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 aqueous solubility. In this research preformulation study, Fourier transform infrared (FTIR) analysis studies were 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 nanoparticles. 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, they have remained largely as unexploited physical dispersions of oil in water, anchored by their latin definition, emulsi, 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.

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.
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 200 mg 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) |
|------------------|-------|-----------------|-----------------------|
|                  |       |                 |                       |
| A1               | 1:09  | 190             | 855                   |
| A2               | 2:08  | 380             | 760                   |
| A3               | 3:07  | 570             | 665                   |
| A4               | 4:06  | 760             | 570                   |
| A5               | 5:05  | 950             | 475                   |
| A6               | 6:04  | 1140            | 380                   |
| A7               | 7:03  | 1330            | 285                   |
| A8               | 8:02  | 1520            | 190                   |
| A9               | 9:01  | 1710            | 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) |
|------------------|-------|-----------------|-----------------------|
|                  |       |                 |                       |
| A10              | 1:09  | 190             | 570                   |
| A11              | 2:08  | 380             | 1013.333333           |
| A12              | 3:07  | 570             | 886.666667            |
| A13              | 4:06  | 760             | 380                   |
| A14              | 5:05  | 950             | 633.333333            |
| A15              | 6:04  | 1140            | 506.666667            |
| A16              | 7:03  | 1330            | 190                   |
| A17              | 8:02  | 1520            | 253.333333            |
| A18              | 9:01  | 1710            | 126.666667            |
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 50°C for 1 min. Then oil added to Smix and vortexed for 5-10 min and placed in oven at 50°C for 1 min, 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 less 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:

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

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 °C in 75% -100% intensity. The samples were analyzed.

3.1 Preformulation 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 200-400 nm scanning range using UV spectrophotometer the absorption maxima of Itraconazole was found to be 262 nm 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

| Formulation code | Ratio | Amount Oil (mg) | Amount Smix (2:1) (mg) | Surfactant | Co surfactant |
|------------------|-------|-----------------|------------------------|------------|--------------|
| A19              | 1:09  | 190             | 1140                   | 570        |              |
| A20              | 2:08  | 380             | 1013.33333             | 506.666667 |              |
| A21              | 3:07  | 570             | 886.666667             | 443.333333 |              |
| A22              | 4:06  | 760             | 760                    | 380        |              |
| A23              | 5:05  | 950             | 633.333333             | 316.666667 |              |
| A24              | 6:04  | 1140            | 506.666667             | 253.333333 |              |
| A25              | 7:03  | 1330            | 380                    | 190        |              |
| A26              | 8:02  | 1520            | 253.333333             | 126.666667 |              |
| A27              | 9:01  | 1710            | 126.666667             | 63.3333333 |              |
Table 4: Absorbance of different concentration solution of Itraconazole in methanol

| Concentration (μg/ml) | Absorbance at 262nm ±STD |
|-----------------------|---------------------------|
| 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 used 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.

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 2: Overlay FTIR spectrum of Optimized formulation

Figure 3: Solubility of Itraconazole in different oils

Among all oil ethyl oleate have higher solubility of itraconazole 19.888±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 different HLB value surfactant

Table 6: Solubility of itraconazole 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±0.038 mg/ml followed by the tween 80 9.87±0.04 mg/ml as compare to other surfactant. In low HLB value surfactant span 80 have maximum solubility of itraconazole 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±2.70 mg/ml followed by Propylene Glycol 18.77±0.11 mg/ml as compare to other Cosurfactant.

On the basis of solubility Ethyl oleate, 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 stable 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, OS5, 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 | Kolliphor EL        | 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 remained 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 10. Pseudoternary phase diagram is used to identify the nanoemulsion region depicted in figure 18-20.

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

| S.No. | Oil:Smix 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:Smix Ratio | 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: Smix | 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 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, R² of responses of the quadratic polynomial response models were relatively high and the predicted R² values were in reasonable agreement with the adjusted R², 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:

\[ Y = 92.908+16.08X_1+6.025X_2-4.54X_1X_2-15.204X_1^2-3.31X_2^2 \]

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.

ANOVA 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. The percentage 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              |
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 IN14 as shown in figure 13.

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, Transmittance % 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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