Available online on 15.01.2023 at http://jddtonline.info

Journal of Drug Delivery and Therapeutics

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Research Article

Formulation and Evaluation of Nanoemulgels for the Topical Drug Delivery of Posaconazole

Priyanka Priyadarshini[1], Preeti Karwa*[2], Ayesha Syed[3], A.N. Asha [2]

[1] *Research Student, Department of Pharmaceutics, Al-Ameen College of Pharmacy, Bengaluru-560027, Karnataka, India*

[2] *Professor, Department of Pharmaceutics, Al-Ameen College of Pharmacy, Bengaluru-560027, Karnataka, India*

[3] *Assistant professor, Department of Pharmaceutics, Al-Ameen College of Pharmacy, Bengaluru-560027, Karnataka, India*

Article Info:

Article History:

Received 08 Nov 2022
Reviewed 19 Dec 2022
Accepted 27 Dec 2022
Published 15 Jan 2023

Cite this article as:

Priyadarshini P, Karwa P, Syed A, Asha AN, Formulation and Evaluation of Nanoemulgels for the Topical Drug Delivery of Posaconazole, Journal of Drug Delivery and Therapeutics. 2023; 13(1):33-43

DOI: http://dx.doi.org/10.22270/jddt.v13i1.5896

*Address for Correspondence:

Dr. Preeti Karwa, Professor and Head, Department of Pharmaceutics, Al-Ameen College of Pharmacy, Hosur road, Bengaluru-560027. Karnataka (State), India

Abstract

Objective: Posaconazole(POS) is an antifungal drug widely used in treatment of fungal infections. However, the drug has very low oral bioavailability due to high first-pass metabolism, thereby limiting its therapeutic effectiveness. The present study is aimed to prepare nanoemulsion of posaconazole to increase its solubility and to develop posaconazole-loaded nanoemulsion based gel for single dose topical administration to sustain the release of the drug, improve patient compliance and avoid repeated administration.

Method:-FTIR-study was performed to determine compatibility of drug with excipients. Pseudoternary-phase diagram was constructed to optimize the Smix ratio. Optimization of nanoemulsion was done by Box-Behnken Design. The optimized formulation was evaluated for particle-size (nm), polydispersity index (PDI), (%) drug content and *in-vitro* drug release. POS-nanoemulgel (FNG) was developed using carbopol-934P (1%), which was evaluated for homogeneity, pH, viscosity, spreadability, percentage-yield and *in-vitro* drug release. The anti-fungal activity of POS-nanoemulgel was evaluated against *Candida Albicans*.

Results: The FTIR-study confirmed that there was no interaction between drug and excipients. The optimized selected nanoemulsion (FN1) exhibited particle size 79.19nm, PDI 0.357, drug content 95.90% and *in-vitro* release of 79.40% at 24 hour. The prepared nanoemulgel was homogenous, opaque with good spreadability 13.91±1.19, viscosity 3760±0.014, pH 5.6±0.06 and the zeta-potential was -29mV, indicating good stability. The drug release at 24h was 72.01%. Further, the developed nanoemulgel formulation exhibited better antifungal activity in comparison to the pure drug gel.

Conclusion: Hence, it can be concluded that nanoemulgel of posaconazole can provide better antifungal activity and improve patient compliance.

Keywords: Nanoemulsion, Nanoemulgel, Posaconazole, Anti-fungal, Box-Behnken Design, Pseudoternary phase diagram.

INTRODUCTION:

The most common cause of skin disease in most age groups is fungal skin infections. Fungal infection that occurs in human are invasive candidiasis, ringworms, *Mucor mycosis*, oral thrush etc., which can be superficial or severe.[1] These infections range from superficial rushes in the mucosa, in the skin or in the nails, to systemic infections, in which the fungal cells disseminate in the bloodstream and may end up colonizing any major internal organ. *Candida* species are among the more relevant etiological agent's causative of superficial and invasive fungal infections. *C.albicans* is an opportunistic pathogen that resides as a harmless commensal in the gut, genitourinary tract, and skin but it becomes an opportunistic pathogen under a number of different host conditions, usually involving reduced immune competence or an imbalance of the competing bacterial microflora. Though *Candida* is the leading cause of the superficial opportunistic infections but there is limited number of availabilities in the antifungal therapy.[2-6] Generally, amphotericin B–based preparations, Azole antifungal agents and Echinocandin antifungal agents are used in the treatment. However, the therapy for these infections is dominated by the azole antifungal agents. Several types of azoles include itraconazole, fluconazole, voriconazole and posaconazole. Among them the second generation anti-fungal triazole named posaconazole (POS) , a sterol C14α demethylase inhibitor with the formula $C_{37}H_{42}F_2N_8O_4$ exhibiting higher potency in *in-vitro* studies against broad range of fungal infections shares a common mechanism of action which involves inhibition of fungal cytochrome P-450 enzymes. These enzymes are required for successful synthesis of ergosterol, a critical component of the fungal cell wall. Henceforth, the depletion of ergosterol results in the fungistatic effect. Posaconazole is currently available mostly in oral and IV formulations. The oral suspension has satiable absorption and variable bioavailability that necessitate the administration in divided doses three to four times per day. The bioavailability is also strongly dependent on the concomitant intake of food, gut motility, and gastric

acidity. However, for chronic diseases the oral route is mostly preferable but due to its first-pass metabolism and low bioavailability nearly half of the medication is hampered.[7-10] The topical route for the management of skin disorders offers several advantages over the oral route and has tremendous potential for successful drug delivery. Topical dosage forms like ointments and cream have disadvantage like irritant to the skin, low spreadability on the skin and less stability[11-13]. Due to some of these drawbacks's preparation of nanoemulsion based gel formulation has taken over pharmaceutical as well as cosmeceutical industry. Nanoemulsion can be either O/W or W/O type of nano sized emulsion behaving like a dispersed system which is a promising alternative to improve drug delivery and solubility. Nanoemulgel is the incorporation of nano-emulsion into the gel matrix which on application releases the oil droplets from the gel, which penetrate the stratum corneum of the skin and delivers the drug to intended site. Further, patient compliance is improved due to increased spreadability, and decreased stickiness compared to creams and ointments. Furthermore, nanoemulsion based gels do not require any expensive sophisticated instruments for preparation and therefore the cost of preparation as well as time required for its preparation is less.[14-19] Hence, the study is aimed to prepare nanoemulsion of Posaconazole to increase its solubility and to develop Posaconazole-loaded nanoemulsion-based-gel for single dose topical administration to sustain the release of the drug, improve patient compliance and avoid repeated administration.

MATERIALS AND METHODS:

Materials:

Posaconazole was received from Mylan Laboratories Ltd as gift sample, Ethanol from Changsu Hong sheng Fine Chemicals Co. Ltd, Methanol, Sodium Hydroxide Pellets from Thomas Baker Chemicals, Oleic Acid from Finar chemicals, Almond Oil from Afghans bazaar pvt. Ltd., Transcutol P from TCI Pharmaceuticals Ltd, Carbopol 934P, Isopropyl Myristate, Tween 20, Propylene Glycol, Octane-1-ol LR from SD fine chemicals Ltd, Tween 80, PEG 400, Potassium Dihydrogen Phosphate from Finar chemicals.

Methodology:

Drug excipient interaction study by Fourier-transform infrared spectroscopy (FTIR):

Physical incompatibilities between drug (posaconazole) and excipients such as oil phase (oleic acid), surfactant (Tween 80), co-surfactant (Transcutol P) and Carbopol 934 P (gelling agent) were investigated by FTIR (Bruker-II) in the IR range of 400 to 4000 cm^{-1}.

Screening of oils, surfactants, and co-surfactants for nanoemulsion:

To find out the suitable oil, surfactant and co-surfactant, the solubility of posaconazole in various oils (oleic acid, almond oil, isopropyl Myristate), surfactants (Tween-20, Tween 80) and cosurfactants (PEG 400, propylene glycol, transcutol-P) were determined. 5 mg of Posaconazole was added in 5 mL of the selected oil, surfactant, and cosurfactant in stoppered vials (capacity 25.0 mL) and then was stirred continuously at 25 ± 0.5ºC for 48 hours to achieve equilibrium. The equilibrated samples were then centrifuged at 3,000 rpm for 15 minutes. The supernatant was separated, filtered and after appropriate dilution solubility was determined by UV Spectrophotometer at 226 nm.

Development of pseudo ternary phase diagram:

The existence of nanoemulsion regions was determined by using pseudo ternary phase diagram. Oleic acid as oil phase, Tween 80 as surfactant and Transcutol-P as co-surfactant were selected from the solubility studies. Pseudo ternary phase diagrams were constructed by titrating the blend of oil and surfactant: co-surfactant mixture (Smix) without drug by incremental amounts of water. Mixture of surfactant and co-surfactant were prepared in different weight ratios (1:1, 2:1, 3:1, 3:2, 4:1). Each Smix was mixed with oil in different weight ratios (oil: Smix) 1:9, 2:8, 3:7, 4:6, 5:5, 6:4, 7:3, 8:2 and 9:1. Each mixture of oil and Smix was subjected to vortex mixing to form homogenous mixture before titration with water. During titration the aqueous phase was added in drop wise until turbidity is formed. These values of oil, surfactant and co-surfactant were used to determine the boundaries of nanoemulsion region identified in the region of the phase diagram which showed the maximum and minimum levels of mixtures for the formulation of NE. The visual appearance of the homogenous mixture was noted down for clear and easily flowable o/w nanoemulsions.[20-21]

Preparation of nanoemulsion Formulation:-

Posaconazole loaded nanoemulsion was prepared by classic titration method by dissolving 1% POS in the oil and Smix ratio, preheated at 75˚C and vortexed for 5 minutes until the drug gets completely dissolved in the mixture. The aqueous phase was added slowly to this oil phase and vortexed for a period of 2 min. Then the whole system was cooled down to room temperature to get transparent nanoemulsion. The droplet size in the emulsion was further reduced by ultra-homogenization.

Optimization of formulation by Box Behnken design:

The optimization of nanoemulsion formulation was performed by three factors, three level Box Behnken Design (BBD)(Design Expert version-13). BBD might be a better option to reduce number of runs and to generate higher order response surface using fewer required runs than a normal factorial technique. The three variables; oil concentration as to Smix ratio (A), homogenization speed (B) and homogenization time (C) were selected as independent variables. The particle size (Y1), PDI (Y2) and % drug content (Y3) were selected as the dependent variables. Based on the design, 18 formulations were prepared. The formulations were coded as F1-F18. Optimization signifies how the responses change when the three factors are changed concurrently. The coded values of independent variables are shown in Table-1. Three-dimensional plots were generated to determine the effect of independent variables on the responses. It signifies the effect of independent variables on the responses by 3D graphical representation. Polynomial models including interactions were generated for all the response variables. Models were evolved by carrying out multiple regression analysis of the data and F- statistics to identify statistically significant terms.

Table 1: Coded values for independent variables

Coded Values	Actual Values		
	Oil: Smix	Homogenization Speed (rpm)	Homogenization Time (mins)
-1	3:7	10000	5
0	5:5	15000	10
+1	8:2	20000	15

Evaluation of optimized Nanoemulsion system:

Based on the desirability factor, optimized formulation (FN1) was formulated and evaluated.

Determination of particle size and PDI:

The estimation of particle size and PDI of FN1 was evaluated by Malvern particle size analyzer which works on the principle of light scattering technique.

Determination of drug content:

An estimated 1 ml of nano-emulsion was dissolved in pH 6.4 phosphate buffer. The solution was then filtered and suitably diluted, and the resulting solution was analyzed at 226nm in the UV-visible Spectrophotometer.

Determination of Percentage transmittance:

To determine transparency, % transmittance was measured on a UV spectrophotometer. All nanoemulsion formulations were diluted 50 times with distilled water. Then the percent transmittance was measured at 650 nm in a UV spectrophotometer using distilled water as blank.

Determination of *In-vitro* drug release of Nanoemulsion:

In-vitro drug release of POS loaded nanoemulsion (FN1) was performed by Franz diffusion cell. Nanoemulsion equivalent to 1g was taken in the donor compartment and the receptor compartment was filled with pH 6.4 phosphate buffer as the receptor medium. Dialysis membrane (molecular weight cut off 12000-14000 Da, (Hi-Media, India) was first washed with running water for 10 mins, then immersed in releasing medium (0.1 N HCl) for 24 h. The donor and the receptor compartment were separated by activated dialysis membrane. The media in the receptor compartment was stirred on a magnetic stirrer at 50 RPM maintained at 37±0.5°C. Approximately, 5 mL of sample was withdrawn from receiver solution at different time intervals and the diffusion cell was replenished to their marked volumes with fresh buffer solution. Obtained solution was analyzed for the % of drug release.

Preparation of Nanoemulgel Formulation:

Carbopol 934 P was selected as gelling base based on the preliminary screening studies to obtain a suitable formulation for topical application. The selected optimized nanoemulsion was incorporated into gel base. The Nanoemulgel of posaconazole (FNG) was prepared in three steps.

Step 1: Preparation of posaconazole optimized Nanoemulsion (FN1) as given by the design software.

Step 2: Preparing gel formulation by dispersing 1% Carbopol 934P in distilled water and was stirred using magnetic stirrer for 30 mins then allowing it to hydrate and swell for 6 hours.

Step 3: The nanoemulsion and gel formulation were mixed in the ratio 1:10. The pH of the resulting formulations was adjusted with triethanolamine and the nanoemulgel formulation data is given in Table-2.

Table 2: Formulation values of nanoemulgel.

Ingredients	Nanoemulgel formulation
Nanoemulsion	10 mL containing 1g POS
Carbopol 934 P	1%
Water	q.s to 100mL
Triethanolamine	0.5%

Evaluation of nanoemulgel formulations:

Determination of homogeneity:

The prepared gels were visually inspected for clarity and color. The prepared gels were also evaluated for the presence of any particles. Smears of gels were prepared on glass slide and observed under the microscope for the presence of any particle or grittiness.

Determination of pH:

The pH of the prepared nanoemulgel was measured using digital pH meter (Micropro Gradmate). The average of three readings was taken.

Determination of viscosity:

The viscosity of gels is dependent on the type and concentration of polymer used. The viscosity of different Nanoemulgel was determined using a Brookfield digital rheometer (DV III+) with spindle #7 at 200 RPM with torque ranging from 10-100%. Average of three determinations was recorded.

Determination of Zeta potential:

The zeta potential of nanoemulsion was determined using nano zeta sizer Horiba scientific SZ-100.

Determination of Spreadability:

Spreadability of the gels was determined by glass slides and wooden block which was provided by a pulley at one end. A ground glass slide was fixed on this block. An excess of gel (about 1 gm) of different formulation were placed on the ground slide. The gel was then pressed between the same shaped slides. Excess of the gels was scrapped off from the edges. The top plate was then subjected to pull 20 gms, lesser the time taken for separation of two slides better the spreadability. Spreadability was then calculated using the following formula:

$$S = \frac{m\,X\,l}{t}$$

Where, S= Spreadability, m= weight tied to upper slides, l= length of the slides and t= time (sec) taken to travel the distance.

Determination of percentage yield of nanoemulgel formulations:

Weight of all the ingredients used was added up theoretically. The percentage yield was calculated by the formula.

$$\% YIELD = \frac{Practical\ Yield}{Theoretical\ Yield} X\ 100$$

***In-vitro* drug release study:**

In-vitro drug release study was determined with a Franz diffusion cells, using dialysis membrane molecular weight cut off 12000-14000 Da, (Hi-Media, India). This was mounted between both chambers of the Franz diffusion cell. The receiver chamber was filled with Phosphate buffer pH 6.4 as diffusion medium and the whole assembly was placed on magnetic stirrer with 50 rpm. 1 gm. of nanoemulgel was placed on the donor chamber equally distributed on the membrane. Samples was withdrawn from the receiver solution at predetermined time intervals of 1st hr., 2nd hr., 3rd hr., 4th hr., 5th hr., 6th hr., 7th hr., 8th hr., 24th hr., and the cell was replenished to their marked volumes with fresh buffer solution. The samples were filtered and analyzed for the % of drug release.

Modelling of Drug Release Kinetics:

The drug release data of the optimized nanoemulgel formulation was fitted into zero order, first order, Higuchi matrix, Hixson-Crowell model and Korsmeyer-Peppas model. Based on the goodness of fit test, the most appropriate model was selected.

Anti-fungal activity by well diffusion method to check the zone of inhibition:

The anti-fungal activity of the samples (Posaconazole nanoemulgel and pure drug gel) were evaluated in duplicates against organisms *Candida albicans* using Potato Dextrose Agar media as culture media.

Different aliquots of the sample were prepared by pipetting 10µL (100µg), 20µL (200µg), 30µL (300µg), 40µL (400µg) of marketed product, posaconazole nanoemulsion, pure drug gel and posaconazole nanoemulgel using Dimethyl sulfoxide (DMSO).

Arrangement of plates for zone of inhibition against organisms was done by taking approximately 25mL of the media and was poured into the sterilized petri plates and allowing it to solidify. 200µL *Candida albicans* was poured respectively on a agar plate and spread thoroughly using a plate spreader. Five wells measuring 0.6cm was made in each plates using the borer and 50µL of prepared samples were loaded into the respective wells and 50µL of DMSO was loaded in the middle well as control blank.

The plates were incubated at 25°C for 72h. Later, zone of inhibition was recorded in mm (Millimeter).

RESULTS AND DISCUSSION:

Compatibility Studies by FTIR:

FTIR techniques have been used to study the physical and chemical interactions between drugs and excipients. The FTIR spectra obtained for the pure Posaconazole showed the presence of its characteristic peaks(Table-14). The peaks observed were, O-H (2884 cm^{-1}), C=O (1644 cm^{-1}), I-F (1003 cm^{-1}), C-N (1290 cm^{-1}), C-O (1243 cm^{-1}), (1105 cm^{-1}) C-N (amine) and C-O (2° alcohol) (1243cm^{-1})(Table-3). IR spectras of pure drug posaconazole was found unchanged in the spectras of mixtures indicating no chemical interaction. Hence, it can be concluded that there is compatibility between the drug and the excipients used, as drug maintains its identity without undergoing any interaction with the excipients.

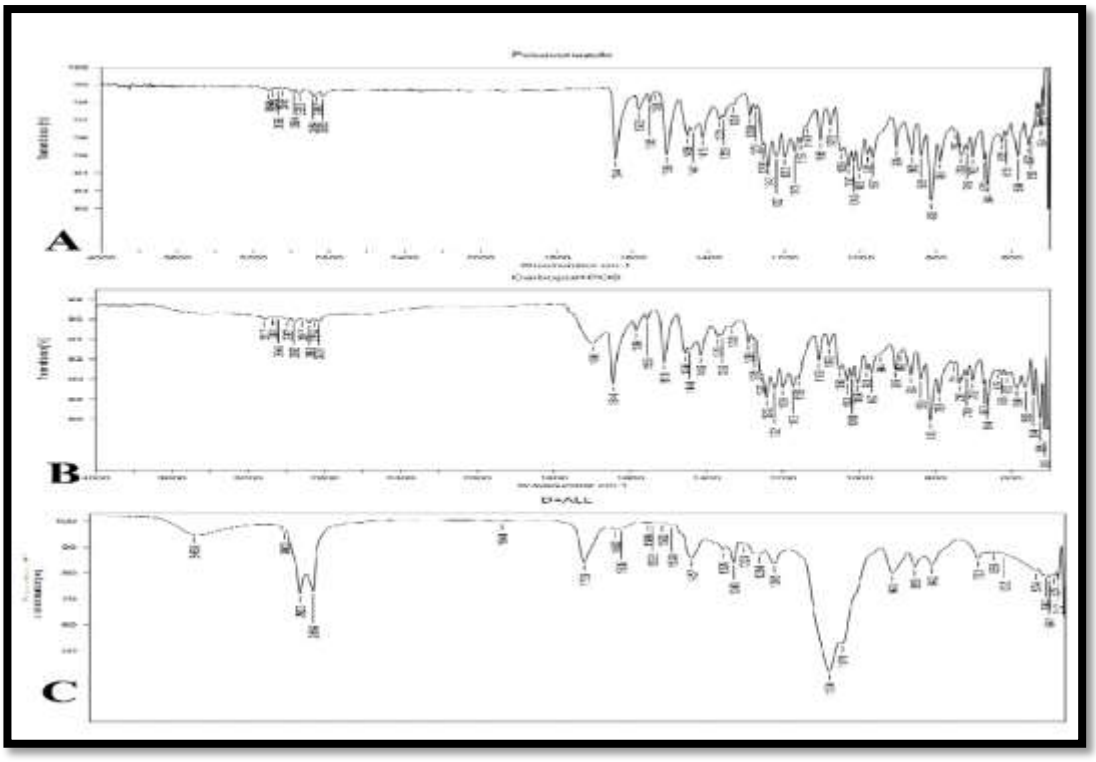

Figure 1: FTIR spectrums of Pure drug and POS with Carbopol and excipients.

Table 3: Characteristics peaks of FTIR of POS and Carbopol 934.

Functional groups	Theoretical Value	Pure drug Wave no. (cm^{-1})	Physical mixture Wave no. (cm^{-1})	POS+Carbopol 934 Wave no. (cm^{-1})
O-H Stretching	3200-2700	2884	2880	2923
C=O (3 amide) stretching	1680	1644	1644	1733
I-F (Fluro compound)	1400-1000	1003	1242	1104
C-N (aromatic amine)Stretching	1342-1266	1290	1290	1284
C-O (alkyl-aryl ether) stretching	1275-1200	1243	1242	1245
C-N (amine) Stretching	1250-1020	1243	1242	1104
C-O Stretching (2 alcohol) stretching	1124-1087	1105	1105	1104

Screening of oils, surfactants, and co-surfactants:

The solubility of Posaconazole in various oils, surfactants, and co-surfactants was determined as shown in the Table-4. Selection of the oil phase is the most important parameter when attempting to achieve a stabilized nanoemulsion with the maximum amount of solubilized drug with highest drug loading. Oleic acid was selected as an oil phase due to high solubility of POS (0.972mg/mL) because oleic acid has a 56%–84% of its total fatty acids and hydrogen bonding was found to be the main driver of the solubilization of API in fatty acids.[68] The HLB value is another significant criterion to choose the surfactant and co-surfactant. Tween 80 having good emulsifying activity with the HLB value of 15 was selected as surfactant, as it showed the highest solubility of POS (0.745 mg/mL). The high solubility of API can also be due to its non-ionic nature of the surfactant which usually found to be least affected by the changes in ionic strength and pH. Similarly, Transcutol P is a good emulsifying agent and penetration enhancer with HLB value of 4.2. It was selected as co-surfactant with highest solubility of POS (0.720 mg/mL).

Table 4: Solubility of Posaconazole in various oils, surfactants, and cosurfactants.

Sl.no.	Components	Use In Nanoemulsion	Solubility (mg/mL)
1	Oleic acid	Oil	0.97291
2	Almond oil	Oil	0.69583
3	Isopropyl Myristate	Oil	0.49583
4	Tween 20	Surfactant	0.72083
5	Tween 80	Surfactant	0.74583
6	PEG 400	Co-surfactant	0.69166
7	Propylene glycol	Co-surfactant	0.50208
8	Transcutol-P	Co-surfactant	0.72083

Development of Pseudoternary phase diagram:

In order to find the optimum nanoemulsion components, pseudoternary phase diagrams were constructed by titrating the blend of oil and Smix without drug by an incremental amount of water. Ternary phase diagram was constructed by selecting Oleic acid as oil phase, Tween 80 as surfactant, Transcutol P as co-surfactant and water as aqueous phase. It was constructed by performing aqueous titration. The area outside the frame indicates a turbid region with multiphase systems. It could be noted that the area of nanoemulsion region was considerably large since Transcutol P acted as a cosurfactant and interacted with the tween 80 as surfactant to increase the flexibility of the interfacial film. Smix were prepared with different weight ratios of surfactant and co-surfactant (1:1, 3:1, 3:2, 4:1) (Figure-2). Each Smix was mixed with oil in different weight ratios (oil: Smix) 1:9, 2:8, 3:7, 4:6, 5:5, 6:4, 7:3, 8:2, 9:1 vortexed and titrated with an aqueous medium until turbidity is formed. The change in composition and visual characterization upon incremental addition of water was noted (Figure-3) and the values were used to determine the boundaries of the nanoemulsion region. The existence of large or small nanoemulsion region depends on the capability of a particular surfactant or co-surfactant mixture to solubilize the oil phase. The extent of solubilization results in a greater area with clearer and homogenous solution. It was seen that when the surfactant (Tween 80) was used alone, the oil phase was solubilized to a lesser extent implying that surfactant alone was not able to reduce the interfacial tension of the oil droplets to a sufficiently low level and thus was not able to reduce the free energy of the system to an ultralow level desired to produce nanoemulsion. When a co-surfactant Transcutol P was added, the interfacial tension was reduced to a very low level and very small free energy was achieved which helps in larger nanoemulsion region. With further increase in surfactant from 1:1 to 3:1, further not much drop in interfacial tension and free energy was seen resulting in decreased region of nanoemulsion formation. Thereby, at Smix ratio 3:1, 3:2 and 4:1 clear nanoemulsion region reduction was observed. Thus, pseudoternary phase diagram indicated Smix ratio of 1:1 can be selected for the formulation of drug loaded nanoemulsion.[22]

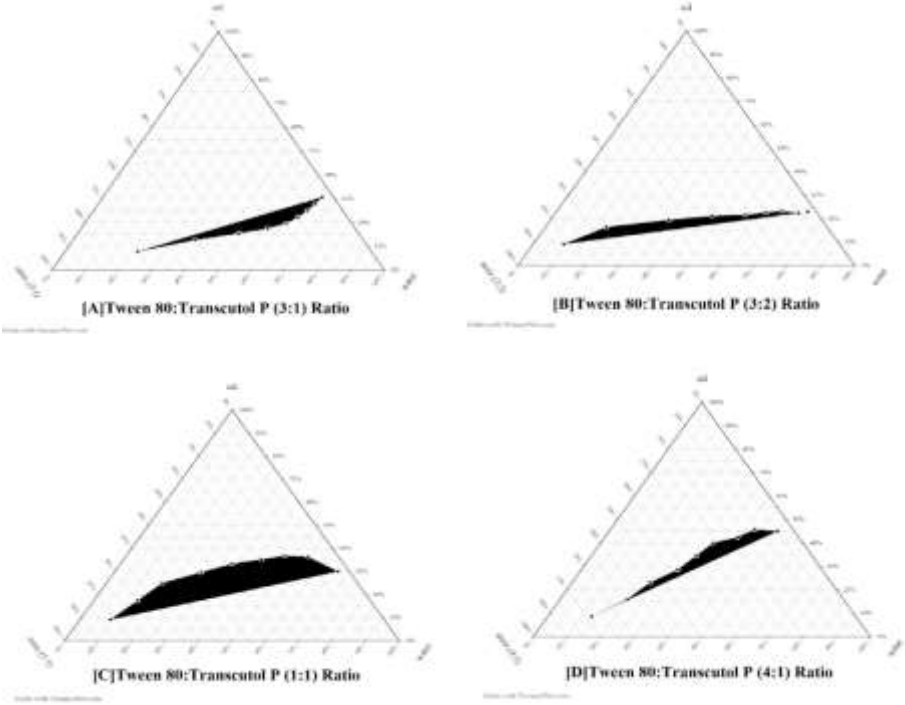

Figure 2: Pseudo ternary plot for Smix ratio A(3:1), B(3:2), C(1:1) and D(4:1)

Visual representation of chosen Smix ratio (1:1)

OIL: SMIX RATIO	OBSERVATIONS MADE AFTER EACH INCREMENT OF WATER DURING TITRATION.			
1:9	Nanoemulsion(NE)	E	E	E
2:8	NE	NE	NE	E
3:7	NE	NE	NE	G
4:6	NE	Emulsion(E)	Cloudy(C)	C
5:5	NE	NE	NE	G
6:4	NE	Gel(G)	G	E
7:3	NE	NE	NE	G
8:2	NE	NE	NE	G
9:1	NE	C	C	C

Visual representation of chosen Smix ratio (3:2)

OIL: SMIX RATIO	OBSERVATIONS MADE AFTER EACH INCREMENT OF WATER DURING TITRATION.			
1:9	-	NE	NE	E
2:8	NE	NE	G	-
3:7	NE	NE	G	-
4:6	NE	NE	NE	E
5:5	C	C	C	C
6:4	C	C	C	C
7:3	C	C	C	C
8:2	C	C	C	-
9:1	C	C	C	-

Visual representation of chosen Smix ratio (3:1)

OIL: SMIX RATIO	OBSERVATIONS MADE AFTER EACH INCREMENT OF WATER DURING TITRATION.			
1:9	E	Phase separation (PS)	PS	-
2:8	E	E	E	-
3:7	NE	NE	NE	-
4:6	NE	G	G	-
5:5	NE	G	G	-
6:4	G	G	G	-
7:3	C	E	E	-
8:2	NE	E	E	-
9:1	E	E	E	-

Visual representation of chosen Smix ratio (4:1)

OIL: SMIX RATIO	OBSERVATIONS MADE AFTER EACH INCREMENT OF WATER DURING TITRATION.			
1:9	NE	NE	PS	PS
2:8	NE	E	-	-
3:7	C	E		
4:6	C	E	-	-
5:5	NE	E	—	-
6:4	C	E	-	-
7:3	NE	E		-
8:2	NE	G	G	-
9:1	C	E	-	-

Figure 3: Visual representation of chosen Smix ratios

Optimization of nanoemulsion formulation by Box-Behnken Design:

Total of 18 runs were carried out by Box- Behnken design to study the effect of Oil:Smix ratio, homogenization speed and homogenization time on particle size, PDI and % drug content. The observed effect of responses are reported in Table-5[23].

Table 5: Experimental measured values for particle size, PDI and %drug content of nanoemulsion by BBD.

Code	oil: Smix	Homogenization Speed (rpm)	Homogenization Time (min)	Particle size (nm)	PDI	Drug content (%)
F1	-1	0	1	155	0.5	93.02
F2	-1	0	-1	104.2	0.7	95.13
F3	0	-1	-1	67.51	0.3	95.18
F4	0	1	1	117	0.5	94.34
F5	0	0	0	148	0.63	93.08
F6	0	1	-1	53.43	0.32	95.44
F7	1	1	0	98.4	0.2	95.08
F8	-1	1	0	143	0.2	93.11
F9	0	-1	1	138.1	0.4	93.18
F10	1	0	1	84.4	0.4	95.69
F11	0	0	0	126.3	0.53	93.58
F12	1	-1	0	127.2	0.492	93.24
F13	0	0	0	104.2	0.571	94.53
F14	0	0	0	123	0.655	93.94
F15	0	0	0	133	0.452	93.22
F16	-1	-1	0	92.65	0.432	95.83
F17	1	0	-1	114.8	0.433	94.53
F18	0	0	0	120.7	0.378	94.26

Data optimization:

For predicting the optimal point, a second-order polynomial model was fitted to correlate relationship between independent variables and response.

- *Effect of critical factors on particle size (A):*

The regression model for predicting the particles size was found to be statistically significant based on the results obtained from ANOVA. The F-value (3.33) of model was significantly higher with P value < 0.05. The lack-of-fit was insignificant (F-value = 2.76, P value = 0.14). If a model shows a significant lack of fit, then the model does not fit well and lacks prediction efficiency. The p-value of the lack of fit test was higher (0.146) than the significance level (0.05). So, it indicates the non-significant lack of fit value that is desirable for an adequate model. The value of regression co-efficient, R^2 adjusted and R^2 predicted for the model was found to be 0.4509 and -0.4383 respectively where a negative sign implies that the overall mean may be a better predictor of the response than the current model.

The significance and effect of each independent variable and their interaction on the response were evaluated by analyzing the coefficient value. According to the polynomial equation [Y1 = +121.65 -8.76 *A -1.70 *B +19.32 *C -19.79 *AB -20.30 *AC -17.34 *C^2] the effect of A, B, AB, AC, C^2 is negative i.e., Oil:Smix ratio, Homogenization speed, interaction of [Oil:Smix ratio and homogenization speed], interaction of [Oil:Smix ratio and homogenization time] and homogenization time^2 increases; particle size decreases. The effect of C is positive i.e., homogenization time increases, particle size increases. Higher the F-value (lower the p value) of the variable higher will be its impact on response. Hence, the effect of homogenization time has significant impact on particle size(Table-6).

From the 3D response plots, the effect of Oil:Smix and homogenization speed (Figure-3A) and the effect of Oil:Smix and homogenization time (Figure-3B) on particle size suggested that as Oil:Smix ratio increases, particle size decreases due to its significant decrease in viscosity that leads to decreased droplet size. Further, as homogenization speed increases, particle size decreases and form smaller particle size and as homogenization time increases particle size increases because if force is induced for longer period of time, particles collide and increases in size.

- *Effect of critical factors on PDI (B):*

The regression model for predicting the PDI was found to be statistically significant based on the results obtained from ANOVA. The F-value (5.78) of model was found to be significantly higher (P value < 0.05) while the lack-of-fit was found to be insignificant (F-value = 1.21, P value = 0.4431). Further, the value of regression co-efficient, R^2 adjusted and R^2 predicted for the regression model were found to be 0.3599 and 0.1755 respectively, which suggest that the Predicted R^2 is in reasonable agreement with the Adjusted R^2 the difference is less than 0.2.

According to the polynomial equation [Y2 = +0.5249 -0.0505 *B -0.1694 *B^2] the effect of B, B^2 is negative i.e., homogenization speed increases PDI decreases. Whereas other factors are having no effect on PDI.

From the 3D response plots, the effect of Oil:Smix and homogenization speed (Figure-3C) on PDI suggested that Oil:Smix, homogenization time has no effect on PDI and as homogenization speed increases PDI decreases because homogenization speed helps to reduce droplet size and disperse it uniformly. The homogenization speed has the only impact on getting desired PDI whereas Oil:Smix ratio and

homogenization time is not significantly affecting PDI (Table-6).

- *Effect of critical factors on % drug content (C):*

The regression model for predicting the % drug content was found to be statistically significant based on the results obtained from ANOVA. The F-value (3.94) of model was found to be significantly higher (P value < 0.05) while the lack-of-fit was found to be insignificant (F-value = 1.95, P value = 0.2403). Further, the value of regression co-efficient, R^2 adjusted and R^2 predicted for the regression model were found to be 0.4635 and 0.2850 respectively, which suggest that The Predicted R^2 is in reasonable agreement with the Adjusted R^2 the difference is less than 0.2.

According to the polynomial equation [Y3 = +94.25 +0.1807 *A +0.0681 *B -0.5055 *C +1.14 *AB +0.8181 *AC] the effect of A, B, AB, AC is positive i.e., as Oil:Smix ratio, Homogenization speed, interaction of [Oil:Smix ratio and homogenization speed], interaction of [Oil:Smix ratio and homogenization time] increases; % drug content increases. The effect of C is negative i.e., as homogenization time increases, % drug content decreases. The coefficient of interaction of Oil:Smix and homogenization speed is significantly higher which indicates that increasing the ratio and speed will increase the yield of the response.

From the 3D response plots, the effect of Oil:Smix and homogenization speed (Figure-3D) and the effect of Oil:Smix and homogenization time (Figure-3E) on % drug content suggested that as Oil:Smix and homogenization speed increases, % drug content increases because Oil:Smix and homogenization speed both helps solubilizing the drug. Further as homogenization time increases % drug content decreases [24-25].

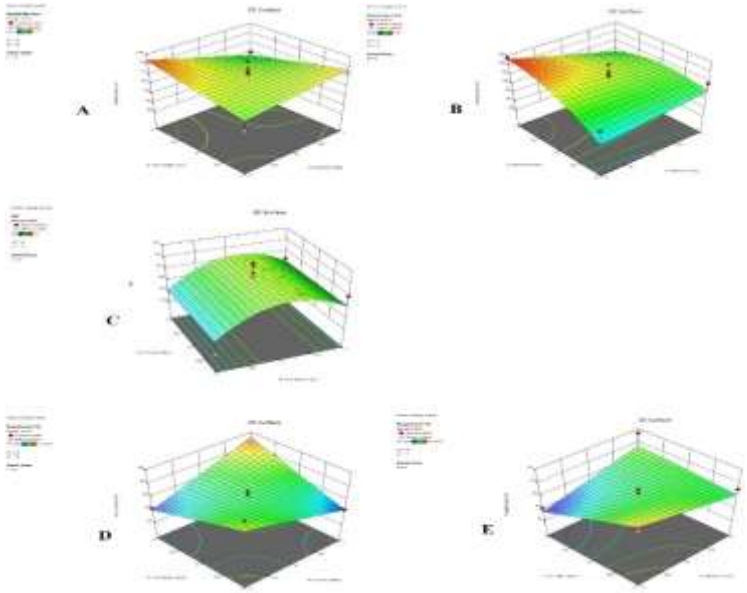

Figure 4: 3D Plot graph showing effect of factor on response particle size (A and B), PDI (C), and % drug content (D and E)

Table-6: Co-efficient value, *F*-value and corresponding *p*-value of independent variable for particle size, PDI and % drug content

Term	Coefficient	F-value	p-value
Particle size			
A-Oil:Smix	-8.76	1.50	0.2466
B-Hmz Speed	-1.70	0.0567	0.8162
C-Hmz Time	**+19.32**	**7.29**	**0.0206**
AB (interaction of Oil:Smix ratio and homogenization speed)	-19.79	3.82	0.0764
AC (interaction of Oil:Smix ratio and homogenization time)	-20.30	4.03	0.0700
C^2	-17.34	3.26	0.0983
PDI			
B-Hmz Speed	-0.0505	1.59	0.2260
B2	**-0.1694**	**9.96**	**0.0065**
% Drug Content			
A-Oil:Smix	+0.1807	0.5045	0.4911
B-Hmz Speed	+0.0681	0.0717	0.7935
C-Hmz Time	-0.5055	3.95	0.0702
AB (interaction of Oil:Smix ratio and homogenization speed)	**+1.14**	**9.99**	**0.0082**
AC (interaction of Oil:Smix ratio and homogenization time)	+0.8181	5.17	0.0421

Evaluation of optimized nanoemulsion:

The optimized nanoemulsion(FN1)was prepared based on the desirability factor. The factor Oil:Smix ratio was selected as the +1 coded value i.e., 8:2 ratio, the homogenization time and speed factor was also selected as +1 coded value i.e., 20,000 rpm for 15 mins. Particle size, PDI, pH, % drug content of the optimized FN1 formulation was evaluated and the data are shown in Table-7, whereas the transmittance percentage was found to be 98.164 which showed the good transparency of the optimized nanoemulsion and The *In vitro* drug release of formulations FN1 at the end of 24 hours was found to be 79.40% as shown in the Figure-5.

Table 7: Evaluation of Nanoemulsion (FN1)

Optimized formulation (FN1)	Particle size (nm)	PDI	pH ± SD	% Drug Content ± SD
	79.19	0.357	5.625 ± 0.30	95.90 ± 0.05

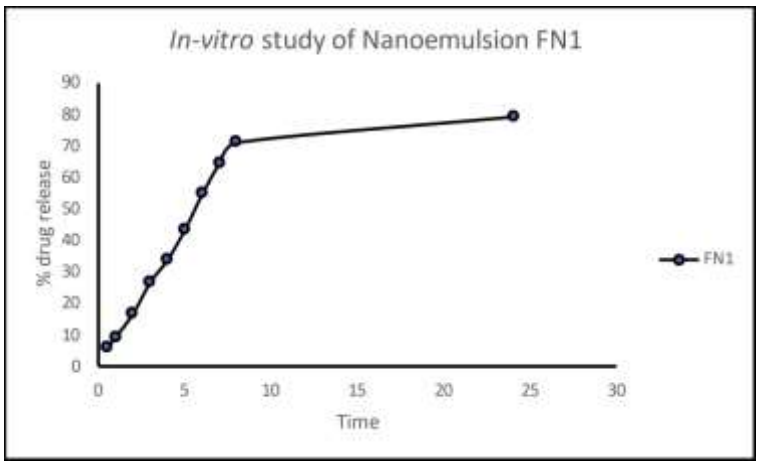

Figure 5: In-vitro drug release study for Nanoemulsion (FN1).

Evaluation of nanoemulgel formulation:

The prepared nanoemulgel (FNG) formulation was evaluated for homogeneity, which was visually inspected for clarity and color. The pH of the formulation was found to be 5.6±0.06 (Table-8) which is in the range of 4.5-7 mimicking the skin pH for topical use. If the pH of the gel goes beyond 7, it reaches to alkaline state which irritates the skin. The viscosity and spreadability was found to be 3760±0.014 cps and 13.91±1.19 respectively (Table-8) indicating ease of application. Further zeta potential was found to be -29.3mV (Table-8) indicating that the FNG formulation is stable. The % drug release at the end of 24 hours was found to be 72.01% (Figure-6). Incorporating nanoemulsion into gel base showed no major difference in drug release, however the nanoemulgel provides better adhesion property to the surface of the skin, high solubilization capacity and better penetration due to its gel matrix.

Table 6: Evaluation of Nanoemulgel (FNG)

Formulation	Homogeneity	pH	Viscosity (cps)	Zeta potential (mV)	Spreadability (gm.cm/sec)	% Yield
FNG	Homogenous	5.6 ± 0.06	3760 ± 0.014	-23.9	13.91 ± 1.19	93

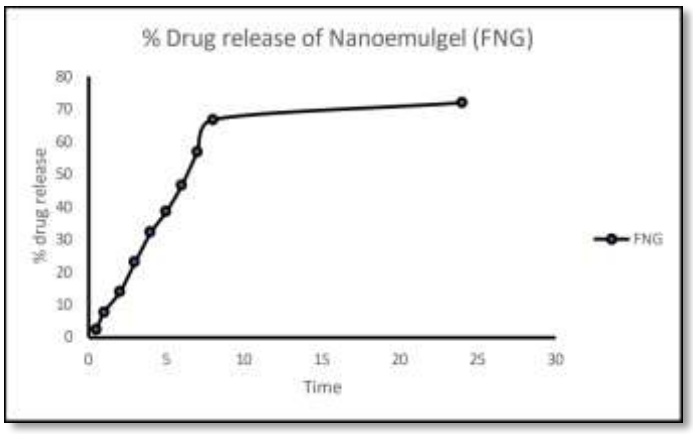

Figure 6: *In-vitro* drug release of Nanoemulgel (FNG) formulation.

Drug release kinetics:

To study the mechanism of drug release, the *in-vitro* drug release values were fitted in the kinetic models of zero order, first order, Higuchi matrix and Hixson-Crowell model where the R^2 was found to be 0.809, 0.7917, 0.9525 and 0.6689 respectively shown in (Table-9). FNG formulation followed zero order release with Higuchi matrix kinetics confirming the drug is released from the polymer gel matrix occur by diffusion mechanism. Moreover, from Korsmeyer Peppas model, 'n' value was found to be 0.95 indicating Case II transport for swellable polymers. Thus, it confirms zero order release with constant rate of drug diffusion due to solvent induced relaxation and swelling in the polymer.[26]

Table 9: Drug release kinetics of Nanoemulgel formulation.

Formulation	Zero Order	First Order	Higuchi Matrix	Korsmeyer Peppas		Hixson Crowell	Best Fit Model
FNG	R^2	R^2	R^2	R^2	n	R^2	Higuchi Matrix
	0.809	0.7917	0.9525	0.9194	0.9513	0.6689	

Anti-fungal Activity:

Anti-fungal activity of marketed product, POS-nanoemulsion (FN1), pure POS-gel and POS-nanoemulgel (FNG) were examined on the test organism *Candida albicans* (Figure-7). The zone of inhibition of FNG nanoemulgel (15±0.03mm) (Table-10) was found to be better in comparison to the marketed product (0 mm), POS nanoemulsion (13mm) and POS-gel (0 mm) (Table-10) indicating enhanced antifungal activity of the nanoemulgel formulation. This improvement could be attributed to posaconazole presence in a soluble form that made it easily diffuse to the medium of agar and affecting the fungus. The reduced particle size increases the penetration of the drug through *Candida albicans* cell membrane where it inhibits ergosterol synthesis.[27]

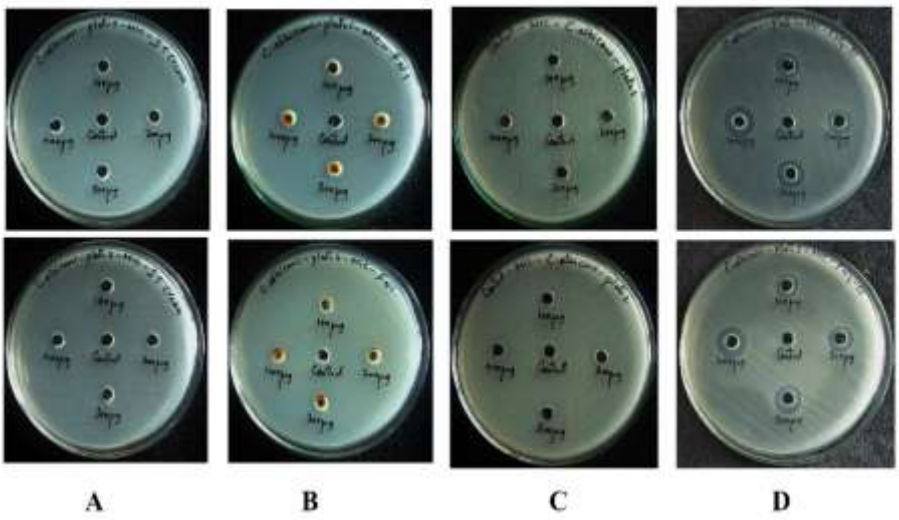

Figure 7: Minimum Zone of Inhibition of Marketed product [A], POS nanoemulsion [B], pure POS gel [C], POS nanoemulgel [D].

Table 10: MIC of Marketed product, POS-nanoemulsion, POS-Gel and POS-nanoemulgel against Pathogens(C.albicans).

Organism	Zone of inhibition of samples against *Pathogens* in mm							
	Concentration in µg							
	100 µg		200 µg		300 µg		400 µg	
Plate	1	2	1	2	1	2	1	2
Marketed product	-	-	-	-	-	-	-	-
POS nanoemulsion	-	-	10	-	12	12	13	13
POS gel	-	-	-	-	-	-	-	-
POS nanoemulgel	11	10	12	12	13	13	14	15

CONCLUSION:

The novel drug delivery system of the anti-fungal drug posaconazole was developed by assortment of the drug into Nanoemulsion formulation. The high solubilization of POS in oleic acid was helpful to encapsulate the solubilized drug in nanosized oil droplets with high loading efficiency. The oil-in-water nanoemulsion was uniformly distributed into the matrix of an aqueous hydrogel system and this formulation presented the highest solubility and nanoscale droplet size, narrow PDI, negative zeta potential indicating improved stability with sustained drug release. Further excellent antifungal activity of POS-Nanoemulgel against *candida albicans* was exhibited in comparison to the POS-nanoemulsion, POS-gel and marketed product. Hence, based on the above study, it can be concluded that POS-Nanoemulgel can be cost effective approach for safe and efficacious delivery of drug against skin fungal infections.

Acknowledgement:

We would like to thank the Principal, Head of the Department, and management of Al-Ameen college of Pharmacy for providing facilities in carrying out the research activity.

Conflict of interest:

There is no conflict of interest associated with this manuscript.

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