RESEARCH ARTICLE

Selection and Characterization of a Nanoemulsion of Poorly Soluble Drug by Applying Box-Behnken Design and Converting it into a Nanoemulgel for Topical Application

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ABSTRACT

In the present work, nanoemulsion (NE) based hydrogel, i.e., nanoemulgel (NEG) of Itraconazole, an antifungal drug was prepared and investigated to study its potential in delivering a drug topically. NE of Itraconazole was prepared by high energy emulsification method employing anise oil as lipid phase; Gelucire 44/14 and PEG 400 are surfactant and co-surfactants, respectively. The construction of ternary phase diagrams determined the concentration of the ingredients. The prepared NE's were thus assessed for various parameters such as pH, electric conductivity, refractive index, viscosity, spreadability, poly-dispersity index (PDI), particle size, zeta potential etc and then distributed into suitable gel bases such as Carbopol 934P and Guar gum to obtain nanoemulsion based hydrogel. Therefore, various quality control tests such as pH, electric conductivity, refractive index, viscosity, spreadability, poly-dispersity index (PDI), particle size, zeta potential, swelling index, drug content estimation, *in-vitro* diffusion and *ex-vivo* permeation studies, antifungal studies etc were tested on the formulated NEG's. The optimized formulation FI2 released 98.09 ± 0.84% of the active ingredient in 12 hours and the pH, viscosity, and spreadability were found to suit the skin requirements. The results prove that topical administration of Itraconazole NEG increases the permeability and diffusibility of the drug.

Keywords: Nanoemulsion, Nanoemulgel, Topical preparation, Itraconazole, Box Behnken.

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INTRODUCTION

Pharmaceutical scientists have been facing remarkable challenges with lipophilic drugs because of their limitations, such as bioavailability and pharmacokinetic variations. A novel nanodrug delivery technology has risen to deliver poorly soluble drugs topically.[1] Delivery of drugs *via* skin refers to the topical drug delivery system (TDDS), which offers advantages due to improved bioavailability, painless administration, and sustained delivery of drugs. A variety of anti-inflammatory, antifungal, and a few of the drugs belonging to other classes are administered as conventional topical drug delivery systems such as ointments, creams,lotions,liniments, poultices, emulsions, gels, dusting powders, etc. Low retention and poor bioavailability are the major pitfalls of conventional TDDS.[2] These pitfalls were resolved by conducting extensive research to develop new drug delivery systems, such as micro sponges, mucoadhesive bioadhesives, nanoemulsions, nanoemulgels etc for topical application.[3]

Emulsion is a biphasic dispersed system consisting of minute droplets well disseminated in an immiscible phase. Depending on the particle size, distributed phase emulsions are divided into conventional emulsion/colloid, microemulsions and nanoemulsions. The droplet size of macroemulsions ranges between 1 to l00 μm, and that of nanoemulsion (NE) is 20 to 200 nm.[4,5]

High kinetic and thermodynamic stability are the key factors in delivering active pharmaceutical ingredients (API) through the skin by means of a vehicle. The concentration of surfactant is greater in an NE compared to an ME, resulting in improved release of API, reduced droplet size of the drug, increased surface area and decreased surface tension.[6] Hence, these properties promote rapid penetration of lipophilic

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moieties transdermally, decreasing the dose and enhancing the safety and efficacy of the drug. Lipid carriers such as liposomes, vesicles etc have limited applications than NE's due to certain drawbacks such as stability and decreased skin permeation. Therefore, improved kinetic stability and non-irritant nature of NE help deliver lipophilic drugs topically. Moreover, NE's can be formulated as foams, creams, sprays, gels etc. Among all these, gel is preferred because of its specific dermatological properties such as non-greasiness, emollient, thixotropy and spreadability.[7]

Gel is a three-dimensional structure and a two-phase system in which particles are dispersed with the interpenetration of liquid. The distribution of nanoemulsion into a gel base is termed as "Nanoemulgel" (NEG). The skin contact time of NEG is greater when compared to NE. It is a semi-solid dosage form in whose dispersed phase droplets are nm in size. The emulsion is either W/O or O/W type. Major ingredients in NEG include oil, a surfactant and a co-surfactant. A permeation enhancer and stability-instilling agent may also be added.[8]

NEG is said to be a promising alternative to conventional TDDS in case of biopharmaceutics classification system (BCS) class II drugs. It is superior because of increased penetration and absorption *via* the skin and greater retention time of active moiety at site of action.[9] This work involves developing itraconazole loaded NEG, designed to deliver the drug topically to treat fungal infections caused by *Candida* species and some *Aspergillus* species. The NEG's so prepared were subjected for pH, refractive index, conductivity, rheological studies, heating cooling cycle, drug release, skin irritation studies, syrengibility *in-vitro and ex-vivo* diffusion studies. The formulations are also analyzed for, polydispersity index (PDI), particle size, zeta potential and transmission electron microscopy (TEM).

MATERIALS AND METHODS

Materials

Itraconazole, and gelucire 44/14 are gift samples obtained from Metrochem API Pvt Ltd, Visakhapatnam, and Gateffosse, respectively. PEG 400 was purchased from Otto Chemical Pvt Ltd; Carbapol 934P and methylparaben were obtained from Yarrow chem, Mumbai.

Methods

The preparation of nanoemulgel includes several steps which are discussed below in detail:

Step 1: Solubility of drug in major ingredients

The first and foremost step in NEG formulation is selecting major components such as lipid vehicle, surfactant and co-surfactant. They were selected by equilibrium solubilization technique. The method involves adding excess amount of itraconazole to 2 mL each of major components singly and kept in water bath shaker for 72 hours at 25 ± 2°C. Then, if any, the equilibrated samples for l5 minutes at 3000 rpm to separate undissolved itraconazole. Whatman's filter paper was used to filter the supernatant and diluted with dichloromethane for quantification of itraconazole by using UV spectrophotometer at 255 nm. The oil that showed maximum solubility of the drug was used further for the formulation of NE.[10]

Step 2: Screening of emulsifiers for nanoemulgel

The emulsification ability of emulsifiers is tested to screen the emulsifier. The surfactants' emulsification ability is determined by adding 300 mg of the surfactant to 300 mg of oil and homogenized. Then 50 mg of the homogenized mixture was thinned to 50 mL with double distilled water, resulting in a fine emulsion observed for any turbidity. Then the emulsion was left aside for about 2 hours and the transmittance was assessed using UV spectrophotometer.

Secondly, the co-surfactants were assessed for their emulsification ability by preparing a mixture of l00 mg of co-surfactant to 200 mg of selected oil and evaluated in the same way as that of the surfactant.[11]

Step 3: Aqueous titration method

The selected emulsifier and co-emulsifier were integrated in 1:1, 1:2, 1:3, 2:1 and 3:1 ratios to generate S_{mix}. Then, oil is mixed with various combinations of S_{mix} in divergent combinations from 1:9 to 9:1 and titrated slowly against water. The choice of ratios is based on the increase in the concentration of co-emulsifier with respect to emulsifier and vice versa to construct phase diagrams. The volume of titrant added was noted down when a clear mixture was obtained. After equilibrium was achieved, the mixture was observed for transparency. Those compositions that are visually clear, isotropic and rich in water can be observed for the nanoemulsion region.[12]

Step 4: Pseudo ternary phase diagram construction

Ternary phase diagrams were constructed to study the nanoemulsion region. Oil, S_{mix} and water are taken on the three axes. The figure in which oil, S_{mix} and water combinations show greater NE areas are used for further study.

Step 5: Preparation of nanoemulsion

High-speed homogenization method was employed to prepare NE's using the calculated quantity of oil, S_{mix} and water. Firstly, the required quantity of oil to which the drug was added and mixed thoroughly was measured. Then, S_{mix} was added and homogenized for 5 minutes at 3000 rpm. Finally, the measured amount of water is transferred dropwise and homogenized once again at 3000 rpm for l5 minutes.[13]

The prepared NE's were Optimized using Statistical Method

Design expert Software was used to produce a 3-factor 3-level Box-Benhnken design (BBD). Though several techniques are available in the response surface method, BBD is the most appropriate approach for studying the effect of response variables as dependent factors. Three factor 3 level statistical design was employed to screen the interaction effects of the variables such as concentration of oil, surfactant and co-surfactant on the responses of formulated NEG's and applying the desired function in optimizing the formulation.

BBD required 15 experimental runs with 3 central points to determine the experimental error and precision of the design. BBD produced a non-linear quadratic model equation which is as follows

$$Y= \alpha_0 \pm \alpha_1 x_1 \pm \alpha_2 x_2 \pm \alpha_3 x_3 \pm \alpha_4 x_1 x_2 \pm \alpha_5 x_2 x_3 \pm \alpha_6 x_1 x_3 \pm \alpha_7 x_1^2 \pm \alpha_8 x^2 \pm \alpha_8 x_2^2 \pm \alpha_9 x_3^2$$

Where,
Y = dependent variables measured response
α_0 = intercept
α_1 to α_9 = Regression coefficient

Step 6: Transformation of nanoemulsion into nanoemulgel

The incorporation of the optimized NE into a suitable gel base produces a NEG. The base was prepared by addition of water to the gelling agent and left aside overnight.[14]

Evaluation

The formulated NEG's are thus evaluated for the following factors:

Appearance and pH

The prepared NEGs were evaluated for their appearance by visual observation. pH of the NEG is determined as it affects the stability of the product. It is measured by pH meter (digital) in triplicate and average results were considered.

Electrical conductivity

It is measured with the help of a conductivity meter that provides data regarding the structure and phase behavior of NEG systems.[15]

Viscosity

Viscosity is a measure of the formulation's rheological properties. Brookfield viscometer was used to determine viscosity. It is measured in triplicate and average is taken into consideration.[16]

Drug content

The amount of drug in the preparation was determined by a UV spectrometer. About 0.5 mg of every formulation was weighed and diluted with 5 mL methanol. Hence, the obtained solutions were spectroscopically analyzed following suitable dilution. The efficiency of drug loading was determined.[17]

$$\text{Drug loading efficiency} = \frac{\text{Amount of drug in known amount of formulation}*100}{\text{Initial drug load}}$$

Refractive index

Refractive index was determined for the NEG's by using Abbe's refractometer.[18]

Particle size and poly dispersity index

In a 100 mL volumetric flask the formulations were diluted in 1:100 ratio and gently assorted by upending the flask. The diluted formulations' mean particle size and PDI was observed by photon correlation spectroscopy using Malvern Zeta sizer. All examinations were repeated in three trials.[19]

Zeta potential

The stability of the nanoemulsion is directly related to the magnitude of the surface charge. Zeta potential of the formulations was measured with a zetasizer using laser diffraction analysis. To guarantee homogeneity, the samples were diluted in 1:50 (v/v) ratio with double distilled water and homogenized using a magnetic stirrer. After homogenization, aliquots were introduced into a folded capillary cell which was loaded into the Zeta sizer for further study. All investigations were repeated 3 times at 25°C.[20]

Heating cooling cycle

About six cycles were performed at 4 and 40°C individually for 48 hours to identify if any instability (phase separation) is present.[21]

Centrifugation

The formulations were centrifuged using a laboratory centrifuge at 3000 rpm for 15 minutes. The resultant was then tested if any instability problem, such as phase separation, persists. The stable preparations were utilized for further studies.[22]

Swelling index

One gm of prepared NEG was taken onto porous aluminum foil and placed in l0 mL of 0.1N NaOH solution. NEG was removed from time to time and weight was determined until a constant weight is obtained.[23]

$$\text{Swelling index (SW) \%} = [(W_t - W_o)/W_o]*100$$

Where (SW) % = Percentage swelling.
W_o, = Original weight of nanoemulgel
W_t = Weight of swollen nanoemulgel at time

Spreadability

Spreadability influences the therapeutic efficacy of the prepared NEG's. The spreading coefficient depends on the viscosity of the formulation as viscosity increases, spreadability increases and *vice-versa*. It is determined by placing 0.5 g of the formulation in a circle of 1-cm diameter previously drawn on a glass plate. Another glass plate was used to cover 1st glass plate. The weight of %g was made to rest on to the covered plate for 5 minutes and the extension in diameter of the formulation due to spreadability was noted.[24]

Extrudability

This test involves the measurement of the force needed to extrude gel out of the tube. The amount of gel extruded from the aluminum tube was determined by the application of weight. The greater quantity of gel extruded, better the extrudability. Extrudability and viscosity are inversely related, i.e., a less viscous preparation is easily extrudable. Therefore, extrudability is usually measured 3 times and calculated by,.[25]

$$E = \frac{M}{A}$$

In-vitro drug release studies

These studies were performed using a Franz diffusion cell in an effective area of 2.27 cm^2, a thermostable method for predicting drug permeation via skin. These studies are conducted using a dialysis membrane that is mounted in between the benefactor and receptor compartments. pH buffer was filled into the receptor compartment and sample was applied onto the dialysis membrane. One mL of the buffer was introverted at a predestined time and an equivalent volume of sample is restored. The samples were analyzed with UV-visible spectroscopy to determine the concentration of the drug.[26]

Ex-vivo diffusion studies

Ex-vivo drug diffusion studies were conducted using Franz diffusion cell in an effective area of 2.27 $cm^{2,}$ which is a the most reliable method for predicting drug permeation *via* skin. These studies use excised goat skin mounted between the benefactor and receptor compartments.[27] pH buffer was filled into the receptor compartment and the sample was applied to the dialysis membrane. 1-mL of the buffer was introverted at a predestined time and an equivalent volume of sample is restored. The samples were analyzed with UV-visible spectroscopy to determine the concentration of the drug.[28]

Comparison between optimized and marketed products

To study the efficacy of the prepared nanoemulgel, the *ex-vivo* diffusion studies were performed in comparison to marketed Itraconazole ointment.

Antifungal activity

The antifungal activity of preparations was studied in sterilized Sabouraud's agar medium using the agar well diffusion method. A measured amount of the organism was introduced into the medium and mixed thoroughly. About 2 mL of this mixture was transferred into a petridish aseptically and left aside for solidification. A sterile cork borer was used to pierce the surface. The pierced dwells were filled with an optimized batch of nanoemulgel and incubated at 18 to 24°C for 72 hours. Fungal growth if any was observed and the inhibition zone was measured by an antibiotic zone reader.[29]

RESULTS AND DISCUSSION

Screening of Components (Figure 1)

The first and foremost step in the development of nanoemulgel of itraconazole for topical delivery is that it should possess maximum solubility in the components, as only solubilized drugs can permeate efficiently through the skin. The solubility of the drug in all the major components of a NEG was studied. itraconazole had the highest solubility in anise oil which is an essential oil. It was used in an emulsion system to enhance the permeation of active ingredients *via* skin and possesses mild antifungal activity. Similarly, based on solubility studies, Gelucire 44/14 and PEG400 were selected as surfactants and co-surfactants, respectively.[28]

Pseudo Ternary Phase Diagram (Figure 2)

CHEMIX school 10 software was employed in constructing pseudo ternary phase diagrams for each S_{mix} ratio to study the nanoemulsion area's extent. The efficiency of S_{mix} to solubilize oil defines the region of nanoemulsion area. In the present study, as the ratio of S_{mix} increases, the isotropic nanoemulsion area also increases. S_{mix} of 1:2 showed a greater nanoemulsion region when compared to other S_{mix} ratios. Therefore, an increasing proportion of glycerol exhibits a larger nanoemulsion area due to its co-solvency with peppermint oil.

Application of Response Surface Methodology for Optimization of NE

BBD was applied for analyzing effect of 3 independent variables on dependent variables. According to BBD, 15 NE's were formulated and evaluated for their particle size (Y_1), PDI (Y_2) and zeta potential (Y_3), i.e., response variables. All data was obtained using Design Expert Software. All the responses were fitted separately into a full quadratic equation. ANOVA used for determining the significance of model, lack of fit and multiple co-relation co-efficient (R^2) test. The model *p-value* to be < 0.005 for the model to fit well into the quadratic equation (significant). Lack of fit test which is insignificant having a *p-value* > 0.05 was applied to analyze data variation. The

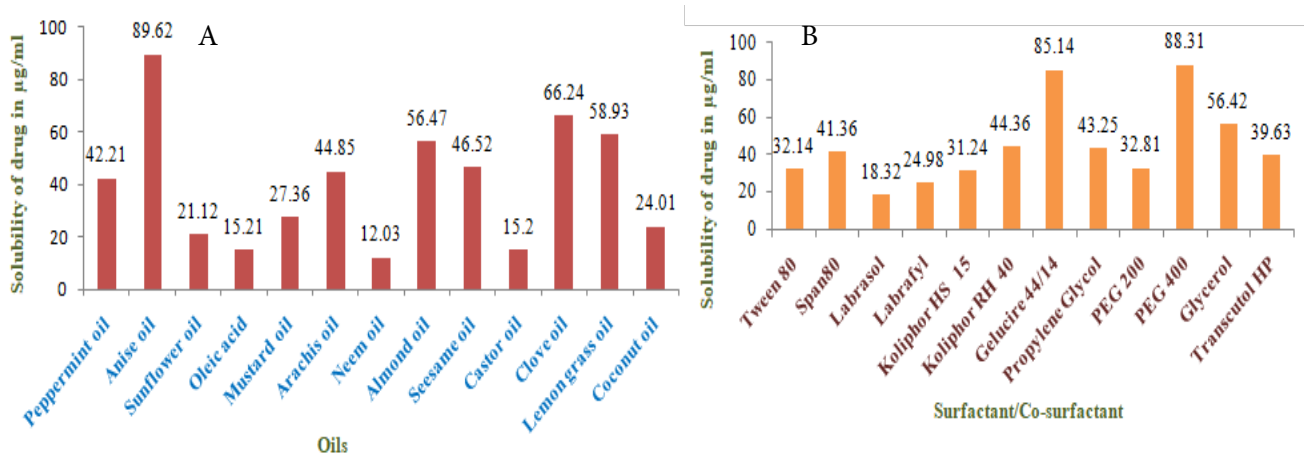

Figure 1: A) Drug solubility in various oils in µg/mL. B) Drug solubility in different surfactants and co-surfactants expressed in µg/mL.

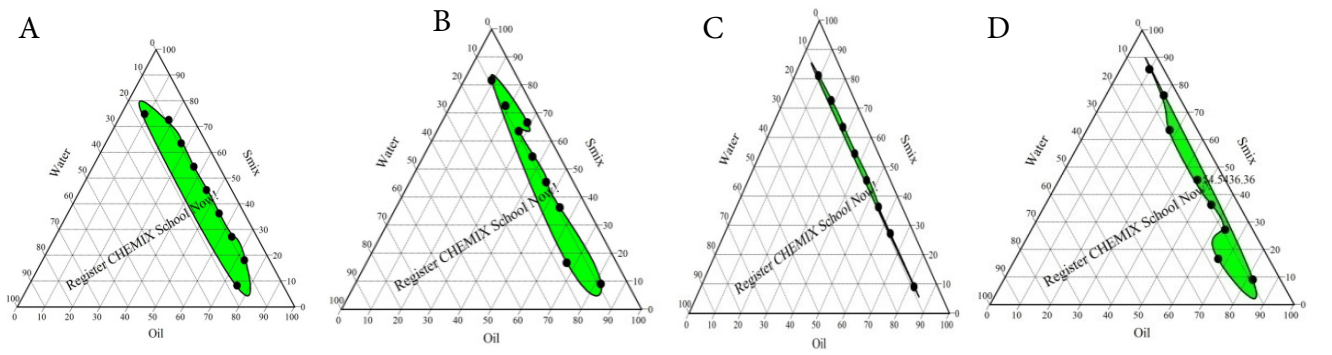

Fig 2: A) Pseudo ternary Phase diagram of S_{mix} ratio 1:1 B) Pseudo ternary Phase diagram of S_{mix} ratio 1:2. C) Pseudo ternary Phase diagram of S_{mix} ratio 1:3 D) Pseudo ternary Phase diagram of S_{mix} ratio 2:1.

multiple correlation coefficient (R^2) expressing the variation amount should be near to 1.

Particle Size (Figure 3, Table 1)

The particle size was found to be in between 95.72 to 190.61 nm. The result of particle size analysis of various formulations proved that an increase in the quantity of surfactant and a decreased ratio of oil resulted in a diminution in globule size.

The independent factors anise oil (X_1), gelucire 44/14 (X_2) and PEG 400 (X_3) resulted in various response variables of globule size (Y_1). The mathematical relationship for globule size in polynomial equation form, is given by.

$$Y_1 = 111.64 \pm 14.11\ X_1 \pm 9.65\ X_2 \pm 4.60\ X_3 - 2.90\ X_1 X_2 - 5.49\ X_2 X_3 \pm 16.53\ X_1 X_3 \pm 16.17 X_1^2 \pm 26.70 X_2^2 \pm 18.80\ X_3^2$$

The equation shows effect of independent variables (X_1–X_3) and their interactions (co-efficient with more than one – factor term, $X_1 X_2$, $X_1 X_3$, $X_2 X_3$) on the response Y_1. The obtained *p-value* for the coefficient is < 0.05, which signifies a collaborative effect whereas the negative sign indicates the opposite effect of independent variables on response. Greater co-efficient value of the factor indicates a substantial effect on the response. All of the responses fitted well into the quadratic model, and the efficiency of the model was substantiated by multiple correlation tests (R^2) and ANOVA.

The p, and R values were <0.05 and 0.6317, which proved that independent variables had a significant effect on predicting the response (Y_1). Multi colinearity of the independent factors noticed no multi- co linearity between independent variables (X1–X3) in the quadratic model. VIF value less than 10 indicates it to be tolerable.

Response surface plot, contour plot and predicted Vs actual plot Y_1 between X_1 and X_2 at middle levels of X_3 are depicted in Figure 3.

Polydispersity index (Figure 4, Table 1)

The polydispersity index, a dimensionless factor, is used to study particle size distribution. Its value should be in between 0 to 1. A greater value represents a heterogeneous system having a wide range of particles. All the formulations have low PDI values describing the particles as almost small and the preparation is monodisperse.

The PDI of the formulations were found to be in between 0.189 to 0.96. The quadratic equation for the measured response, i.e., PDI is given by

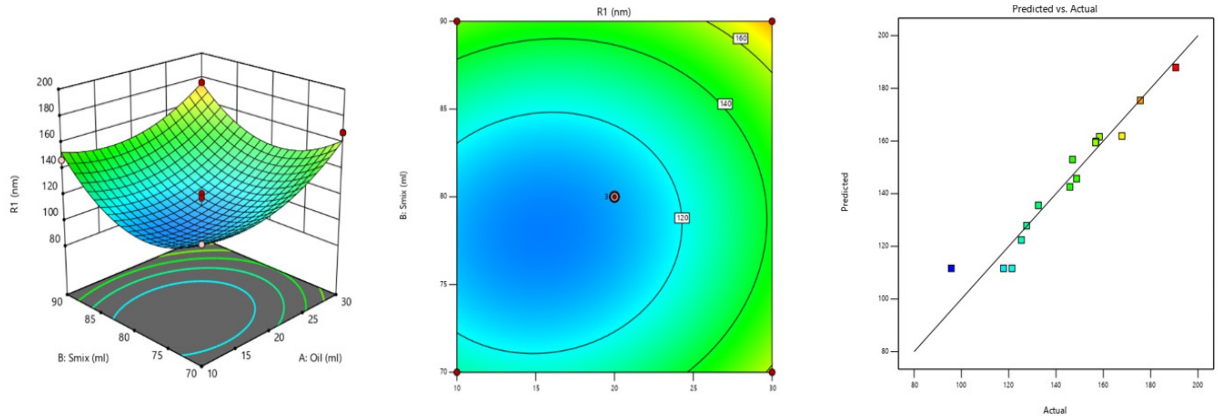

Figure 3: Response surface plot, countour plot and predicted vs actual plot showing the effect of oil, S_{mix} (Gelucire 44/14 and PEG 400) and water on particle size

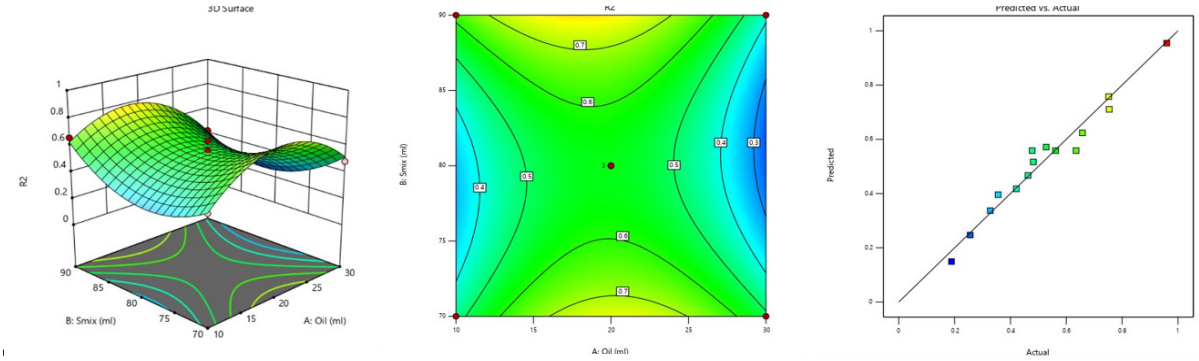

Figure 4: Response surface plot, contour plot and predicted vs actual plot showing the effect of oil, S_{mix} (Gelucire 44/14 and PEG 400) and water on polydispersity index.

$Y_2 = 0.5583\text{-}0.0391\ X_1 \pm 0.0145X_2 \pm 0.0841X_3\text{-}0.0640X_1X_2 \pm 0.0098\ X_2X_3 \pm 0.1075\ X_1\ X_3\text{-}0.2593X_1^2 \pm 0.2070X_2^2\text{-}0.0168\ X_3^2$

The above equation is a polynomial determining the quantitative effect of independent variables on measured response (Y_2). R^2 value of 0.6973 is in consonance with the adjusted R^2 value, and their difference in <0.2. Hence, the polynomial equation fit the response variable (Y2) well. The resultant equation for the analysis of regression for Y_2 produced negative sign for oil (X_1), a positive for variables X_2 and X_3. Therefore the equation suggests that the PDI decreases with increase in the concentration of $S_{mix.}$ The ANOVA study suggested that the independent variable largely affects the prediction of response $Y_{3.}$ Coefficient terms with *p-value* < 0.05 affect the prediction efficacy of model significantly. The result of the contour plot, response surface plot and predicted vs actual plot were given in the following Figure 4.

Zeta potential (Figure 5, Table 1)

The zeta potential values obtained for various preparations are in the range of to -26.32 to -38.22 mV. The quadratic equation for the measured response zeta potential is given by

$Y_{3\,=}$ -29.27 - 0.5275 $X_1 \pm 1.62\ X_2 \pm 2.10\ X_3$- 0.6000 $X_1\ X_2$ – 0.7850 X_2X_3 -2.57 $X_1\ X_3 \pm 0.6379X_1^2$ -3.03$X_2^2 \pm 0.3154\ X_3^2$

The equation obtained for zeta potential depicts a good fit to the response variable (Y_3) as the R^2 of 0.8163 is in congruence to the adjusted R^2 of 0.9445. Regression analysis of Y_3 depicted negative sign for X_1 (oil) and positive signs for X_2 (surfactant) and X_3 (co-surfactant). Decrease in quantity of lipid and increase in S_{mix} concentration, the zeta potential value decreases. The model equation generated by ANOVA analysis proved that independent variables showed affect on $p < 0.05$ in predicting response (Y_3). F exhibited a zeta potential value of -20.2 mV. The resultant response surface plot, contour plot and predicted vs actual plot are given in the Figure 5.

From the above studies, NE containing oil and S_{mix} in the 1:1 ratio is used for the preparation of NEG.

Thus the NE is converted into NEG by dispersing it into a suitable gel base such as carbapol 934P and guar gum in 2, 3, and 4%% concentration. The results obtained are as follows: (Table 1)

- *Appearance and pH*

The prepared NEG's appeared to be clear and homogeneous preparations having pH in the range of 4.78 to 4.96 to avoid discomfortability and irritation.

- *Electrical conductivity*

The conductivity values describing the NEG's phase system was measured using the conductivity meter and ranged from 0.12 to 0.67.

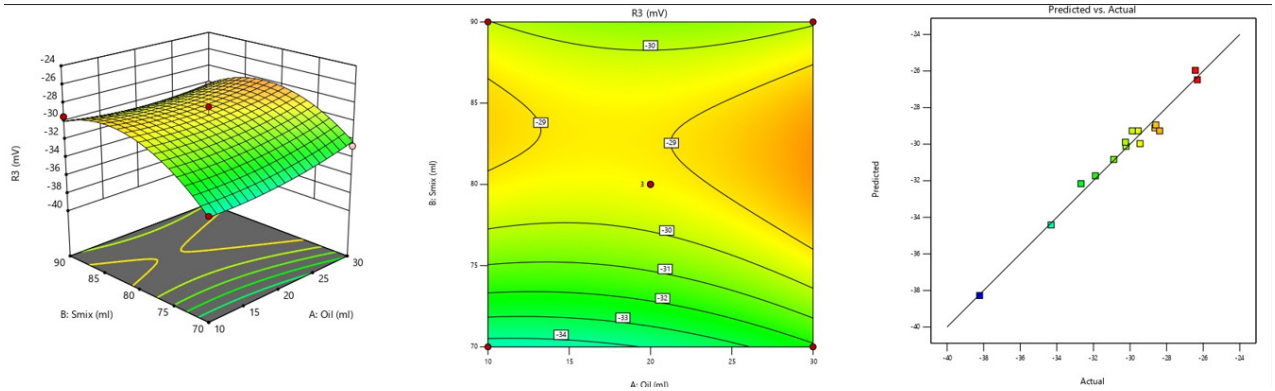

Figure 5: Response surface plot, contour plot and predicted vs actual plot showing the effect of oil, S_{mix} (gelucire 44/14 and PEG 400) and water on zeta potential

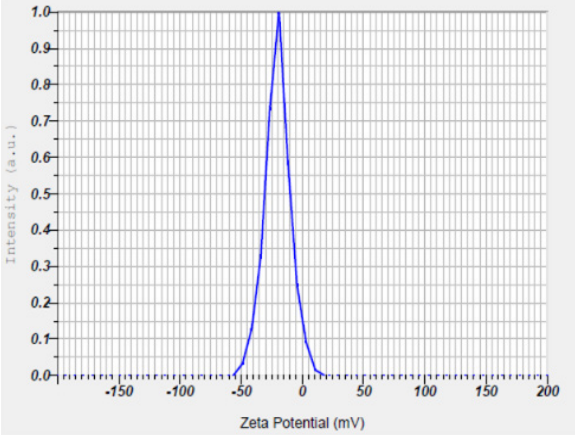

Figure 6: Zeta potential graph of optimized formulation

- *Viscosity*

The viscosity was found to be in the range of 3.9 to 4.3cP

- *Refractive index*

Refractive index of the prepared NE's was between 1.36 to 1.39, almost equal to water thus indicating the formation of a clear and transparent nanoemulgel.

- *Polydispersity index and particle size*

The mean particle size of nanoemulgel was in the range of nm, and polydispersity index results prove the NEGs to be monodisperse.

- *Zeta potential (Figure 6)*

Zeta potential data of the NEG does describe the preparations to be stable.

- *Drug content release*

The drug content release was determined spectrophotometrically for formulations and was found to be in the range of 62.6 ± 0.3 to 82.4 ± 0.2%

- *Physical stability of NEG's*

NEGs are kinetically stable dosage forms when formed at a particular proportion of oi1. The optimized NEG did not show any phase separation, creaming, cracking or Ostwald ripening when subjected to stability confirmation tests like freeze thaw cycle, heating cooling cycle, and centrifugation.

- *Swelling index*

%Swelling index of the formulated NEGs was found to be 10.54 to 18.83%

- *Spreadability*

The spreading coefficient values of the formulations ranged between 4.2 to 5.1 gcm/s. Formulations with greater viscosity has larger spreading co-efficient.

- *Extrudability*

The extrudability of the formulations FI1 to FI6 in the range of 82 to 221.25 g/cm^2.

- *In-vitro drug release studies (Table 2)*

In-vitro drug release studies were performed using pH 7.2 phosphate buffer in Franz diffusion cell. These studies showed that formulation F2, containing 3% Carbopol 934P as the gelling agent, released 98.09 ± 0.84% of the drug in 12 hours.

- *Ex-vivo diffusion studies (Table 3)*

Drug release studies for the optimized formulation *(ex-vivo)* were performed in pH 7.2 phosphate buffer using Franz diffusion cell. From these studies it was noticed that optimized formulation FI2 made containing 3% Carbopol 934P as the

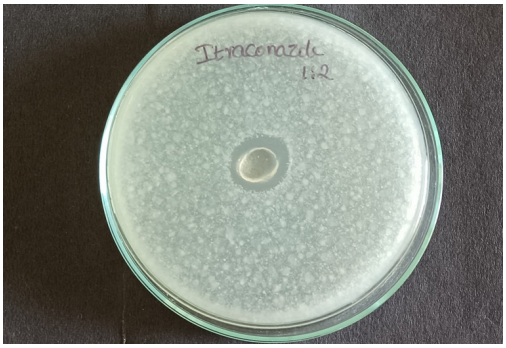

Figure 7: Antifungal activity of itraconazole nanoemulgel

Table 1: Evaluation parameters of formulations F1 to F6

Evaluation factor	*FI1*	*FI2*	*FI3*	*FI4*	*FI5*	*FI6*
pH	5.47 ± 0.03	4.85 ± 0.04	4.96 ± 0.03	5.03 ± 0.05	4.78 ± 0.04	4.67 ± 0.02
Electric conductivity	4.74 ± 0.03	4.85 ± 0.04	4.88 ± 0.03	4.69 ± 0.05	4.78 ± 0.04	4.67 ± 0.02
Refractive index	1.34 ± 0.03	1.36 ± 0.04	1.39 ± 0.03	1.38 ± 0.05	1.57 ± 0.03	1.35 ± 0.04
Centrifugation	Pass	Pass	Pass	Pass	Pass	Pass
Heating cooling cycle	Pass	Pass	Pass	Pass	Pass	Pass
Drug content estimation	78.52 ± 0.4	84.4 ± 0.3	82.15 ± 0.3	83.3 ± 0.4	81.4 ± 0.5	68.8 ± 0.2
Swelling index	12.44 ± 0.2	13.24 ± 0.3	11.53 ± 0.4	12.38 ± 0.3	16.87 ± 0.3	17.65 ± 0.2
Particle size (nm)	94.78 ± 0.3	137.26 ± 0.1	115.32 ± 0.3	147.21 ± 0.5	139.98 ± 0.2	96.32 ± 0.6
Zeta potential	-27.59 ± 0.2	-31.24 ± 0.4	-30.86 ± 0.2	-28.36 ± 0.1	-27.38 ± 0.3	-28.54 ± 0.4
Polydispersity index	0.57 ± 0.03	0.58 ± 0.03	0.63 ± 0.02	0.75 ± 0.05	0.55 ± 0.04	0.45 ± 0.02

Table 2: Drug release studies (*In-vitro*) of the formulations FI1 to FI6

Time	*FI1*	*FI2*	*FI3*	*FI4*	*FI5*	*FI6*
0.25	28.19 ± 0.41	22.21 ± 0.41	18.23 ± 0.36	10.21 ± 0.64	9.36 ± 0.36	7.45 ± 0.89
0.5	34.15 ± 0.60	24.15 ± 0.56	21.46 ± 0.54	14.11 ± 0.58	12.41 ± 0.62	10.68 ± 0.65
0.75	40.21 ± 0.58	26.18 ± 0.89	25.13 ± 0.87	19.24 ± 0.74	16.15 ± 0.41	13.32 ± 0.44
1	46.87 ± 0.69	44.62 ± 0.77	34.32 ± 0.45	23.36 ± 0.63	21.23 ± 0.68	18.43 ± 0.49
2	52.31 ± 0.72	50.25 ± 0.58	40.21 ± 0.89	28.45 ± 0.49	26.18 ± 0.72	24.19 ± 0.56
3	60.45 ± 0.55	56.59 ± 0.69	46.82 ± 0.52	34.93 ± 0.67	32.55 ± 0.25	28.48 ± 0.78
4	68.72 ± 0.21	61.53 ± 0.74	52.38 ± 0.58	39.84 ± 0.41	38.74 ± 0.33	34.71 ± 0.95
5	78.12 ± 0.98	64.22 ± 0.85	57.89 ± 0.74	44.67 ± 0.52	42.07 ± 0.86	39.54 ± 0.19
6	86.23 ± 0.45	73.75 ± 0.63	62.15 ± 0.92	50.02 ± 0.93	48.85 ± 0.75	44.30 ± 0.37
7	94.15 ± 0.65	77.06 ± 0.66	66.35 ± 0.43	54.78 ± 0.74	51.62 ± 0.98	48.58 ± 0.49
8	98.23 ± 0.87	82.75 ± 0.57	70.95 ± 0.28	59.82 ± 0.49	56.34 ± 0.41	53.74 ± 0.66
9		88.31 ± 0.80	74.11 ± 0.14	64.28 ± 0.52	61.44 ± 0.74	58.65 ± 0.54
10		91.78 ± 0.47	78.54 ± 0.35	71.54 ± 0.41	64.19 ± 0.37	61.87 ± 0.88
11		93.40 ± 0.39	80.25 ± 0.78	75.22 ± 0.85	68.35 ± 0.59	64.35 ± 0.97
12		96.09 ± 0.84	84.39 ± 0.65	78.14 ± 0.75	70.15 ± 0.29	68.21 ± 0.54

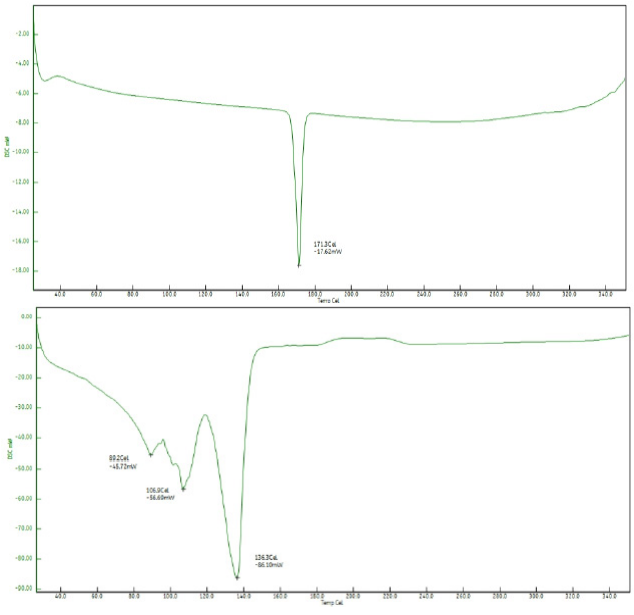

Figure 8: DSC graph of A) Drug B) Optimized formulation

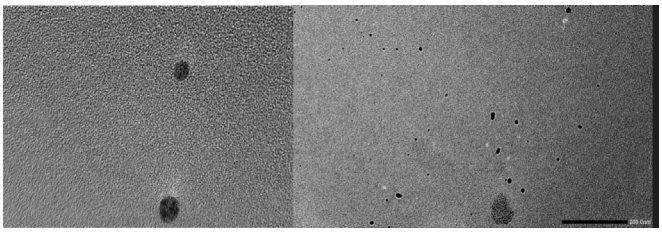

Figure 9: TEM images of optimized itrconazole nanoemulgel

Table 3: Drug release studies of optimized formulation. (*In-vitro* and *Ex-vivo*)

Time (hours)	*Optimized formulation (In-vitro studies)*	*Optimized formulation (Ex-vivo studies)*
0.25	22.21 ± 0.41	22.65 ± 0.240
0.5	24.15 ± 0.56	24.40 ± 0.31
0.75	26.18 ± 0.89	29.59 ± 0.23
1	44.62 ± 0.77	34.46 ± 0.29
2	50.25 ± 0.58	42.12 ± 0.35
3	56.59 ± 0.69	47.28 ± 0.42
4	61.53 ± 0.74	53.76 ± 0.18
5	64.22 ± 0.85	57.21 ± 0.23
6	73.75 ± 0.63	62.25 ± 0.46
7	77.06 ± 0.66	64.43 ± 0.31
8	82.75 ± 0.57	68.03 ± 0.52
9	88.31 ± 0.80	75.37 ± 0.51
10	91.78 ± 0.47	78.43 ± 0.27
11	93.40 ± 0.39	82.03 ± 0.35
12	96.09 ± 0.84	88.65 ± 0.41

gelling agent released 88.65 ± 0.41% of the drug release in 12 hours.

- *Comparison between optimized and marketed products (Table 4)*

From the *ex-vivo* diffusion studies performed for selected formulation (FI2) and branded itraconazole ointment, it was

Table 4: Drug release studies of optimized formulation and marketed formulation (*Ex-vivo*)

Time (hours)	*Optimized formulation*	*Marketed formulation*
0.25	22.62 ± 0.240	8.34 ± 0.69
0.5	24.40 ± 0.31	11.18 ± 0.55
0.75	29.59 ± 0.23	19.26 ± 0.50
1	34.46 ± 0.29	23.29 ± 0.38
2	42.12 ± 0.35	27.51 ± 0.47
3	47.28 ± 0.42	32.49 ± 0.23
4	53.76 ± 0.18	38.29 ± 0.12
5	57.21 ± 0.23	44.21 ± 0.57
6	62.25 ± 0.46	50.22 ± 0.63
7	64.43 ± 0.31	56.37 ± 0.24
8	68.03 ± 0.52	60.38 ± 0.41
9	75.37 ± 0.51	64.71 ± 0.51
10	78.43 ± 0.27	68.14 ± 0.34
11	82.03 ± 0.35	72.14 ± 0.32
12	88.65 ± 0.41	76.14 ± 0.44

noted that FI2 released 98.09 ± 0.84% of the drug release in 12 hours, whereas marketed formulation released only 72.23 ± 0.21 in 12 hours.

- *Antifungal activity (Figure 7)*

In-vitro antifungal studies depict that after 24 hours of incubation the formulation showed similar fungal activity against *Candida* sp. The area of inhibition for the test preparations was about 16 mm.

- *Differential scanning calorimetry (Figure 8)*

Thermal property of pure itraconazole and optimized formulation were studied by DSC. The spectra showed a sharp endotherm at melting point at 171.3ºC with respect to its melting point but when incorporated into the nanoemulgel the melting endotherm was significantly decreased to 136.3ºC. The study also revealed that in the optimized formulation, the endothermic peak sharpness corresponding to melting point of pure drug was reduced which indicates the amorphous nature of itraconazole.

- *Transmission electron microscopy (Figure 9)*

The morphology and size distribution of particles in NEG systems was obtained by performing transmission electron microscopy (TEM). The particles in NEG system were round having a size range of 200 nm or less.

CONCLUSION

The above research concluded that the prepared nanoemulgel of itraconazole has been a promising candidate to deliver the drug topically for a stipulated time. The dosage frequency was well reduced because of sustained and prolonged systemic absorption of itraconazole.

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