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Research Article

Development and *In-Vitro* Evaluation of Itraconazole Loaded Nanoemulsion

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Abstract

Nanoemulsions are one of the major popular formulation systems in the pharmaceutical and cosmeceutical fields. Nanoemulsions are generally composed of a dispersed oil phase within a continuous aqueous phase. Itraconazole is an antifungal medication used to treat a number of fungal infections. It is in the triazole family of medications. Itraconazole, antifungal agent has poor bioavailability due to low aqueos solubility. In this research preformulation study, Fourier transform infrared (FTIR) analysis studies was conducted for studying the compatibility. In preparation of Itraconazole loaded nanoemulsions from the ternary phase diagram ratio of surfactant to co-surfactant (Smix) was optimized with broad area. Optimized surfactant and co surfactant are accurately weighed and then vortexed for 5-10 min for Smix preparation. Particle size and value of PDI was found to be 159.21nm and zeta potential demonstrated the stability of prepared nanoparticles was found to be -15.9mv .Transmission electron microscope indicated a homogeneous distribution of small, spherical optimized Itraconazole loaded nanoemulsion formulation. These studies were aimed to improve the oral bioavailability of Itraconazole through nanoemulsions.

Keywords: Nanoemulsion, Itraconazole, Surfactant, cosurfactant, Nanoparticles

1 Introduction

1.1 Nanoemulsions

Nanoemulsions have been at the other end of the drug delivery sophistication spectrum. While emulsions have a history of safe use in nutrition cosmetics and drug formulation [1], they have remained largely as unsophisticated physical dispersions of oil in water, anchored by their latin definition, emulge, meaning literally 'to milk'. The view of emulsions as being relatively uninteresting soft matter has meant that methods for encoding advanced functionality are only now emerging. Nanoemulsions are generally composed of a dispersed oil phase within a continuous aqueous phase and have a radius of less than 1000 nm, though the upper boundary is variable with some validity to assertions that an upper limit of 100–200 nm better defines a nanoemulsion to the exclusion of microemulsions.[2,3]

Itraconazole

Itraconazole, sometimes abbreviated ITZ, is an antifungal medication used to treat a number of fungal infections . It is in the triazole family of medications. It stops fungal growth by affecting the cell membrane or affecting their metabolism. Itraconazole has a broader spectrum of activity than fluconazole (but not as broad as voriconazole or posaconazole). In particular, it is active against Aspergillus, which fluconazole is not. It is also licensed for use in blastomycosis, sporotrichosis, histoplasmosis, and onychomycosis. Itraconazole is over 99% protein-bound and has virtually no penetration into cerebrospinal fluid.[4]

2. Experimental Work

2.1 Preformulation study

2.1.1 Organoleptic properties (API)

The identification of itraconazole was done by checking the physical appearance i.e. colour, odour, taste and state.

2.1.2 Melting point

The melting point of the drug was determined by using capillary method with melting point apparatus.

2.1.3 Partition coefficient

$$\text{Partition coefficient} = \frac{Concentration\ of\ drug\ in\ organic\ phase}{Concentration\ of\ drug\ in\ aqueous\ phase}$$

API in both phases was determined by UV spectroscopy and partition coefficient was calculated using the equation.

2.1.4 Determination of absorption maxima

Methanol was selected as ideal solvent for spectrophotometric analysis of Itraconazole. The UV spectrum is generally recorded as a plot of absorbance versus wavelength.

2.1.5 Drug and excipients compatibility studies

These studies were conducted to determine the compatibility of the excipients with the drug for the preparation of formulation. Fourier transform infrared (FTIR) analysis studies was conducted for studying the compatibility.

2.1.6 Solubility Studies

The solubility of Itraconazole in various oils, surfactants and co-surfactants was determined by dissolving an excess amount of telmisartan in 500 mg of each of selected oils, surfactants and co-surfactants in stoppered vials.

2.1.7 Preliminary screening of surfactants for emulsification efficiency

Screening of surfactant was done on the basis of percent transmittance. Emulsification ability of surfactants was assessed by adding .

2.1.9 Preliminary screening of co-surfactants for emulsification efficiency

For this study, 100 mg of oil and 200mg of surfactant were added to 300 mg of cosurfactant phase and then this mixture was heated at 50ºC for homogenization of the components

2.2 Preparation of ternary phase diagram

Pseudo-ternary phase diagram using oil, surfactant and consurfactant was prepared by aqueous titration method at room temperature, using selected oil, surfactant, co-surfactant and DM water as an aqueous phase.

Table 1: Composition of different ratio of oil, Smix (1:1) of Pseudo-ternary phase diagram

Formulation code	Ratio	Amount Oil (mg)	Amount Smix (1:1) (mg)	
			Surfactant	Co surfactant
A1	1:09	190	855	855
A2	2:08	380	760	760
A3	3:07	570	665	665
A4	4:06	760	570	570
A5	5:05	950	475	475
A6	6:04	1140	380	380
A7	7:03	1330	285	285
A8	8:02	1520	190	190
A9	9:01	1710	95	95

Table 2: Composition of different ratio of oil, Smix(1:2) of Pseudo-ternary phase diagram

Formulation code	Ratio	Amount Oil (mg)	Amount Smix (1:2) (mg)	
			Surfactant	Co surfactant
A10	1:09	190	570	1140
A11	2:08	380	506.6666667	1013.333333
A12	3:07	570	443.3333333	886.6666667
A13	4:06	760	380	760
A14	5:05	950	316.6666667	633.3333333
A15	6:04	1140	253.3333333	506.6666667
A16	7:03	1330	190	380
A17	8:02	1520	126.6666667	253.3333333
A18	9:01	1710	63.33333333	126.6666667

Table 3: Composition of different ratio of oil, Smix(2:1) of Pseudo-ternary phase diagram

Formulation code	Ratio	Amount Oil (mg)	Amount Smix (2:1) (mg)	
			Surfactant	Co surfactant
A19	1:09	190	1140	570
A20	2:08	380	1013.333333	506.6666667
A21	3:07	570	886.6666667	443.3333333
A22	4:06	760	760	380
A23	5:05	950	633.3333333	316.6666667
A24	6:04	1140	506.6666667	253.3333333
A25	7:03	1330	380	190
A26	8:02	1520	253.3333333	126.6666667
A27	9:01	1710	126.6666667	63.33333333

2.3 Preparation of Itraconazole loaded nanoemulsions

From the ternary phase diagram ratio of surfactant to co-surfactant (Smix) was optimized with broad area. Optimized surfactant and co surfactant are accurately weighed and then vortexed for 5-10 min for Smix preparation. After that, Smix was placed in oven at 50ºC for 1min.. Then oil added to Smix and vortexed for 5-10 min and placed in oven at 50ºC for 1min, with the purpose of an isotropic mixture was formed. Drug was loaded to these isotropic formulations at the end and vortexed by vortex shaker until clear solution was obtained. The isotropic mixture was diluted with water in order to form nanoemulsion.

2.4 Optimization of Itraconazole loaded nanoemulsions using central composite design

A design with a central composite of two factors was applied to optimize the effect of amount of oil and amount of Smix over the drug solubilization involving the amount of oil (X1), amount of Smix (X2). It has been determined that each one of the effects of these two parameters in the response variable, namely percentage drug content (Y1) of nanoemulsion containing itraconazole. Thirteen experimental runs according to the central composite design (CCD) was utilized to determine the optimized levels of significant factors, and the interactions of these variables in a process developed by the Design Expert version 6.0.6 software (Stat-Ease Inc., Minneapolis, USA). Two independent variables were carried out at two different levels for every individual variable. The central composite design les us study the impact of variables and interaction between variables in the results independently.

2.5 In vitro characterization of Nanoemulsion

2.5.1 Percentage Drug content

Accurately weighed quantities of nanoemulsion were mixed with 100 ml of methanol. The filtrate was analysed spectrophotometrically at 262 nm for drug content against methanol. Corresponding drug concentrations in the samples were calculated from the calibration plot generated by regression of the data. Drug content was calculated as detected amount of Itraconazole with respect to theoretical amount of drug used for the preparation of nanoemulsion. Each determination was carried out in triplicate. The amount of the drug content in the nanoemulsions was calculated using the formula:

$$\% \text{ Drug content} = \frac{Amount\ of\ drug\ actually\ present\ in\ supernatant}{Theoretical\ drug\ load\ expected} \times 100$$

2.5.2 Particle size analysis and zeta Potential

The particle size, polydispersity index and zeta Potential of nanoemulsion was measured by photon correlation spectroscopy using a Malvern Zetasizer. Samples were diluted appropriately with the aqueous phase of the formulation to get optimum kilo counts per second (Kcps) of 50 - 202.8 for measurements, and the pH of diluted samples ranged from 6.9 to 7.2. The measurements were carried out at 25 ºC in 75% - 100% intensity. The samples were analyzed.

3.1Preformulation studies

3.1.1 Organoleptic Parameters: visual observation demonstrated that Itraconazole was white, crystalline powder with odorless powder.

3.1.2 Melting Point

Melting point of Itraconazole in bulk form was found to be 167°C±0.78-167°C±0.38, lies close to the reference value of 166.4°C.

3.1.3 Standard calibration curve of Itraconazole in methanol

On scanning of certain concentration of 5µg/ml solution of Itraconazole in methanol in 200-400nm scanning range using UV spectrophotometer the absorption maxima of Itraconazole was found to be 262nm similar to value of mentioned in literature.[6,7]

Standard calibration curve

A range of concentration 1-10µg/ml was selected for preparation of standard calibration curve because this concentration range follows the lambert beer law. A line graph was prepared between concentration and absorbance and linear equation was generated. The value of regression equation was found to be Y = 0.0903x+ 0.004 and R^2 value 0.999, showed good linearity.[8]

Table 4: Absorbance of different concentration solution of Itraconazole in methanol

Concentration (µg/ml)	Absorbance at 262nm
0	0±0
1	0.096±0.0020
2	0.183±0.0026
3	0.279±0.003
4	0.367±0.0030
5	0.450±0.0017
6	0.549±0.0015
7	0.632±0.0028
8	0.720±0.040
9	0.817±0.0025
10	0.913±0.0032

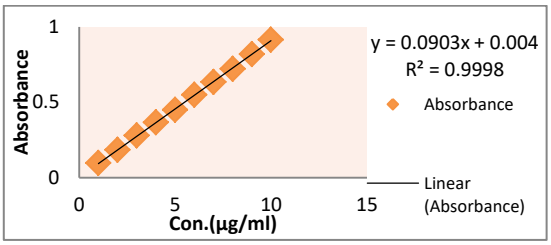

Figure 1: Linear response standard calibration curve of different concentration of itraconazole in methanol vs absorbance in methanol

3.1.4 Partition coefficient of drug

A mixture of hydrophilic and lipophilic solvent was use for determination of partition coefficient. For current activity the mixture n-octanol: water mixture was used for determination of partition coefficient of itraconazole. The value of itraconazole was found to be 5.34±0.727 close to the value mentioned in literature 5.66, indicate the lipophilic nature of the itraconazole.[5]

3.1.5 FTIR of Itraconazole and Itraconazole loaded nanoemulsions

The FTIR spectrum of pure drug itraconazole showed the characteristic peaks of itraconazole which occurred at 3157.14, 2926.23, 1632.75, 1510.59, 1409.26 cm^{-1}. The absorption bands between 2800 and 3200 $cm^{-1}$was attributed to the alkane, aromatic CH and amine groups. The wave numbers observed at 1632.75 and 1409.26 may be assigned to the C=N and C-N bonds, respectively. This is in agreement with the previously recorded spectra of the pure drug. Furthermore FTIR spectrum of Itraconazole loaded nanoemulsions containing demonstrated very less characteristic peak of Itraconazole indicated the solubilization of Itraconazole in nanoemulsions.

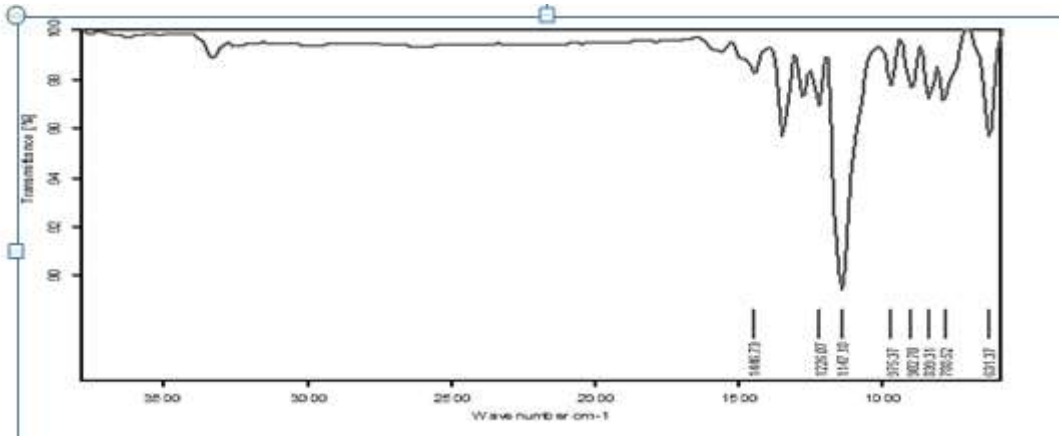

Figure 2: Overlay FTIR spectrum of Optimized formulation

3.1.6 Solubility in itraconazole in oils

Table 5: Solubility of itraconazole in different oils

Names of Oils	Concentration (mg/ml) ±STD
Oleic acid	0.599±0.050
Labrafil 2155	14.666±1.452
Ethyl oleate	19.888±0.293
Labrafil 1944	0.306±0.006
Sunflower oil	0.178±0.021
Coconut oil	2.977±0.048

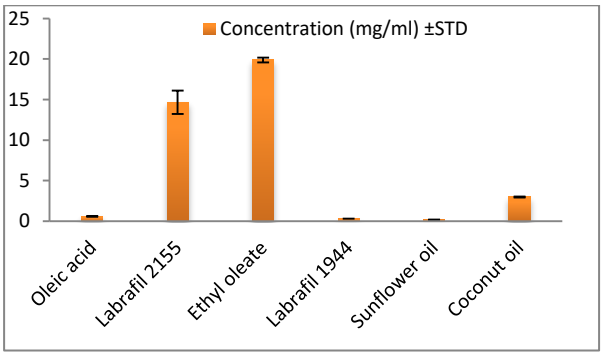

Figure 3: Solubility of Itraconazole in different oils

Among all oil ethyl oleate have higher solubility of itracaonzole19.888±0.293mg/ml followed by the Labrafil 2155 14.666±1.452mg/ml as compare to other oils (Figure 3).

3.1.7 Solubility of itraconazole in in different HLB value surfactant

Table 6: Solubility of itraconazole in in different HLB value surfactant

Names of Surfactant	Concentration (mg/ml) ±STD
Kolliphor EL	9.53±0.038
Tween 60	4.95±0.38
Kolliphor RH 40	8.43±0.41
Tween 80	9.87±0.04
Tween 20	4.82±0.030
Span 80	7.11±0.031

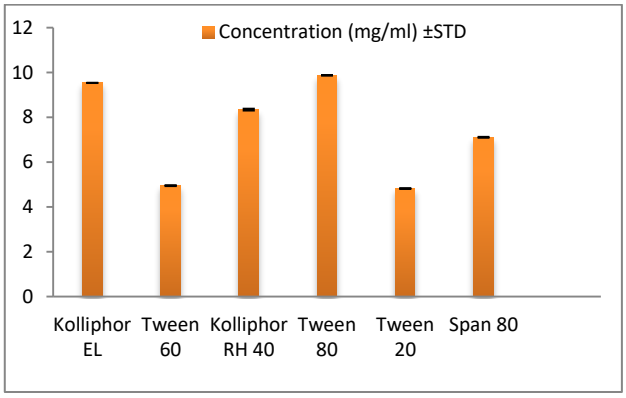

Figure 4: Solubility of itraconazole in different HLB value surfactant

Among all surfactant Kolliphor EL have higher solubility of itraconazole 9.53±0.038mg/ml followed by the tween 80 9.87±0.04mg/ml as compare to other surfactant. In low HLB value surfactant span 80 have maximum solubility of itracaoznole was 7.11±0.031,g/ml.

3.1.8 Solubility of itraconazole in different Cosurfactant

Table 7: Solubility of itraconazole in different Cosurfactant

Names of Cosurfactant	Concentration (mg/ml) ±STD
PEG 200	3.87±0.089
PEG 400	4.98±0.340
Ethanol	27.18±2.70
Glycerol	2.42±0.13
Propylene Glycol	18.77±0.11

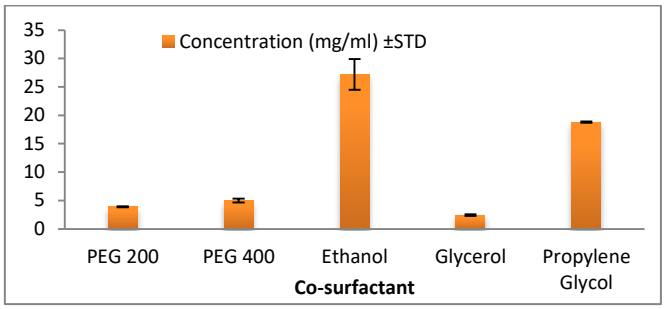

Figure 5: Solubility of itraconazole in different Cosurfactant

Figure 5 demonstrated that among all cosurfactant ethanol have maximum solubility of itraconazole 27.18±2.70mg/ml followed by Propylene Glycol 18.77±0.11mg/ml as compare to other Cosurfactant.

On the basis of solubility Ehtyloleate, Labrafil 2155 was selected as oil, Tween 80, Kolliphor EL and span 80 was selected as surfactant and Ethanol and Propylene glycol was selected as Cosurfactant for further screening activity.

3.1.9 Screening of oil and surfactant through emulsification study

Screening of oil and surfactant was performed to determine the stabile combination of oil and surfactant through emulsification study.

Table 8: Screening of Surfactants

Formulation Code	Oils (in mg)	Surfactants (in mg)	Appearance	% Transparency	Appearance after 24 hr
OS1	Ethyl oleate	Kolliphor EL	Clear Bluish transparent	89.33±5.85	Clear Bluish transparent
OS2	Ethyl oleate	Span 80	Turbid	74±3.60	Turbid
OS3	Ethyl oleate	Tween 80	Clear Bluish transparent	90.33±1.52	Clear Bluish transparent
OS4	Labrafil 2155	Kolliphor EL	Clear Bluish transparent	87.34±2.08	Clear Bluish transparent
OS5	Labrafil 2155	Span 80	Turbid	52.67±3.78	Turbid
OS6	Labrafil 2155	Tween 80	Clear Bluish transparent	83.34±2.52	Clear Bluish transparent

Among six combinations of oil and surfactant, combination of ethyl oleate and Labrafil 2155 shared good emulsification with surfactant Kolliphor EL and tween 80, thus both surfactant and oil were selected for further screening of cosurfactant. Among all six combinations, combination OS2, OS3, O5, OS6 were selected. Although the HLB values of the used surfactants were close in the range of 13–16, the difference observed in their emulsifying ability could be attributed to the difference in their structure and chain length.

3.1.10 screening of oil, surfactant and co-surfactant through emulsification study

Screening of surfactant with the combination of oil and surfactant was performed to determine the most suitable oil, surfactant and Cosurfactant for the ternary phase diagram.

Table 9: Screening of Co-Surfactants

Formulation Code	Oils	Surfactants	Co surfactant	Appearance	% Transparency	Appearance after 24 hr
OSC1	Ethyl oleate	Kolliphor EL	Propylene glycol	Clear Bluish transparent	90.33±1.52	Clear Bluish transparent
OSC2	Ethyl oleate	Kolliphor EL	Ethanol	Clear Bluish transparent	86.67±2.51	Clear Bluish transparent
OSC3	Ethyl oleate	Tween 80	Propylene glycol	Clear transparent	97±1.73	Clear transparent
OSC4	Ethyl oleate	Tween 80	Ethanol	Clear Bluish transparent	98.67±1.53	Clear Bluish transparent
OSC5	Labrafil 2155	Kolliphor EL	Propylene glycol	Clear Bluish transparent	52.67±3.78	Clear Bluish transparent
OSC6	Labrafil 2155	KolliphorEL	Ethanol	Clear Bluish transparent	96.34±3.51	Clear Bluish transparent
OSC7	Labrafil 2155	Tween 80	Propylene glycol	Clear Bluish transparent	89.67±3.21	Clear Bluish transparent
OSC8	Labrafil 2155	Tween 80	Ethanol	Clear Bluish transparent	83.67±2.51	Clear Bluish transparent

Among all eight combination of oil, surfactant and Cosurfactant combination OSC3 was formed clear transparent emulsion and it remain transparent after 24 hr, thus this combination of oil, surfactant and Cosurfactant was selected for further preparation of pseudo ternary phase diagram.

3.2 Preparation of Pseudo ternary phase diagram

The detailed composition of the nanoemulsion formulations used to construct the phase diagram are depicted in Table 14. Pseudoternary phase diagram is used to identify the nanoemulsion region is depicted in figure 18-20.

Table 10: Visual Observation of nanoemulsion formulation prepared from Smix ratio (1:1)

S.No.	Oil: S_{mix} Ratio	Formulation code	Appearance
1.	1:9	A1	Transparent
2.	2:8	A2	Transparent
3.	3:7	A3	Bluish Transparent
4.	4:6	A4	Turbid
5.	5:5	A5	Turbid
6.	6:4	A6	Turbid
7.	7:3	A7	Turbid
8.	8:2	A8	Turbid
9.	9:1	A9	Turbid

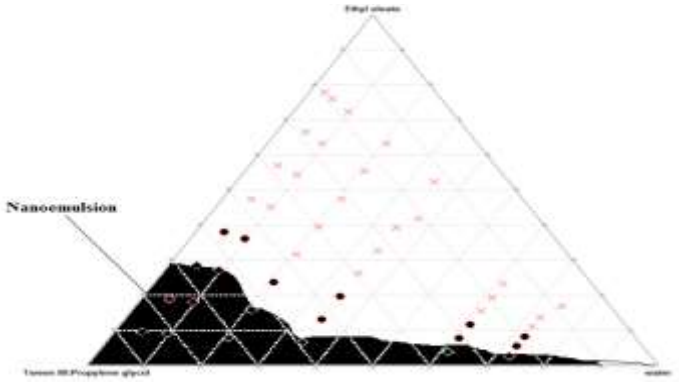

Figure 5: Ternary phase diagram of formulation preparation from Smix ratio (1:1)

Table 11: Visual Observation of nanoemulsion formulation prepared from Smix ratio (1:2)

S.No.	Oil: S_{mix}	Formulation Code	Appearance
1.	1:9	A10	Transparent
2.	2:8	A11	Transparent
3.	3:7	A12	Bluish Transparent
4.	4:6	A13	Turbid
5.	5:5	A14	Turbid
6.	6:4	A15	Turbid
7.	7:3	A16	Turbid
8.	8:2	A17	Turbid
9.	9:1	A18	Turbid

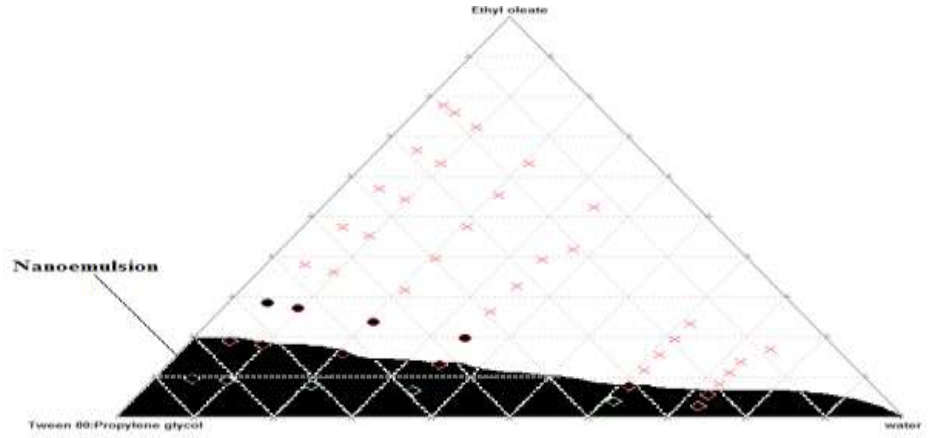

Figure 6: Ternary phase diagram of formulation preparation from Smix ratio (1:2)

Table 12: Visual Observation of nanoemulsion formulation prepared from Smix ratio (2:1)

S.No.	Oil: S_{mix}	Formulation code	Appearance
1.	1:9	A19	Transparent
2.	2:8	A20	Transparent
3.	3:7	A21	Transparent
4.	4:6	A22	Turbid
5.	5:5	A23	Turbid
6.	6:4	A24	Turbid
7.	7:3	A25	Turbid
8.	8:2	A26	Turbid
9.	9:1	A27	Turbid

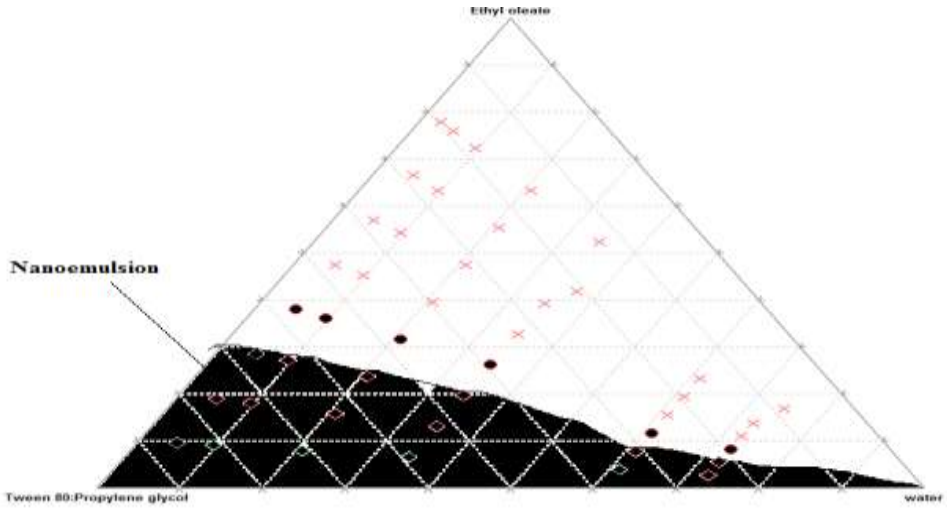

Figure 7: Ternary phase diagram of formulation preparation from Smix ratio (2:1)

Figure no 5-7 displayed that nanoemulsion region was found to be maximum for Smix ratio 2:1 thus this composition was selected for determination of minimum and maximum concentration of oil and Smix for further optimization process.

Table 13: Minimum and maximum value of component for optimization process.

S.No.	Component	Minimum amount(%w/w)	Maximum amount (%w/w)
1.	Oil	2	20
2.	Smix	40	90

3.3 Optimization of Itraconazole loaded nanoemulsion formulation

During the optimization, RSM played a helpful mathematical and statistical role in the selection of formulation and preparation process, understanding the relationship between independent variables and response variables. CCD consisting of 2 factors and 2 levels was seriously employed to develop a second order polynomial regression model for predicting percentage drug content of Itraconazole nanoformulation.

The effect of factors over the response was shown in table

Table 14: Summary of central composite design

Factor	Name	Units	Low Actual	High Actual
X1	Concentration of oil	%w/w	2	20
X2	Concentration of Smix	%w/w	40	90
Response (Y) : Percentage drug content Model : Quadratic				

Table 15: Composition of different formulation with response as per CCD design

Formulation code	X1:Concentration of oil (%w/w)	X2 :Concentration of Smix (%w/w)	Percentage drug content (%)
IN1	11	65	84.37
IN2	23.73	65	86.66
IN3	20	90	88.66
IN4	11	65	95.14
IN5	11	65	96.29
IN6	11	65	94.14
IN7	2	40	50.66
IN8	11	29.64	74.77
IN9	11	65	94.6
IN10	2	90	67.33
IN11	20	40	90.18
IN12	-1.73	65	38.7
IN13	11	100.36	98.14

The effect on Percentage drug content (Y) was observed to be significant by ANOVA. Analysis of variance (ANOVA) obviously implied that the quadratic polynomial model for response Y1 was strongly related to the Model F values of 40.61 which indicated that there was only a 0.01% probability could occur due to noises. Furthermore, R^2 of responses of the quadratic polynomial response models were relatively high and the predicted R^2 values were in reasonable agreement with the adjusted R^2, which was 88% of the response variations of the independent variables could be described by the polynomial model. Lack of fit F-values for response Y1 was 0.66, which implied a non-significant relative to the pure-error. The model can be used to navigate the design space. Final equations in term of coded factors for responses y1 were generated the following polynomial formulas:

$$Y= 92.908+16.08X1+6.025X2-4.54X1X2-15.204X1^2-3.31X2^2$$

The Positive sign for coefficient of X1 & X2 indicates that as the percentage drug content increase with increase the concentration of both factor X1 and X2.

ANNOVA profile of the design shown that The "Pred R-Squared" of 0.886 is in reasonable agreement with the "Adj R-Squared" of 0.9428.

3D plots shows the response surfaces with greater significance for the percentage drug content of itraconazole in the nanoemulsion using the interactions of two variables.Thepercentage drug entrapment increases on increasing concentration of both factor concentration of oil and concentration of Smix.

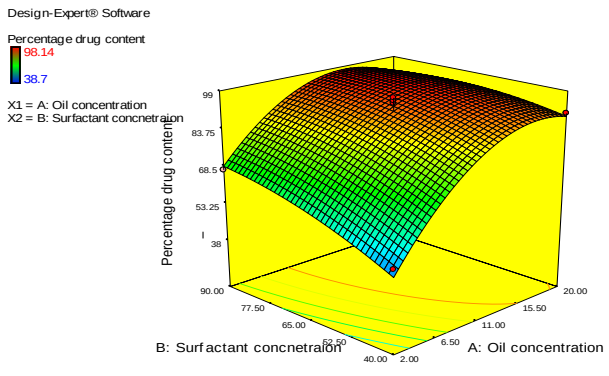

Figure 8: 3D plot graph of Itraconazole Loaded nanoemulsion

3.4 Evaluation of Itraconazole Loaded nanoemulsion

3.4.1 Visual appearance and Transmittance

Visual observation, self emulsification time and Percentage transmittance of all prepared formulation is as follows.

Table 16: Visual observation and Percentage transmittance of all prepared nanoemulsion formulation

Formulation code	Self emulsification time	Percentage transmittance	Appearance
IN1	Within 2-3 sec.	95.33±2.08	Clear, Homogenous, Transparent solution
IN2	Within 2-3 sec.	94.66±1.52	Clear, Homogenous, Transparent solution
IN3	Within 2-3 sec.	98±1	Clear, Homogenous, Transparent solution
IN4	Within 2-3 sec.	98±1	Clear, Homogenous, Transparent solution
IN5	Within 2-3 sec.	98.66±1.5	Clear, Homogenous, Transparent solution
IN6	Within 2-3 sec.	99.33±1.15	Clear, Homogenous, Transparent solution
IN7	Within 2-3 sec.	82.33±1.52	Clear, Homogenous, Transparent solution
IN8	Within 2-3 sec.	89±1	Clear, Homogenous, Bluish Transparent solution
IN9	Within 2-3 sec.	77±2.64	Clear,Homogenous, Transparent solution
IN10	Within 2-3 sec.	67.33±0.29	Turbid solution
IN11	Within 2-3 sec.	96.66±2.08	Clear,Homogenous, Transparent solution
IN12	Within 2-3 sec.	62.66±2.08	Turbid solution
IN13	Within 2-3 sec.	78.33±1.52	Clear,Homogenous, Transparent solution

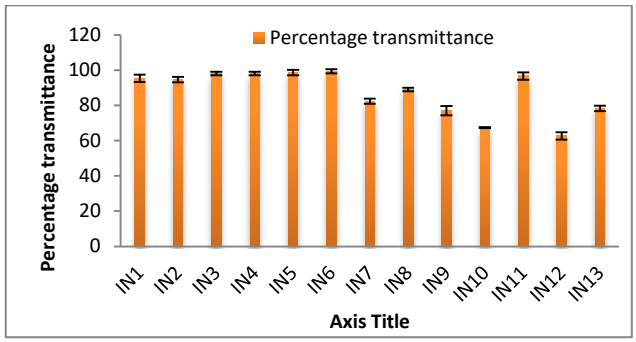

Figure 9: Bar graph of Visual observation and Percentage transmittance of all prepared nanoemulsion formulation

All prepared formulations were clear, homogenous and transparent solution except formulation code IN10 and IN12. Both formulations were turbid. Similarly all above isotropic mixture of oil, surfactant and Cosurfactant mixture immediately form nanoemulsion upon addition of water.

4.4.2 Percentage drug content

Percentage drug content of all prepared formulation was shown in table

Table 17: Percentage drug content of all prepared formulation

Formulation code	Percentage drug content
IN1	84.37±1.00
IN2	86.66±0.50
IN3	88.66±0.29
IN4	95.14±0.90
IN5	96.29±0.23
IN6	94.14±0.33
IN7	50.66±0.58
IN8	74.77±1.23
IN9	94.62±0.32
IN10	67.33±0.29
IN11	90.18±0.16
IN12	38.70±1.26
IN13	63.74±0.78

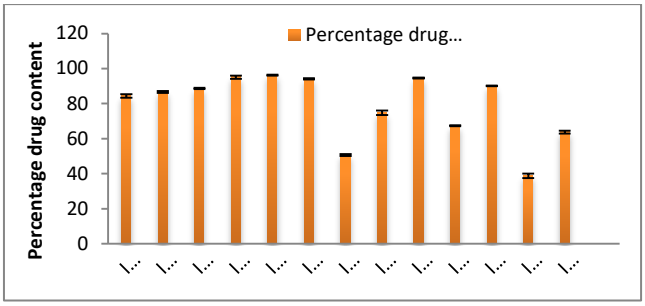

Figure 10: Bar graph of Percentage drug content of all prepared formulation

Percentage drug content of all itraconazole loaded formulation was found to be in a range of 67.33±0.29 to 96.29±0.23.

3.4.3 Optimization of formulation

In validation option of central composite design two formulations was optimized for further evaluation, which composition is as follows.

Table 18: Composition of optimized nanoemulsion formulation

Formulation code	Oil (%w/w)	Smix (%w/w)	Percentage drug content	Desirability
IN14	15.01	81.4	98.2523	1
IN15	15.55	81.93	98.1559	1

3.5 Evaluation of Optimize formulation

3.5.1 Visual appearance and Transmittance

Visual observation, self emulsification time and Percentage transmittance of all prepared formulation is as follows

Table 19: Visual observation and Percentage transmittance of all prepared nanoemulsion formulation

Formulation code	Self emulsification time	Percentage transmittance	Appearance
IN14	Within 2-3 sec.	99.33±1.15	Clear, Homogenous, Transparent solution
IN15	Within 2-3 sec.	96.34±2.08	Clear, Homogenous, Transparent solution

Both formulation IN14 and IN15 were clear, transparent, homogenous solution with percentage transmittance more than 95% and isotropic mixture of oil, surfactant and Cosurfactant form immediate nanoemulsion upon addition of water.

3.5.2 Percentage drug content

Percentage drug content of both prepared formulation was shown in table no 17.

Table 20: Percentage drug content of all optimized prepared formulation

Formulation code	Percentage drug content
IN14	98.25±0.33
IN15	96.66±0.29

Percentage drug content of both itraconazole loaded formulation was found to be in a range of 96.66±0.29 to 98.25±0.33.

3.5.3 Thermodynamic stability using centrifugation study

Thermodynamic stability of both prepared nanoemulsion formulation were determined by using cooling centrifuge. In this activity the both formulation was visually observed after centrifuge at certain rpm to determine any ppt. of drug and phase separation.

Table 21: Visual observation of both optimized prepared formulation after centrifugation

Formulation code	Visual observation
IN14	Clear transparent solution
IN15	Transparent solution but some particles of drug were observed.

Formulation code IN14 displayed no sign of phase separation and ppt. of drug.

On the basis result of above parameter the formulation code IN14 was selected for further evaluation.

3.5.4 Globule size and Zeta Potential

Table 22: Particle size, PDI and Zeta Potential of optimized formulation IN14

S.No.	Formulation code	Particle size (nm)	PDI	Zeta Potential (mv)
1	IN14	159.21	0.180	-15.9

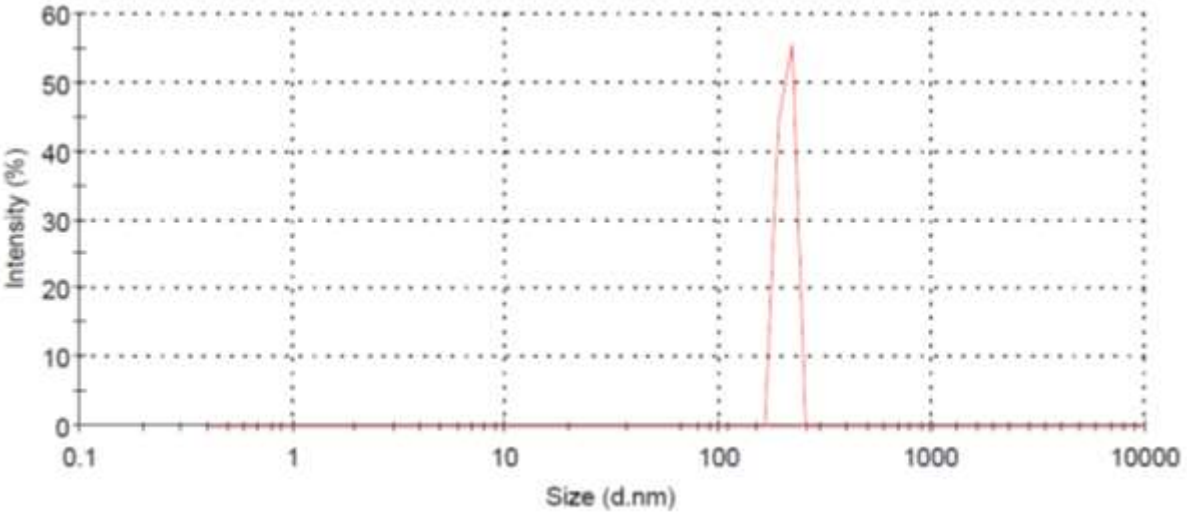

Figure 11: Globule size distribution graph of optimized Itraconazole loaded nanoemulsion formulation IN14

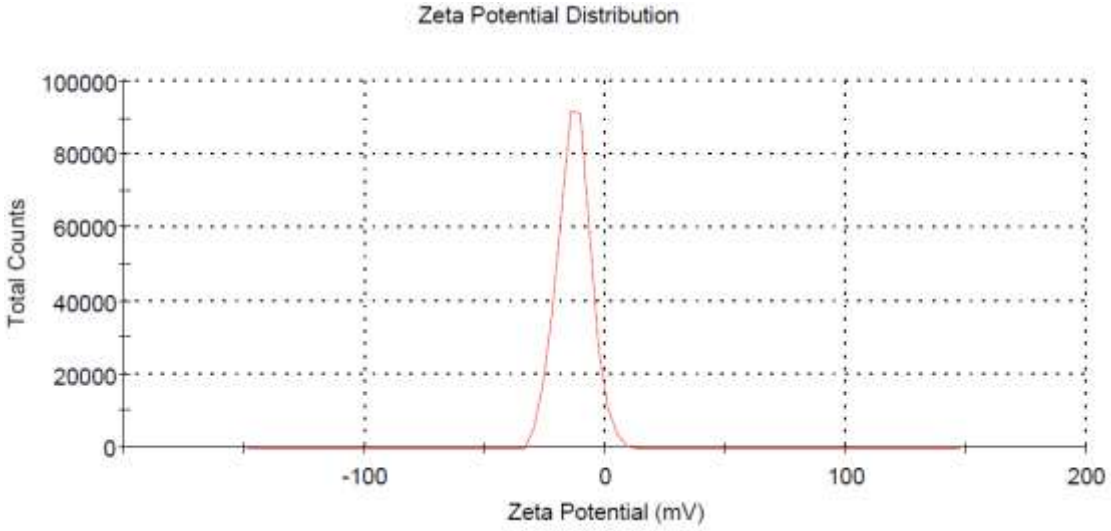

Figure 12: Zeta Potential distribution graph of optimized Itraconazole loaded nanoemulsion formulation IN14

Particle size and value of PDI was found to be 159.21nm and 0.180 as shown in figure no 11. In addition zeta potential demonstrated the stability of prepared nanoparticles was found to be -15.9mv as shown in figure no 12.

3.5.5 Transmission electron microscopy

TEM micrograph indicated a homogeneous distribution of small, spherical optimized Itraconazole loaded nanoemulsion formulation IN14as shown in figure 13.

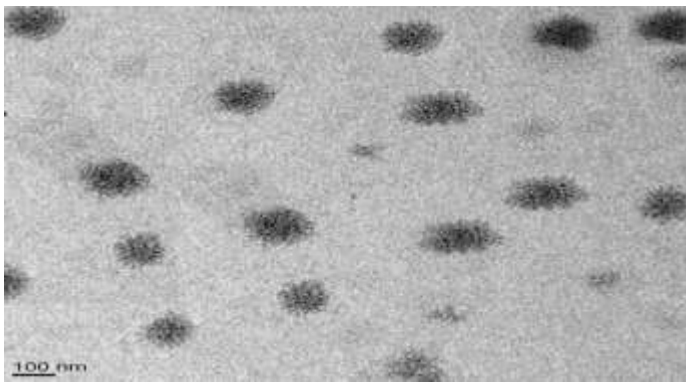

Figure 13: TEM micrograph of optimized Itraconazole loaded nanoemulsion formulation IN 14

CONCLUSION

Melting point of Itraconazole in bulk form was found to be 167°C±0.78-167°C±0.38.Itraconazole loaded Nanoemulsion were prepared by self-emulsification method, and on the basis of evaluation result IN4 ,IN5 shows higher % drug content as compare to other formulation. Formulation IN4 ,IN5 shows higher % drug content as compare to other formulation on the bases of above % drug content result. These two formulation were consider for evaluation and optimization on the bases of result of visual appearance, Tranmittance % drug content and thermodynamic stability.IN14 formulation of nanoemulsion was higher % drug content , transmittance and having stability on thermodynamic stability. All prepared formulations were clear, homogenous and transparent solution except formulation code IN10 and IN12. Percentage drug content of all Itraconazole loaded formulation was found to be in a range of 67.33±0.29 to 96.29±0.23. Particle size and value of PDI was found to be 159.21nm and 0.180. In addition zeta potential demonstrated the stability of prepared nanoparticles was found to be -15.9mv. TEM micrograph indicated a homogeneous distribution of small, spherical optimized Itraconazole loaded nanoemulsion formulation IN14. In this work, we aimed to improve the oral bioavailability of itraconazole through nanoemulsion.

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