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Original Article

Characterization of Oxiconazole Nanoemulsion Using Box-Behnken Design and Transformation of the Selected Nanoemulsion into Nanoemulgel

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ABSTRACT

Background: The present work covers the preparation and investigation of Nanoemulsion (NE) based hydrogel i.e., Nanoemulgel (NEG) of an Antifungal drug Oxiconazole delivered topically. **Materials and Methods:** High-speed homogenization was used to prepare the NE using Clove oil, Tween 80 and Propylene Glycol as oil phase, surfactant and co-surfactant respectively. Ternary phase diagrams are constructed to determine the quantity of ingredients used to formulate nanoemulsion. The prepared NEs were thus assessed for parameters such as pH, refractive index, electric conductivity, viscosity, spreadability, ploy dispersity index, Zeta potential etc., The NE possessing the best characteristics is optimized and converted into a Nanoemulgel (NEG) by distributing the NE into a suitable gel base such as Carbopol 934P and Guar gum. The gel base was prepared by distributing 2%, 3% and 4% of the gelling agent into water and kept aside overnight. The NEG's formulated are thus evaluated for physicochemical characteristics, *in vitro* diffusion, *ex vivo* permeation studies and anti-fungal studies. **Results:** FO1 made up of 2% Carboplol 934P released 97.38±0.64% of the drug in 12 hr is optimized as better formulation based on physico-chemical and drug release characteristics.

Keywords: Nanoemulgel, Surfactant, Co-Surfactant Patrice size, Poly dispersity index, Zeta potential.

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INTRODUCTION

Skin, the largest organ covering the entire body is hostile towards microorganisms and environmental elements such as heat, invasion of toxins, chemicals and loss of water from the body. Nowadays dermatological disorders and diseases are emerging and afflicting millions of people every day. History reveals that skin disorders are a result of various pathogenic micro-organisms, putting forward a number of challenges.[1] Diseases of the skin encompass a wide range of situations based on severity from being minute to life threatening. So, these disorders can be treated by administration of drugs topically on the infected area i.e., local delivery or by systemic application. The route of administration depends on the type and severity of the disease.[2]

The topical route of administration is the most opted route for dermatological disorders. It involves the direct application of the dosage forms containing API to the skin to produce a local effect of the drug. This route is generally preferred in order to treat local infections of the skin, especially fungal infections. The spread of fungal infections on the skin is increasing drastically, where about 40 million people around the world affected every year.

Percutaneous absorption of drug through the skin occurs via stratum corneum which is 10μm thick and is made up of dead, keratinized epidermal cells. It acts as a barrier to the permeation of drugs.[3] Therefore, the percutaneous absorption of drug molecules is a difficult task. The aim of the administration of the drug through the skin is for topical treatment of skin disorders or to absorb the drug transdermally into the systemic circulation.[4]

Most of the active pharmaceutical ingredients developed are lipophilic in nature. So, in order to administer lipophilic drugs by topical route the drugs are formulated into novel drug delivery systems such as Emulsified gel.[5] O/W systems entrap a lipophilic drug which gets captured into the oil phase and slowly released to the skin through the external phase.

Since the mid-1980's, emulsion-based gels have been gaining importance in topical drug delivery systems, one is concerned with the topical delivery of poorly water-soluble drugs, and emulgels serve as a better option. Emulgels have been used for

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the treatment of various viral, bacterial and fungal disorders of the skin.[6] Scientists have been studying various possibilities to improvise emulgels and develop a novel drug delivery system known as nanoemulgels. Nanoemulgels are dispersions of a nanoemulsion in a gel base. The particle size is in the range of 20-200 nm.

MATERIALS AND METHODS

Materials

Oxiconazole, a low soluble drug was the drug of choice and was obtained as a gift sample from Metrochem API Pvt. Ltd., Visakhapatnam, Tween 80, Propylene glycol, Carbapol 934P, Guar gum and Methylparaben were obtained from Yarrow Chem Ltd., Mumbai.

Methods

The formulation of a NEG includes a sequence of steps which include:

Step1: Solubility of drug in major components of NE

The vital components of NEG such as oil, surfactant and co-surfactant were chosen based on their potential to solubilize the active ingredient. The method includes addition of excess amount of Oxiconazole to 2 mL each of oil, surfactant and co-surfactant separately and kept in a water bath shaker for 72 hr at 25+2°C.[7] Then the equilibrated samples were centrifuged at 3000rpm for l5 min to separate undissolved Oxiconazole. Followed by filtration of supernatant using Whatman's filter paper and diluted with dichloromethane for quantification of by using UV Spectrophotometer at 231 nm. The oil, surfactant and co-surfactant that can solublize maximum quantity of the drug were used in the formulation of NE.[8]

Step 2: Screening of surfactant and Co-Surfactant for NE

The surfactant and co-surfactant were screened based on their emulsification ability. The emulsification ability of the surfactants was determined by the addition of 300 mg of the surfactant to 300 mg of oi1 and homogenized. 50mg of the homogenized mixture was taken and diluted up to 50 mL with double distilled water to yield a fine emulsion and was observed for any turbidity. Then the emulsion was left aside for about 2 hr and the transmittance was assessed using a UV Spectrophotometer at 231 nm.[9]

Secondly, the co-surfactants were assessed for their emulsification ability by preparing a mixture of 100 mg of co-surfactant and 200 mg of selected oil. The mixture was then evaluated in the same way as that of the surfactant.

Step 3: Aqueous titration method

The selected surfactant and co-surfactant were homogenized in different ratios i.e., 1:1, 1:2, 1:3, 2:1 and 3:1 to generate S_{mix}. Then oi1is integrated with various combinations of S_{mix} in divergent combinations from 1:9 to 9:1 and titrated against water slowly. The selection of ratios is based on an increase in the concentration of surfactant to co-surfactant and vice versa to construct phase diagrams.[10] The volume of titrant run down was noted down when a clear mixture was obtained. After equilibrium was achieved the mixture was observed for transparency. Those compositions that were visually clear, and isotropic can be observed for the nanoemulsion region.

Step 4: Construction of Pseudo ternary phase diagram

The nanoemulsion region was obtained by constructing Pseudo ternary phase diagrams. Oil, S_{mix} and water were taken on the three axes. The ratio in which combinations of oil, S_{mix} and water show greater NE areas are screened to prepare NE's.[11]

Step 5: Preparation of Nanoemulsion

Calculated quantities of oil, S_{mix} and water were utilized to formulate NE using High speed homogenization method. The calculated quantity of oil was taken; drug was added and mixed thoroughly. Then S_{mix} was added and homogenized for l5 min at 3000 rpm. Finally measured amount of water is transferred drop wise and is homogenized once again at 3000rpm for 15 min.[12]

The prepared NE's were optimized using statistical method

A 3 factor 3 levels Box-Behnken statistical Design Expert Software was used in a quest to explore quadratic response surfaces and construct a second-order polynomial equation for optimization of nanoemulsion system because of its accuracy and high efficiency.[13]

Statistical evaluation of main effects, quadratic effects and interaction effects of various independent variables such as particle size, polydispersity index and Zeta potential was made. A design matrix comprising 15 experimental runs was constructed. Thus, the obtained non-linear equation is in a quadratic model and is given by,

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

Where y is the dependent or measured response of dependent variables,

α_0=intercept,

α_1 to α_9=Regression coefficient.

Step 6: Transformation of nanoemulsion into nanoemulgel

The distribution of oil droplets into a gel base increases the stability of a nanoemulsion because of the enhanced affinity of the drug towards oil. Therefore, NE was transformed into NEG by incorporation of the optimized NE into a suitable gel base. The gel base is prepared by soaking the gelling agent in water and left aside overnight.

Evaluation

NEG's are thus evaluated for the following factors

Appearance and pH

The formulated NEGs were evaluated by a vision for their appearance. pH of the NEG was studied to know the stability of the product. pH was determined thrice using a digital pH meter and the average results were taken into consideration.[14]

Electrical Conductivity

Electrical conductivity measured using a conductivity meter is used to study the structure and phase behavior of NEG systems.

Viscosity

Brookfield Rotational Viscometer was used to record the rheological behaviour of the formulations. It was measured three times, and the average was taken into consideration.[15]

Drug content

The drug content in the formulation was determined UV-Spectrophotometrically. 0.5 mg of each formulation was weighed accurately and diluted to 5 mL with methanol. Resultant solutions were analyzed spectroscopically following dilution if necessary.

Drug loading efficiency was calculated by equation

Refractive index

The refractive index of prepared NEG's was studied using Abbe's refractometer.

Particle size and polydispersity index

HPLC-grade water was used to dilute the formulation in the ratio1:100 (*v/v*) in a volumetric flask (100 mL) and gently mixed by overturning the flask. Malvern Zeta sizer Nano-ZS instrument that works on the principle of photon correlation spectroscopy was used to determine particle size and PDI of the diluted formulations.[16] All studies were repeated three times.

Zeta potential

The stability of a NE is a result of magnitude of the surface charge. Laser diffraction analysis performed using a Zeta- sizer helps to determine Zeta potential. The process involves dilution of the samples with water that is distilled twice in a ratio of 1:50 (v/v) and blended with a magnetic stirrer to ensure homogeneity.[17] On complete dispersion; aliquots were transferred into a folded capillary cell and loaded into the apparatus for investigation. All studies were repeated in triplicate at 25°C.[18]

Heating cooling cycle

To identify any instability (phase separation) 6 heating-cooling cycles were performed between 4°C and 40°C at each temperature for not less than 48 hr.

Centrifugation

Centrifugation, used to study the existence of instability was performed using a laboratory centrifuge operated at 3000 rpm for 15 min. The formulation that remains stable was optimized for further studies.

Swelling index

Approximately 1 g of nanoemulgel was taken on porous aluminium foil and placed on l0 mL of 0.1N NaOH solution. At fixed time intervals the weight of the sample was removed and its weight was noted until a constant weight was achieved.[19]

Spreadability

The therapeutic efficacy of the NEGs is dependent on its ability to spread. The spreading coefficient is related to the viscosity of the formulation. It was measured by placing 1g of the NEG in a circle of diameter 1 cm drawn on the glass plate. A second glass plate was used to cover the glass plate. About 125 g of the formulation was made to rest onto the covered plate for 5 min and the extension in diameter of the formulation due to spreadability was noted.[20]

Extrudability

Extrudability is the force required to remove the ge1 from the tube. The quantity of gel extruded from collapsible tube was determined by the application of weight. 0.5 g of NEG should be extruded in the form of ribbon from the Aluminium tube in 10 sec. The greater the quantity of gel extruded, the better the extrudability whereas viscosity is inversely related to extrudability, i.e., a less viscous preparation is easily extrudable.[21] Therefore, extrudability is generally measured in triplicate and calculated using,

In vitro drug release studies

Franz diffusion cell consisting of a dialysis membrane mounted in between the benefactor and receptor compartments is the most reliable apparatus for studying the permeation of drug via skin. The sample was employed onto the dialysis membrane and the receptor compartment was filled with pH buffer. The quantity of drug released *in vitro* was estimated by introverting 1 mL of the buffer at specified time intervals and restoring with an equivalent volume of buffer.[22] The introverted samples were analyzed with UV-visible spectroscopy to study the drug release.

Anti-fungal activity

The agar well diffusion method using Sabouraud's agar medium was used to study Anti-fungal activity. A measured quantity of organisms was added to the medium and blended precisely. Then about 2 mL of the suspension was transferred into the petri dish aseptically and left aside to solidify. A sterile cork borer was used to prick wells in the agar plates. The pricked wells were then pervaded with optimized sample of nanoemulgel and incubated for 72 hr at 18-24°C.[23] Later, the petri dish was observed to study fungal growth if and the zone of inhibition was measured.

RESULTS

Screening of components

To develop Oxiconazole nanoemulgel for topical application, the drug should possess maximum solubility in oil, emulsifier and co-emulsifier, because solubilized drug permeates via skin more efficiently. The drug's solubility in various components was studied and observed that it showed the highest solubility in Clove oil, an essential oil, possessing mild anti-fungal properties.[24] Therefore, Tween 80 and Propylene Glycol were selected as surfactant and co-surfactant respectively based on solubility studies. The results were depicted in Figure 1.

Pseudo-ternary phase diagram

CHEMIX school 10 software was employed for constructing pseudo-ternary phase diagrams. Hence the constructed phase diagrams were represented in Figure 2

Application of response surface methodology for optimization of NE

ANOVA, used for determining the significance of the 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). The lack of fit test applied to analyze data variation was insignificant having a *p* value >0.05. The multiple correlation coefficients (R^2) that express the amount of variation should be near 1.[25]

Particle Size

The particle size was found to be between 121.26±0.4 to 154.33±0.6. The study of particle size of the prepared formulations resulted in globule size diminution with increase in the quantity of surfactant and a decreased ratio of oil.

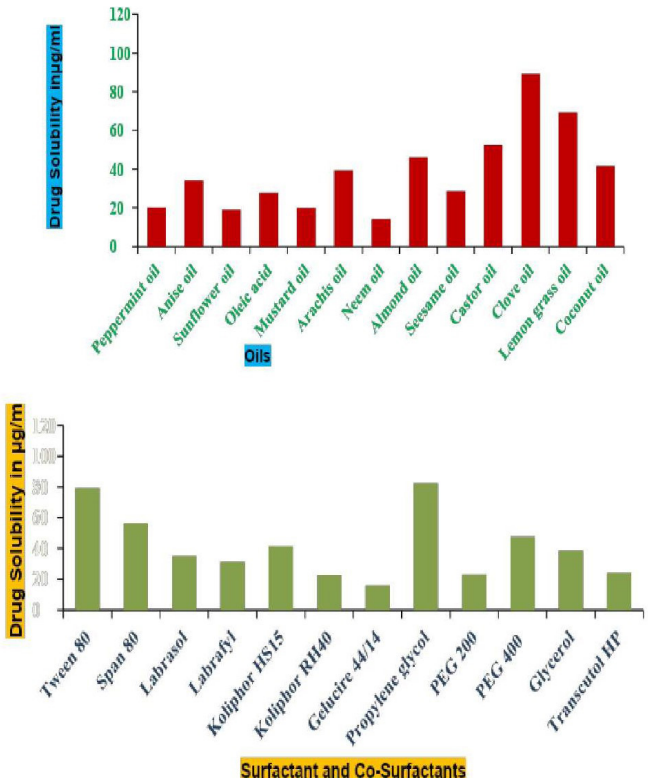

Figure 1: Solubility of Oxiconazole in oils, surfactants and co-surfactants.

Table 1: Evaluation parameters of formulations FO1 to FO6.

Evaluation Factor	FO1	FO2	FO3	FO4	FO5	FO6
pH	4.0±0.4	5.47±0.02	4.36±0.5	4.52±0.6	5.28±0.4	4.41±0.3
Electrical conductivity	4.84±0.05	4.75±0.02	4.78±0.04	4.96±0.02	4.58±0.03	4.87±0.05
Refractive Index	1.36±0.04	1.39±0.03	1.38±0.05	1.36±0.02	1.37±0.04	1.35±0.05
Centrifugation	Pass	Pass	Pass	Pass	Pass	Pass
Heating cooling cycle	Pass	Pass	Pass	Pass	Pass	Pass
Drug content Estimation	68.3±0.3	82.4±0.2	73.5±0.4	76.3±0.4	81.4±0.5	62.6±0.3
Swelling index	10.54±0.3	11.21±0.2	14.35±0.4	13.18±0.2	18.83±0.4	17.65±0.2
Particle size (nm)	133.32±0.2	121.26±0.4	137.98±0.3	123.88±0.5	122.13±0.2	154.33±0.6
Zeta potential	-28.759±0.2	-30.21±0.4	-31.48±0.2	-30.46±0.1	-29.35±0.3	-26.45±0.4
Polydispersity Index	0.55±0.05	0.59±0.03	0.440±0.02	0.733±0.05	0.440±0.04	0.479±0.02

Table 2: Drug release studies (in vitro) of the formulations FO1 to FO6.

Time (hr)	FO1	FO2	FO3	FO4	FO5	FO6
0.25	11.12±0.39	18.32±0.36	16.23±0.15	8.31±0.44	7.42±0.3	7.45±0.89
0.5	17.15±0.65	22.60±0.34	24.85±0.41	13.16±0.56	13.18±0.50	10.68±0.65
0.75	21.81±0.45	27.34±0.75	32.13±0.75	18.42±0.44	16.54±0.18	13.32±0.44
1	28.62±0.58	38.26±0.54	38.32±0.45	24.56±0.47	20.38±0.48	18.43±0.49
2	40.25±0.77	48.19±0.89	46.67±0.89	28.55±0.36	25.30±0.24	24.19±0.56
3	48.59±0.74	55.29±0.74	52.28±0.25	33.93±0.73	31.65±0.35	28.48±0.78
4	56.53±0.69	68.84±0.58	58.38±0.48	38.45±0.48	37.45±0.48	34.71±0.95
5	64.22±0.63	75.43±0.44	63.56±0.41	45.63±0.29	43.27±0.34	39.54±0.19
6	72.75±0.63	84.65±0.28	70.15±0.22	51.26±0.34	48.65±0.57	44.30±0.37
7	79.06±0.66	90.35±0.36	76.54±0.35	56.65±0.24	51.62±0.98	48.58±0.49
8	85.57±0.36	96.95±0.58	85.37±0.55	62.26±0.38	57.48±0.31	53.74±0.66
9	89.781±0.75		88.16±0.32	67.55±0.25	62.47±0.65	58.65±0.54
10	92.31±0.47		93.54±0.33	70.42±0.34	66.86±0.74	61.87±0.88
11	94.04±0.42			74.67±0.76	69.45±0.33	64.35±0.97
12	97.38±0.64			79.11±0.53	72.35±0.93	68.21±0.54

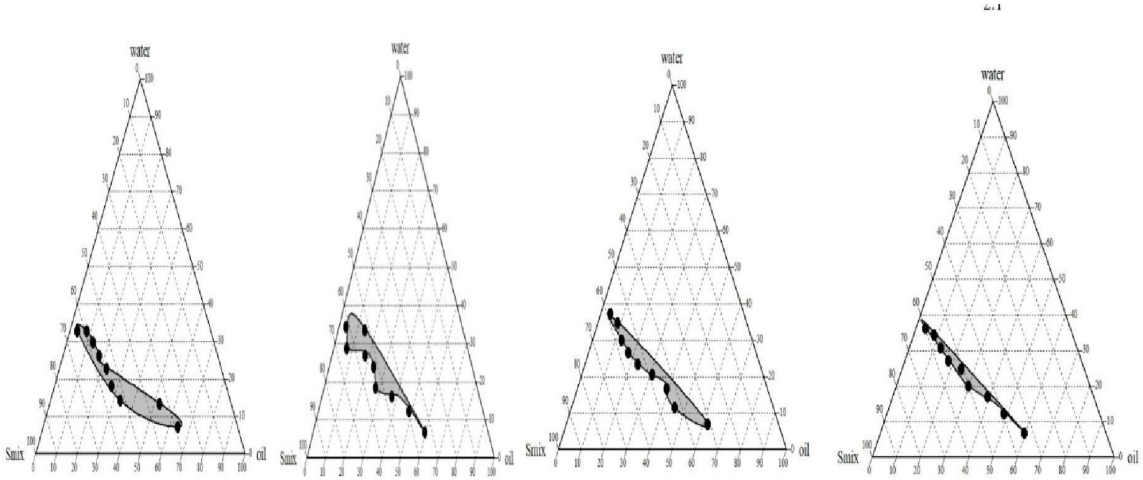

Figure 2: A) Pseudo ternary Phase diagram of S_{mix} ratio 1:1 B) Pseudo ternary Phase diagram of S_{mix} ratio 1:2. C) Pseudo ternary Phase diagram of S_{mix} ratio 1:3 D) Pseudo ternary Phase diagram of S_{mix} ratio 1:4.

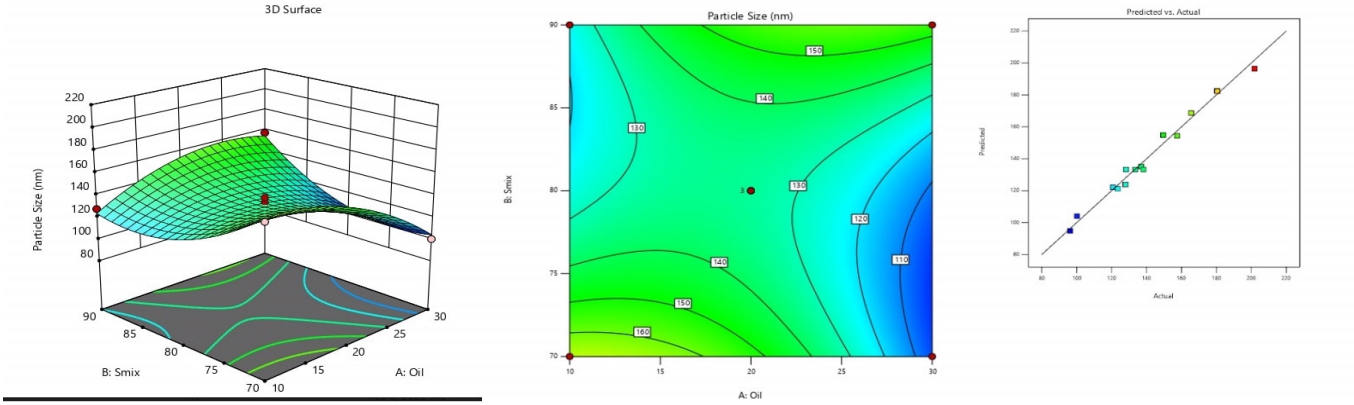

Figure 3: Response Surface Plot, Countour plot and Predicted Vs Actual plot showing the effect of Oil, S_{mix} (Tween 80 and Propylene glycol) and water on particle size.

Table 3: *In vitro* and *Ex vivo* drug release studies of optimized formulation

Time(hrs)	Optimized formulation (*In vitro* studies)	Optimized formulation (*Ex vivo* studies)
0.25	11.12±0.39	8.24±0.65
0.5	17.15±0.65	14.25±0.23
0.75	21.81±0.45	20.28±0.36
1	28.62±0.58	26.23±0.18
2	40.25±0.77	34.34±0.98
3	48.59±0.74	40.42±0.27
4	56.53±0.69	46.84±0.47
5	64.22±0.63	52.11±0.49
6	72.75±0.63	56.64±0.61
7	79.06±0.66	60.41±0.29
8	85.57±0.36	69.82±0.96
9	89.781±0.75	75.31±0.54
10	92.31±0.47	79.24±0.48
11	94.04±0.42	84.22±0.56
12	97.38±0.64	89.76±0.65

Table 4: *Ex vivo* drug release studies of optimized formulation and marketed formulation.

Time(hrs)	Optimized formulation	Marketed formulation
0.25	8.24±0.65	19.76 ± 0.37 6 ± 0.3
0.5	14.25±0.23	20.19 ± 0.24
0.75	20.28±0.36	25.83 ± 0.61
1	26.23±0.18	31.45 ± 0.45
2	34.34±0.98	39.68 ± 0.36
3	40.42±0.27	43.71 ± 0.17
4	46.84±0.47	47.58 ± 0.38
5	52.11±0.49	49.34 ± 0.72
6	56.64±0.61	54.65 ± 0.51
7	60.41±0.29	58.71 ± 0.26
8	69.82±0.96	63.16 ± 0.39
9	75.31±0.54	67.53 ± 0.46
10	79.24±0.48	68.84 ± 0.63
11	84.22±0.56	71.49 ± 0.12
12	89.76±0.65	73.56 ± 0.19

The independent factors Clove oil (X_1), Tween 80 (X_2) and Propylene glycol (X_3) resulted in various response variables of globule size (Y_1). Mathematical relationship for globule size in polynomial equation form is given by

$$Y_1 = 133.33 - 8.51X_1 + 1.38X_2 - 22.14X_3 + 23.77X_1X_2 + 21.70X_2X_3 + 8.46X_1X_3 - 16.26X_1^2 + 20.70X_2 + 13.15X_3^2$$

The equation shows effect of independent variables (X_1-X_3) and their interactions (co-efficient with more than one factor term, (X_1X_2, X_1X_3, X_2X_3) on the response Y_1. The obtained p value for the coefficient is <0.05 signifies collaborative effect whereas negative sign indicates opposite effect of independent variables on response. Greater co-efficient value of the factor indicates substantial effect on the response. All of the responses were fitted well into quadratic model and the efficiency of the model was substantiated by multiple correlation test (R^2) and ANOVA.[26]

The *p*, R^2 values were <0.05 and 0.7991, which proved that independent variables had a significant effect on predicting the response (Y_1). Multi co-linearity of the independent factors

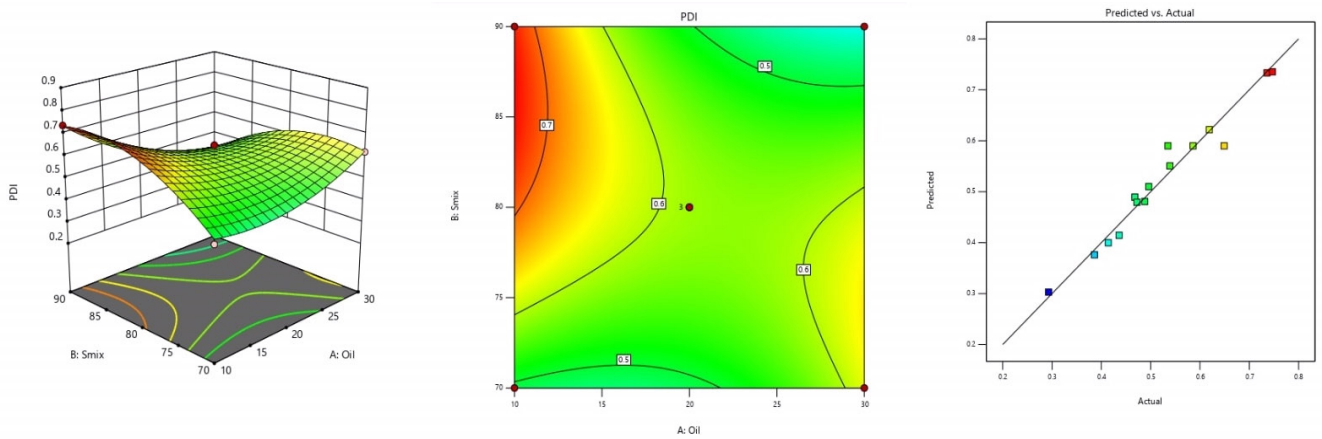

Figure 4: Response Surface Plot, Contour plot and Predicted vs. Actual plot showing the effect of Oil, S_{mix} (Tween 80 and Propylene glycol) and water on polydispersity index.

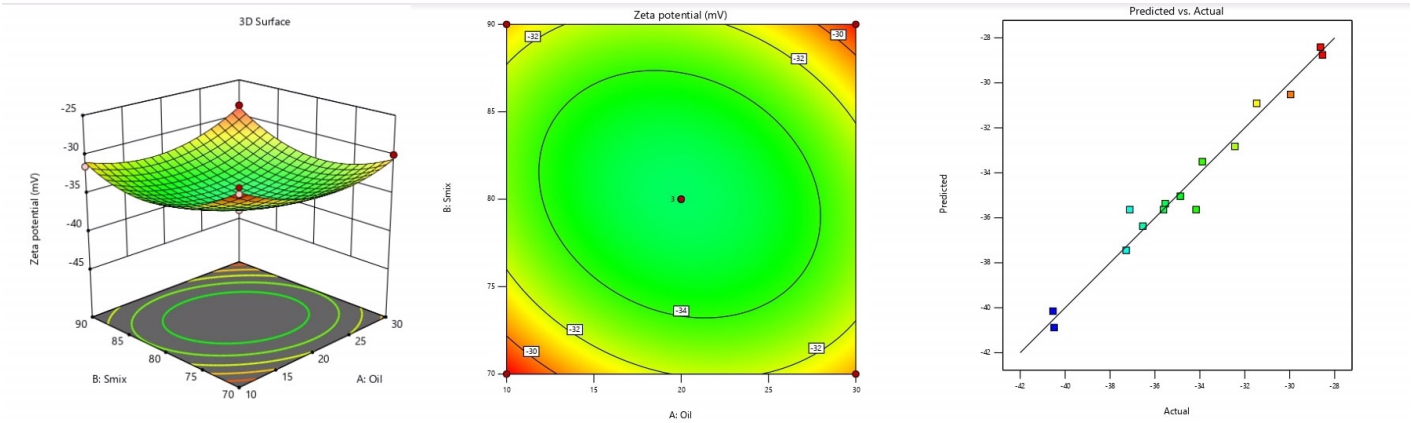

Figure 5: Response Surface Plot, Contour plot and Predicted Vs Actual plot showing the effect of Oil, S_{mix} (Tween 80 and Propylene glycol) and water on Zeta potential.

noticed that there was no multi-co linearity between independent variables (X_1-X_3) in quadratic model. VIF value less than 10 indicating 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

A dimensionless factor used to study particle size distribution is polydispersity index. PDI value between 0 to 1 describes the particles of the sample are almost of the same size and the preparation is monodisperse. A greater value represents a heterogeneous system having a wide range of particles. The PDI of the formulations were found to be in between. The quadratic equation for the measured response i.e. PDI is given by,

$$Y_2=0.5900-0.0466\ X_1+0.00920X_2-0.0459X_3-0.1127X_1X_2+0.0815\ X_2X_3-0.0577\ X_1X_3-0.0695X_1^2-0.0948X_2^2-0.0980X_3^2$$

The above equation is a polynomial determining the quantitative effect of independent variables on measured response (Y_2). The R^2 value of 0.7991 aligns with the adjusted R^2 value of 0.8947 and their difference is <0.2. Hence the above polynomial equation showed good fit towards the response variable (Y_2). The resultant equation for analysis of regression for Y_2 produced a negative sign for oil (X_1), a positive for variables X_2 and X_3. Therefore, the equation suggests that the PDI decreases with an increase in concentration of $S_{mix.}$ ANOVA study suggested that independent variable to a large extent affects prediction of response Y_3. Coefficient terms with p value <0.05 effect prediction efficacy of model significantly.[27] The result of contour plot, response surface plot and predicted Vs actual plot were given in the following Figure 4.

Zeta Potential

The Zeta potential values obtained for various preparations are in the range of -26.45±0.4 to-31.48±0.2mV. The quadratic equation for the measured response Zeta potential is given by:

$$Y_3 = -35.64-0.0125\ X_1+0.1862\ X_2+0.3513\ X_3+1.06\ X_1\ X_2+3.68\ X_2X_3-0.8525\ X_1\ X_3+2.60X_1^2+3.38X_2^{2-}3.81\ X_3^2$$

The above equation for zeta potential showed a good fit to the response variable (Y_3) as the R^2 of 0.8351 is in congruence with the adjusted R^2 of 0.9183. Regression analysis of Y_3 depicted negative signs for X_1 (oil) and positive signs for X_2 (surfactant) and X_3

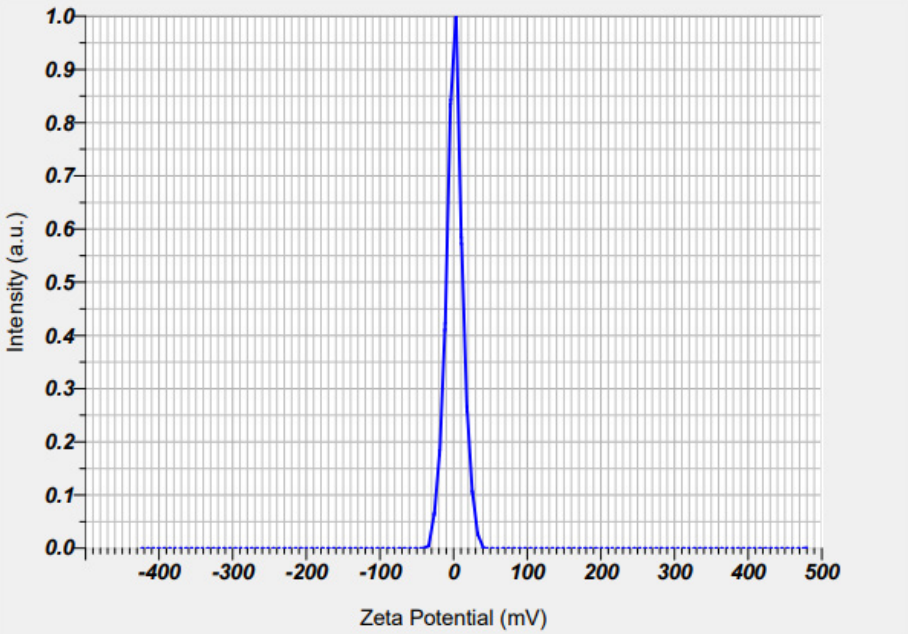

Figure 6: Zeta potential graph of the optimized formulation.

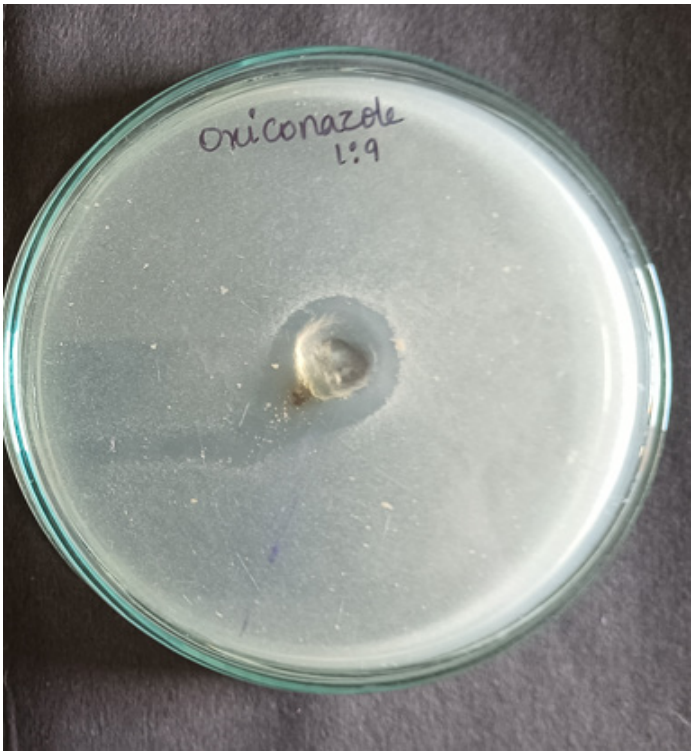

Figure 7: Anti-fungal activity of Itraconazole Nanoemulgel.

(co-surfactant). Decrease in the quantity of lipids and an increase in S_{mix} concentration, the zeta potential value decreases. The model equation generated by ANOVA proved that independent variables showed an effect on $p<0.05$ in predicting response (Y_3). F exhibited a Zeta potential value of -. The resultant response surface plot, contour plot and predicted Vs actual plot are given in the Figure 5. Zeta potential graph for optimized formulation was represented in Figure 6.

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

Thus, the optimized 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 were described below and tabulated in Table 1.

Appearance and pH: The prepared NEGs were clear and homogeneous possessing pH in the range of 4.0±0.4 to 4.52±0.6 which is in line with skin pH.

Electrical Conductivity: The conductivity values determined using a conductivity meter to describe the phase system of the NEG were in the range of 4.75±0.02 to 4.96±0.02.

Viscosity: The viscosity of the NEGs was found to be in the range of 23.29±0.05 to 34.46±0.03 cP.

Refractive index: The refractive index of NEGs was in between 1.35±0.05 to1.39±0.03 which is similar to that of water thus indicating the formation of a clear and transparent nanoemulgel.

Polydispersity index and Particle size: The mean particle size of nanoemulgel was in standard nm range and polydispersity index values were less than 1 describing the NEGs to be monodisperse.

Zeta Potential: Zeta potential data of the NEG describes the preparations to be stable.

Drug content release: The drug content release was determined by UV spectrophotometer for all the preparations and was found to be in the range of 62.6±0.3 to 82.4±0.2%.

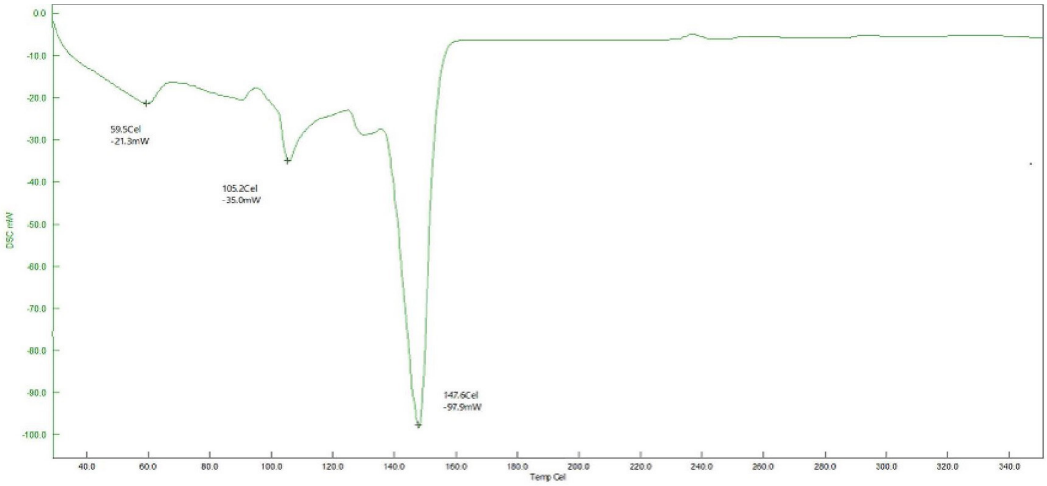

Figure 8: FTIR Spectra of Oxiconazole optimized formulation.

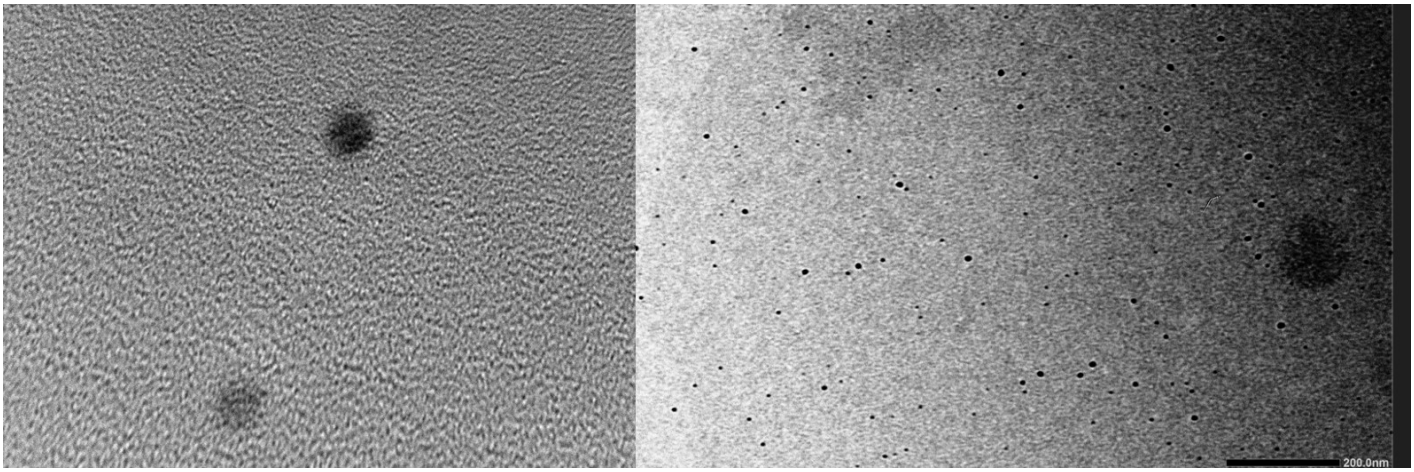

Figure 9: TEM images of optimized Oxiconazole nanoemulgel.

Physical Stability of NEGs: NEGs are kinetically stable when prepared at a definite proportion of oil. The stability confirmation tests like freeze-thaw cycle, heating cooling cycle and centrifugation did not show any phase separation, creaming, cracking or Ostwald ripening in optimized NEG.

Swelling index: %swelling index of the prepared NEGs was found to be between 10.54±0.3 to 18.83±0.4%.

Spreadability: The spreading coefficient values of the formulations ranged between 4.46 to 5.92 gcm/s. Formulations with greater viscosity has larger spreading co-efficient.

Extrudability: The extrudability of the formulations FO1 to FO6 in the range of 92.2±0.2 to 212.81±0.3g/cm^2. Formulation F has greater extrudability and least viscosity.

In vitro drug release studies

In vitro drug release studies were performed using pH 7.2 phosphate buffer in Franz diffusion cell. From these studies, it was observed that Formulation FO1 made containing 2% Carbopol 934P as the gelling agent released 97.38±0.84% of the drug release in 12 hrs. The *in vitro* and *ex vivo* data of optimised formulation is given in Table 3.

Drug release studies (*in vitro*) of the formulations FO1 to FO6 were given in Table 2.

Comparison between optimized and marketed products

From the *in vitro* drug release studies performed for selected Formulation (FO1) and branded.

In oxiconazole nitrate cream it was noted that FO1 released 97.38±0.64% of the drug release in 12 hrs whereas marketed formulation released only 73.56±0.19 in 12 hrs.

Ex vivo diffusion studies

The optimized preparation from *in vitro* study is further used for *ex vivo* studies with pH 7.2 phosphate buffer using a Franz diffusion cell. From these studies, it was noticed that optimized Formulation FO1 made containing 2% Carbopol 934P as the gelling agent released 93.40±0.39 of the drug release in 12 hrs. The *ex vivo* data was given in Table 4.

Antifungal activity

In vitro anti-fungal studies depicted in Figure 7 reveals that after 24 hr of incubation the formulation showed similar anti-fungal activity against *Candida* sps. The area of inhibition for the test preparations was about 16 mm.

Differential Scanning Calorimetry (DSC)

The thermal property of pure Oxiconazole and optimized formulation were studied by DSC. The spectra showed a sharp endotherm at melting point with respect to its melting point but when incorporated into the nanoemulgel the melting endotherm was significantly decreased to. 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 Oxiconazole. This was shown in Figure 8.

Transmission Electron Microscopy (TEM)

The morphology and size distribution of particles in nanoemulgel systems was obtained by performing TEM, as indicated in Figure 9. The particles in NEG system were round having a size range of 200nm or less.

CONCLUSION

From the above research, it was concluded that the prepared nanoemulgel of Oxiconazole 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 Oxiconazole.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

ABBREVIATIONS

NE: Nanoemulsion; **NEG:** Nanoemulgel; **DSC:** Differential Scanning Calorimetry, **FTIR:** Fourier Transform Infrared Spectroscopy; **TEM:** Transmission Electron Microscopy.

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