.04	24.81	72.5%
	69.23	7.69	23.08	68%

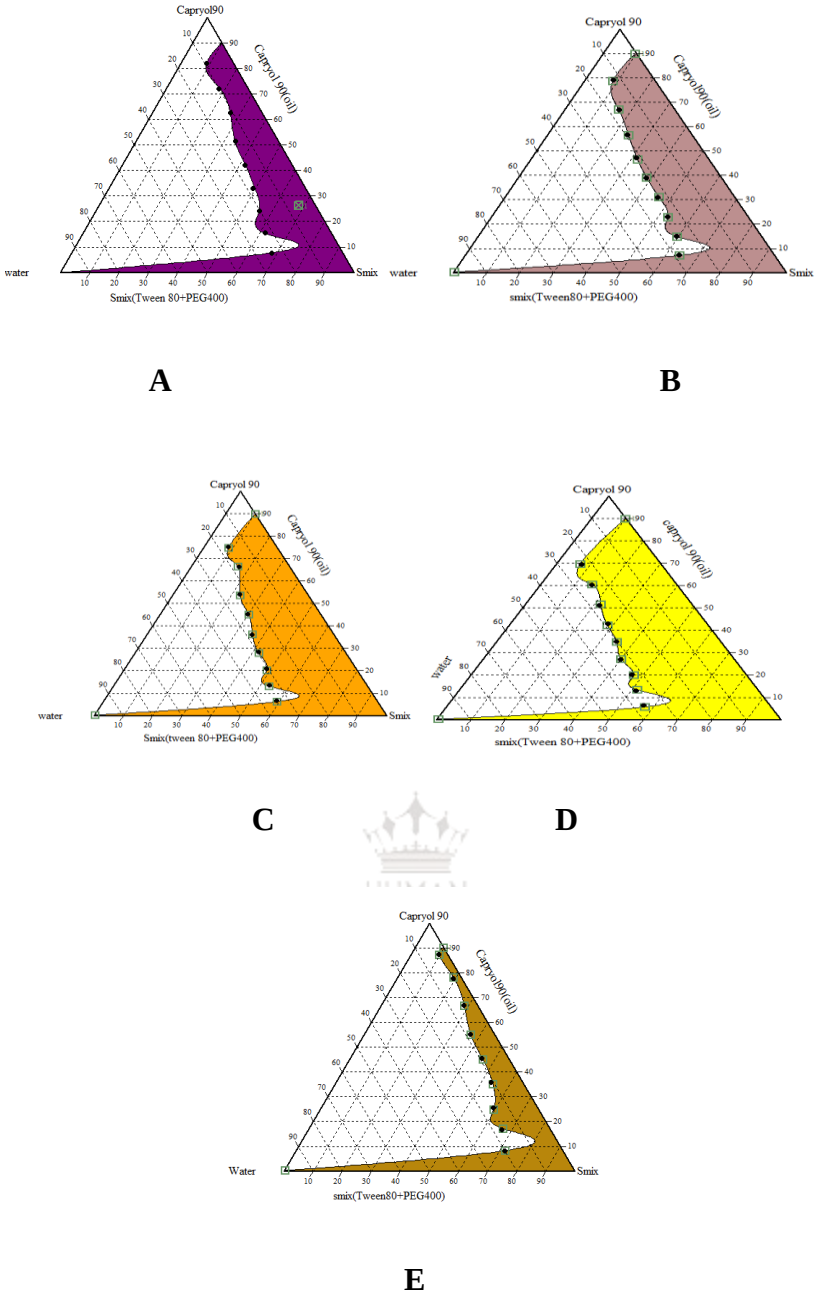


Figure 2: Pseudoternary phase diagram using capryol 90 as sthe oil phase and tween80/PEG400 as the S/Cos rept (A=Smix1:1,B= Smix2:1,C=Smix 3:1,D=Smix 4:1,E= Smix 1:2)

- **Formulation of final batches (preliminary trial batches)of ITZ loaded ME:**

The preliminary batches was taken after screening and construction of pseudoternary phase diagram to optimize various types and levels of variables for DoE study their results are shown in table 5 & 6, The levels selected for DoE study is shown in table 7

Table 5: Preliminary Trial Batches Based On Pseudoternary Phase Diagram

Batch code	Composition of microemulsion					
	Oil (%w/w)	Smix (%w/w)	Water (%w/w)	Oil(ml)	Smix(ml)	Water(ml)
TIM1	6.33	56.96	36.71	0.63	5.69	3.67
TIM2	12.90	51.61	35.49	1.29	5.16	3.54
TIM3	20	46.67	33.33	2	4.66	3.33

Table 6: Characterization Of batch TIM1-TIM3

Batch code	Viscosity(cps)	%Transmittance	%CDR		
			Time (hr)		
			1	2	3
TIM1	142	98	11.11	15.48	30.51
TIM2	148	97.5	7.56	12.41	19.91
TIM3	153	95.3	5.41	12.96	17.06

- **Formulation optimization:**

Formulation and Development of Itraconazole Microemulsion by using Design of Experiment [DoE] Approach:

Various batches of Itraconazole microemulsion by using DoE approach was prepared according to 3^2factorial designs which are as follow

Table 7: 3^2 Factorial Design

Independent variables of formulations			
Independent variables	Low (-1)	Medium (0)	High (1)
Oil concentration(%) (X_1)	5%	10%	15%
Smixconcentration(%) (X_2)	50%	55%	60%
Dependent variables			
Y_1 = %Transmittance			
Y_2 = viscosity			
Y_3 = %Drug release			

Compositions of Factorial Batches in Coded Form

Various batches of Itraconazole Microemulsion with capryol 90, tween 80 and PEG 400 was prepared according to **3 ^{2}**factorial designs which are shown in table 8& 9.Characterization of all factorial batches results were shown in table 10.

Table 8: Compositions of Factorial Batches in Coded Form

ITZ Microemulsion3^2= 9 Batches		
Batch No	**Variable level in coded form**	
	Oil Concentration (X1)	**Smix Concentration (X2)**
F1	-1	-1
F2	-1	0
F3	-1	+1
F4	0	-1
F5	0	0
F6	0	+1
F7	+1	-1
F8	+1	0
F9	+1	+1

➢ **Formulation Design by 3^2 Factorial Design**

Table 9: Compositions of Factorial Batches in Actual Form

Batch No	ITZ Microemulsion3^2= 9 Batches			
	Actual value			
	Oil Concentration (%) (X1)	Smix Concentration (%) (X2)	Amount of Oil (ml) (X1)	Amount of Smix (ml) (X2)
F1	5	50	0.5	5
F2	5	55	0.5	5.5
F3	5	60	0.5	6
F4	10	50	1	5
F5	10	55	1	5.5
F6	10	60	1	6
F7	15	50	1.5	5
F8	15	55	1.5	5.5
F9	15	60	1.5	6

➢ **Characterization of batches from F1 to F9**

Table 10: Characterization of batches from F1 to F9

Batches	%Transmittance(Y1)	Viscosity(Y2) (cps)	%CDR(Y3) (%)
F1	99.5	149	25.20
F2	99.7	143	28.56
F3	99.9	140	31.21
F4	99.1	155	10.72
F5	99.4	153	14.73
F6	99.5	150	19.51
F7	96.2	167	8.54
F8	97.5	162	11.84
F9	97.9	159	17.85

- **Statistical Analysis:**

➢ formulations were prepared as per the experimental design and characterized for various responses like %transmittance, Viscosity and %CDR within 3 hr.The response surface analysis was carried out to understand the effect of selected independent variables on the observed response. Countour plots and 3D plots of all three depended variables are shown in figure 3,4&5.

1) Polynomal equation of X1 and X2 on %transsmitance (Y1)

%transmittance= +98.74-1.25* X1+0.42* X2

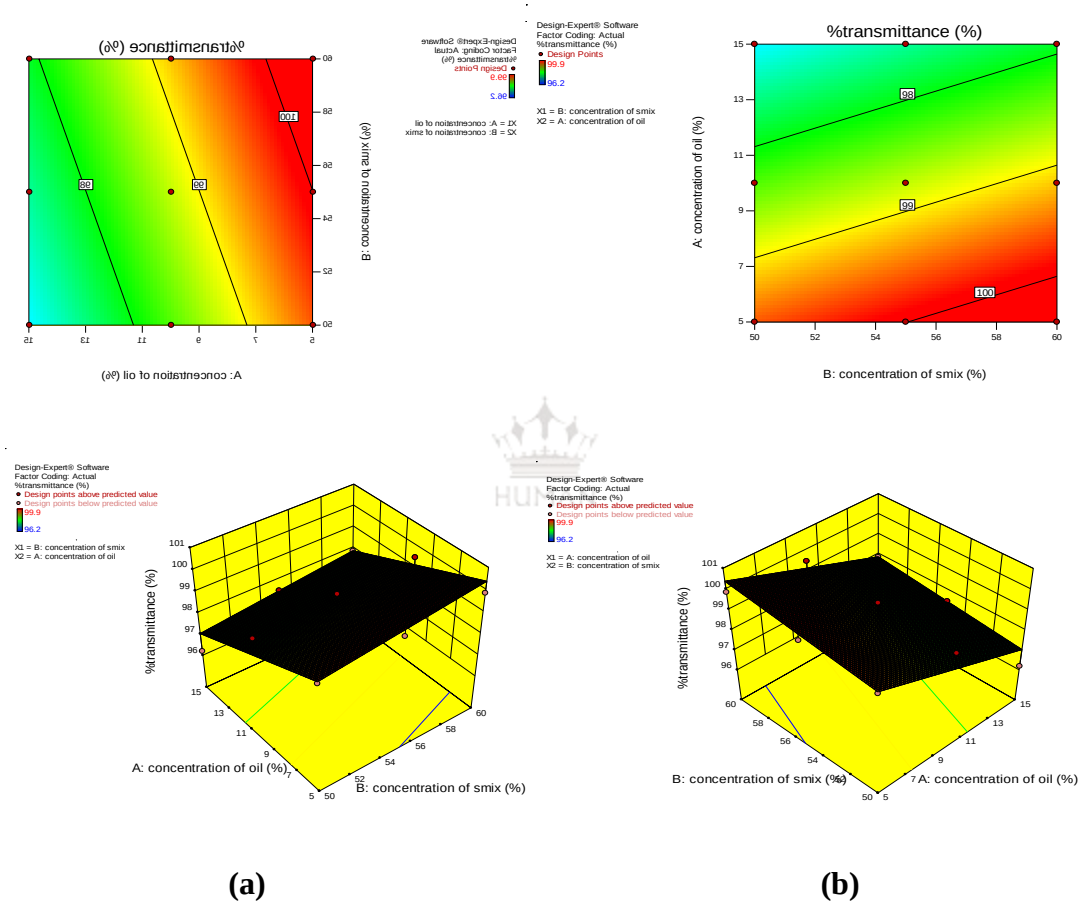
2) Polynomal equation of X1 and X2 on viscosity (Y2)

Viscosity = +153.11+9.33* X1-3.67* X2

3) Polynomal equation of X1 and X2 on %CDR (Y3).

%CDR= +18.68-7.79* X1+4.02* X2

➢ Criteria considered of % Transsmittance (Y1), viscosity(Y2), and %CDR(Y3)is between 96.2-99.9%, 140-150,and 19.51% - 31.21% respectively. Design space shown in figure 6also called as overlay plot which is shaded region with yellow color indicates that region of successful operating ranges. From the design two overlay plots obtained and on the basis of that reading, prepared microemulsion for checkpoint analysis as well as predicted and experimental values were compared and test out the effect on depended variables and determined optimize formula of microemulsion.



(a) **(b)**

Figure 3: Response Surface Plot & 3D Surface Plot: (a) Concentration of oil (b) concentration of Smix on% Transmittance (Y1)

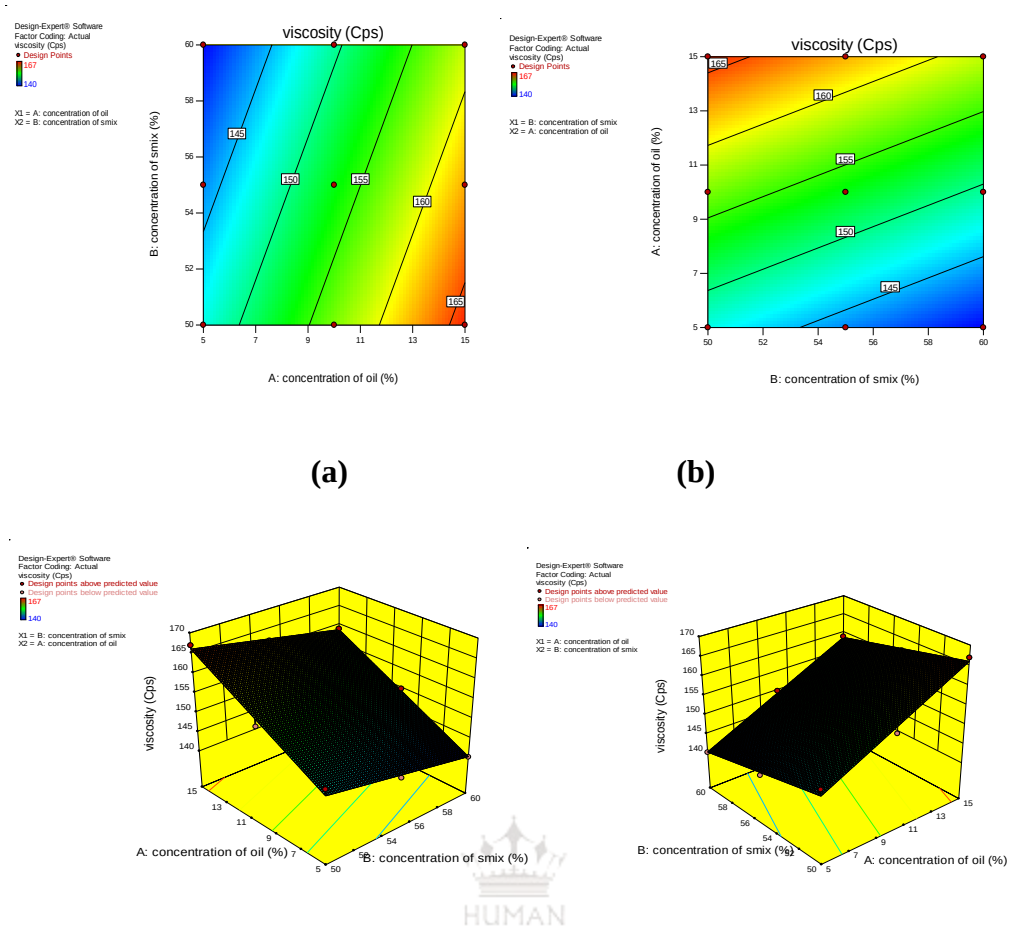
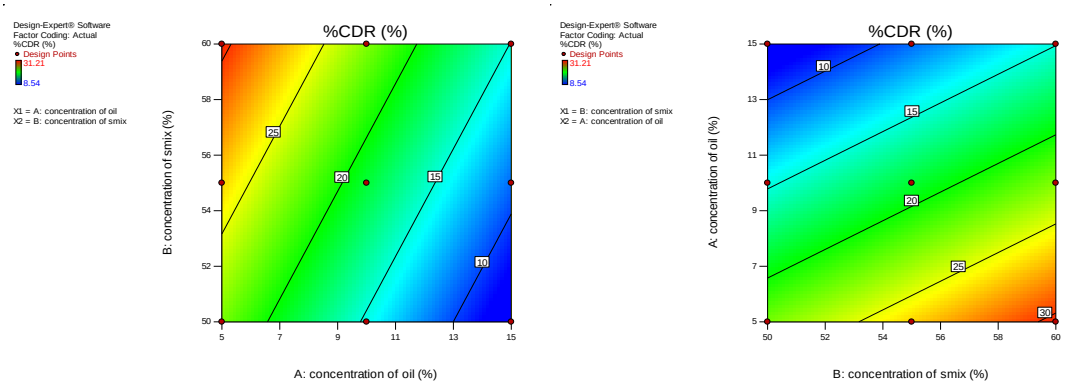
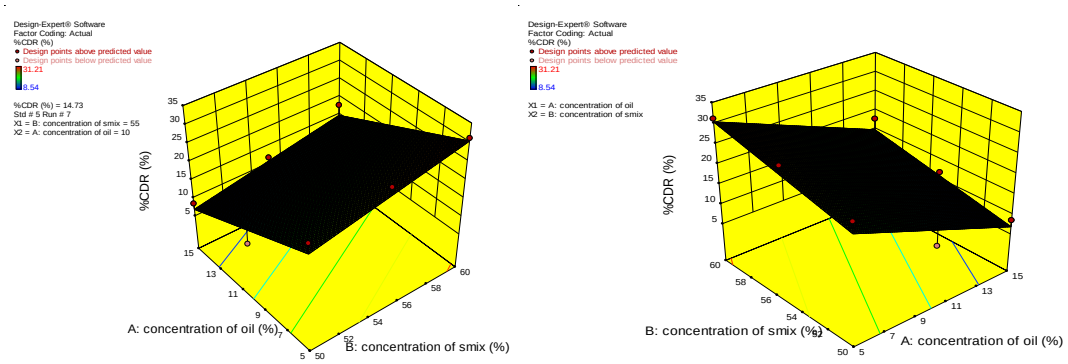


Figure 4: Response Surface Plot &3D Surface Plot: (a) Oil Concentration and (b) Smixconcentration on Viscosity (Y2)





(a) **(b)**

Figure 5: Response Surface Plot & 3D Surface Plot: (a) Oil Concentration and (b) Smix Concentration On % CDR (Y)

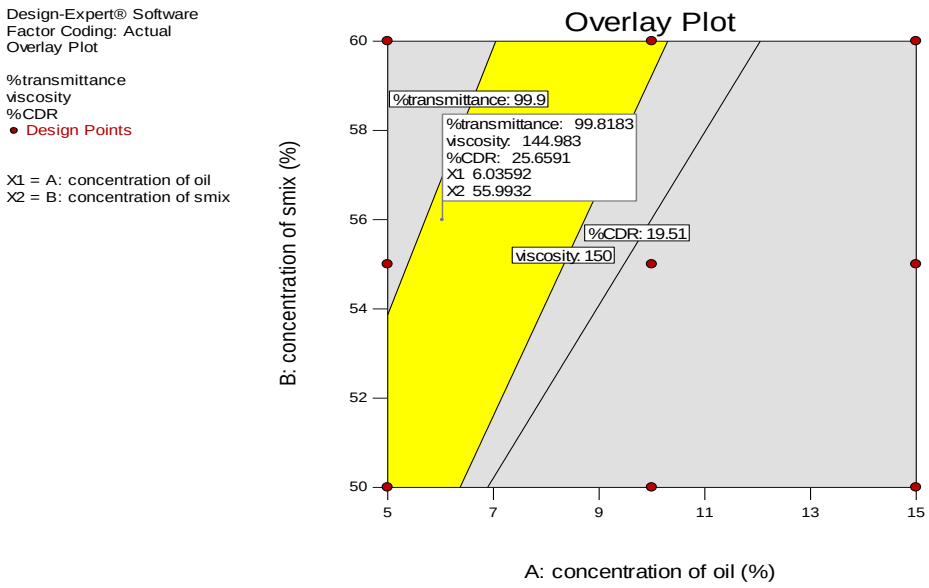


Figure 6: Overlay plot

- **Evaluation of Microemulsion system**

1) General dividing line between stable and unstable microemulsion is generally taken at either +30 or -30mV. At this point Particles with zeta potential more positive than +30mV or more negative than -30 mV are normally considered stable. Optimized batch zeta potential was found to be -28.5 which shows good stability of microemulsion.

➢ Optimized formulation has small droplet size (119.2 d. nm).Smaller size droplets are capable of better drug release.

➢ Polydispersity index (PDI) indicate uniformity of droplet size within the formulation and its stability and it ranges from 0.0 to 1.0. The closer to zero the PDI value, the more homogenous are the particles. Optimized microemulsion formulations showed their PDI is 0.287that indicates acceptable homogeneity of formulation.

2) pH, density, conductivity of the prepared microemulsion formulation results shown in table 11.

3) The prepared microemulsion formulation was diluted in 1:10, 1:50and 1:100 ratio with distil water the system don't show any sign of separation and found to be clear. So it's confirmed that prepared microemulsion is o/w type.

4) Developed formulation have Refractive index and surface tension were found to be 1.45±0.23 (Mean±S.D, n=3) &57.03± 0.03(dynes/cm). So the microemulsion is called thermodynamically stable and forms spontaneously.

5) TEM study was done of optimized batch. It showed the spherical shape and homogeneous droplet size of microemulsion in the range of less than 200nm which shown in figure 7.

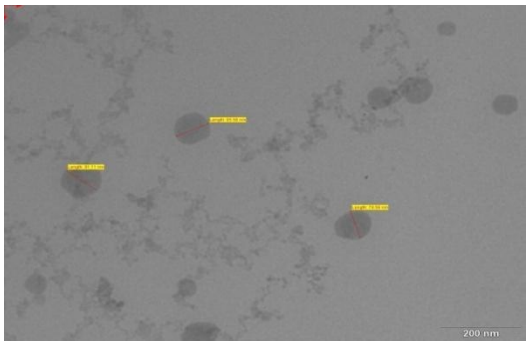


Figure 7: TEM study of microemulsion

6) Drug content of the optimized formulation was found to be 97.34±0.02%.According to result indicates that the drug is distributed almost uniformly throughout the formulation.

7) Optimized ME formulation % cumulative dug release after 9 hr was 90.84 ± 0.56% (Mean± S.D, n=3).

8) Thermodynamic stability studies of prepared formulations including heating, cooling cycles, freeze and thaw cycles showed that all the formulations have good physical stability without any phase separation, creaming and cracking

Table 11: pH, Density and Conductivity of optimized microemulsion

Parameters	Result(Mean±S.D)(n=3)
pH	6.46±0.06
Density (gm/ml)	1.12±0.04gm/ml
Conductivity(µs)	52.3 ±0.02µs

Table 12: Formula for Itraconazole Microemulgel

Sr.no	Ingredients	Quantity
1	Oil	0.6ml
2	Smix	5.59ml
3	Water	4.9 ml
4	Drug (Itraconazole)	200mg
5	1% carbopol 934 gel	10 gm

- **Characterization of microemulgel:**

1. Physical evaluation

➢ Prepared itraconazole microemulgel was inspected visually for their color, odor, texture, homogeneity, consistency. The color of formulation was pale yellow and odorless. Developed formulation was visually inspected and it showed good consistency and excellent homogeneity with absence of any lumps also found no grittiness. Themicroemulgel pH measured by pH meter.The pH value varied from 6.5±0.09 (Mean ± S.D,(n = 3) those were within the acceptable range for skin preparations. The external skin surface has pH between 5-7.

➢ Electrical conductometry is a useful tool for evaluation of conductive behavior of microemulgel. Water phase contains more concentration of ions which proportional to its conductivity means that higher ion concentration, higher the conductivity. Because of this, if we measure the conductivity of obtaining formulation, we have some estimate of the amount of present ion concentration. Here, The measurement of conductivity of the prepared gel was done optimized formulation have 49.5 ±0.06 µs (Mean ± S.D.,n = 3) conductivity. Rate of drug release and stability are closely linked to the viscosity of the formulation. Generally, the viscosity of a formulation increases with the increases in the concentration of the gelling agent.

➢ The measurement of viscosity of the prepared gel was done with a Brookfield viscometer at different rpm by using spindle no 61.Results were shown in table 13.

Table 13: Physical Evaluation Of microemulgel

Properties	**Observation**
Colour	Pale yellow
Odour	Odourless
Texture	Smooth
Feel on application	Cooling sensation
Consistency	Good
Homogeneity	Very good
Grittiness	Not found
pH	6.5±0.09
Conductivity	49.5±0.06
Viscosity	9470±0.034 cps

2. Spreadability study Extrudability Study, Syneresis measurement test and drug content of Topical microemulgel.

➢ Spreadability is important parameter for uniform and ease of application of topical preparation from patient compliance point of view. The spreadability indicates that itraconazole topical gel is easily spreadable by small amount of shear.

➢ During the test, Optimized batch 15.4 ±0.5 gm/cm weight required to extude 0.5 or 1 cm ribbon of microemulgel in 10 sec from aluminium collapsible tube, From the result consider that more quantity of microemulsion based gel extrude at little applied pressure on tube which shows better patient compliance and indicated that microeulgel have a good extrudability.

➢ The stability of gel can be conducted on some parameters like synersis and texture. When subject centrifugation, that did not show separation of more quantity of liquid from gel, so on the basis of result considered that no syneresis was detected on emulgel. This implies that developed formulation thermodynamically stable, having good gel strength and has strong water holding capacity.

➢ According to the result also judge that formulation have better drug loading capability and necessary dose of drug was presented for the pharmacological action. All end results are shown in table 14.

Table 14: Spreadability study Extrudability Study, Syneresis measurement test and drug content of Topical microemulgel.

Parameters	**Observation (Mean ± S.D.) (n=3)**
Spreadability	20±0.75 gm.cm/sec
Extrudability study	15.4 ±0.5 gm/cm
Syneresis measurement	no syneresis found
Drug content	98.18% ± 0.8

3. **Release Kinetic study of Itraconazole microemulsion and microemulgel.**

The release data of formulation for different kinetic models were shown in table 15.According to result indicated that in-*vitro* release of itraconazole microemulsion and microemulgel formulation followed Higuchi model have r^2 value 0.991 and 0.996.

Table 15 Release Kinetic study of Itraconazole microemulsion and microemulgel.

Model	Parameter	Optimized ITZ microemulsion	Optimized ITZ Microemulgel (ITZCG1)
Zero Order	R2	0.98	0.991
	Slope	11.98	9.307
	Intercept	-5.293	-1.341
First Order	R2	0.94	0.968
	Slope	-0.083	-0.058
	Intercept	2.06	2.024
Higuchi Model	R2	0.991	0.996
	Slope	3.53	3.53
	Intercept	9.021	9.021
Hixon Crowell	R2	0.957	0.981
	Slope	3.53	0.182
	Intercept	-0.159	-0.061
Kors-meyerPeppas	R2	0.92	0.943
	Slope	79.12	61.42
	Intercept	-1.62	1.505

4. ***Ex-vivo* permeability study**

The *ex-vivo* study was done with optimized microemulgel and marketed formulation which results is shown in table16. It shows *ex–vivo* release of optimized formulation was found to be

less than in-*vitro* release study. It may be due to the fat content and thickness of rat skin.in figure 8 revealed that marketed formulation gives highest release as compare to optimized batch.

Table 16: *Ex-vivo* permeability study

Time (hr)	%CDR of Optimized ITZ Microemulgel formulation (Mean ± S.D.) (n=3)	%CDR of Marketed Itraconazole Formulation (Mean ± S.D.) (n=3)
0	0	0
1	7.45%	7.91%
2	17.67%	18.03%
3	21.27%	25.32%
4	27.67%	37.30%
5	43.37%	49.28%
6	56.70%	58.76%
7	61.97%	72.26%
8	67.93%	83.35%
9	77.90%	88.14%

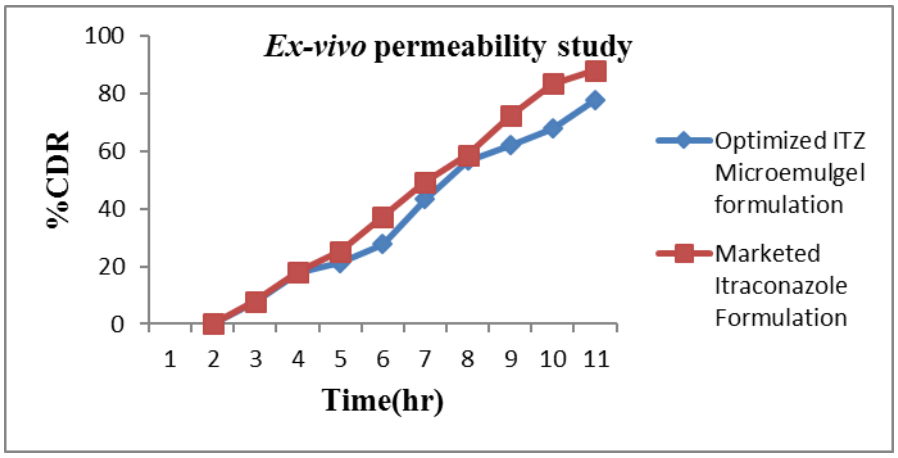


Figure 8: *Ex-vivo* permeability study

5. **J-flux & Permeability Coefficient**

After 9 hr J flux and permeability coefficient of ITZ microemulgel was found to be 0.2301 ($mg/cm^2/hr$) and 0.001151(Kp). Result shown in table 17.

Table 17: J-flux & Permeability Co-efficient

Time(hr)	Flux *J* ($mg/cm^2/hrs$)	Permeability co-efficient (Kp)
0	0.0000	0
1	0.1823	0.000912
2	0.0685	0.000343
3	0.0209	0.000105
4	0.1417	0.000709
5	1.4140	0.00707
6	0.4331	0.002166
7	0.2866	0.001433
8	0.5414	0.002707
9	0.2301	0.001151

6. **Skin irritation study of itraconazole microemulgel.**

From the result which shown in figure 9 indicate that there is no serious apparent sign of edema, erythema(redness) on rat skin in either of the optimized itraconazole microemulgel. It represents that the safety of the formulation.So the optimized formulation appears to be safe for topical use.

TIME	DAY 1 skin irritation study on rat at 24 hours	DAY 2 skin irritation study on rat at 48 hours
Marketed itraconazole Formulation		
Optimized Itraconazole microemulgel Formulation		

Figure 9: Skin irritation photographs of marketed ITZ formulation and optimized ITZ Microemulgel formulation

7. *In-Vitro* Antifungal activity study

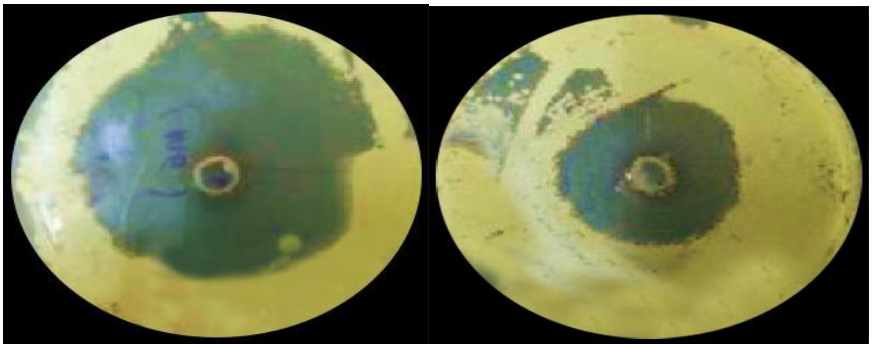


Figure 10: Photograph showing Zone of inhibition of optimized batch ITZ microemulgel and marketed formulation of ITZ

In figure 10 show antifungal activity study, in which indicated more zone of inhibition found in ITZ microemulgel 5.2 cm as compared with marketed product. This proved that prepared microemulgel of itraconazole is better than marketed formulation.

8. Stability Analysis.

Table 18: Stability Analysis of optimized batch at Room Temperature for 1 Months

PARAMETER	Optimized Itraconazole (ITZ) microemulgel			
	Room Temperature			
	0 Day	10 Day	20 Day	30 Day
Clarity	Opaque	Opaque	Opaque	Opaque
Odour	Odourless	Odourless	Odourless	Odourless
pH	6.95	6.83	6.78	6.70
Spreadability	20gm.cm/sec	20.10gm.cm/sec	20.18gm.cm/sec	20.22gm.cm/sec
Viscosity(cps)	9470	9480	9488	9491
% Drug content	98.18%	97.04%	95.89%	93.87%

➢ At fixed time interval drug content determination of these formulations shows no significant changes when compared to the initial formulations. All parameters result shown in table 18.

CONCLUSION

The present work shows that microemulsion is promising transdermal drug delivery vehicle and enhance the solubility of itraconazole drug was done by using this approach. Itraconazole containing microemulsion was formulated for topical application. Microemulgel appears better & effective drug delivery system as compared to other conventional topical drug delivery system. Many ingredients used in the formulations are highly stable and safe for the topical delivery and that pharmaceutical ingredient into emulgels is used in treatment of various diseases like fungal infection, as topical anti-inflammatory infection, psoriasis etc.

MicroEmulgel has advantages in term of better spreadability, adhesion, viscosity and extrusion, this type of drug delivery system will become a popular drug delivery system. Regarding various advantages of microemulgel offer a wide utility in derma care.

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