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Original Article

Statistical Optimization of Nano Emulsion-Based Gel of Efinaconazole for Onchomycosis

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ABSTRACT

Background: The objective of this study is to create a nano emulsion-based gel, of efinaconazole, a class II (BCS) antifungal medication for onchomycosis, by increasing solubility. **Materials and Methods:** Solubility studies in oils, surfactants were conducted. Medication and excipients compatibility was studied by FTIR analyses. Emulsion zone for certain emulgent, oil and water components was determined by Ternary phase diagrams. Statistical optimization by D-optimal design with oil phase (oleic acid), emulgent (Transcutol), water phase as X1, X2, X3 (respectively) dependent variables and responses drug content (R1), drug release percentage (R2) as independent variables. Physico-chemical parameters of nanoemulsion and gel were evaluated along with zeta potential for optimized formulation. **Results and Discussion:** Dependent variables, drug content (R1) follows reduced quadratic model and linear model for % drug release (R2) from lack of fit summary. The optimized nanoemulsion formulation NEP-17 containing Oil (31.29 mL), emulgent (58.70 mL), water (10.00 mL) showed 82.19±0.01% of drug release after 8 hr, drug content 100.7%, 229.7nm Globule size, PDI of 0.284, Zeta-potential of -42.1 mV was formulated into carbopol gel G2. It followed anomalous transport mechanism and was stable. Antifungal studies against *Candida albicans* and *Candida glabrata* of G2 were compared with marketed Luliconazole solution 1% (LUZU). **Conclusion:** The results suggest that nano emulsion gel has enhanced solubility to boost bioavailability.

Keywords: Antifungal agent, Carbopol, Efinaconazole, Emulgents, Luliconazole, Nanoemulsion.

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INTRODUCTION

A system with two immiscible phases is referred to as an emulsion, and it consists of an external phase that is continuous and an internal phase that is scattered as droplets. In order to properly transport the medicine, nanoemulsion as a topical drug delivery method faces various problems (Che Marzuki *et al.*, 2019). An essential nanoemulsion feature is rheology. The nanoemulsion formulation's low viscosity and spreadability make it difficult to employ. Therefore, the restriction has limited the use of nanoemulsion in therapeutic settings. Therefore, the strategy of combining a nanoemulsion with a gelling system can aid in solving this issue. Oil in water (O/W) or water in oil (W/O) nanoemulsions with gelling agent are gelled (Costa and Santos, 2017). Fungal infection of nail is referred to as onychomycosis or *Tinea ungium*. Nail discolouration (White or yellow), nail detachment and nail thickness are symptoms. Nails or fingernails may be impacted; however, toe nails are more frequently impacted. The lower leg could develop cellulitis as a complication. Onychomycosis can be brought on by a variety of fungi, including *Fusarium* and dermatophytes (Boonme *et al.*, 2009; Barry, 1991). Athlete's foot, other nail conditions, contact with others who have the ailment, peripheral vascular disease, and weakened immune system are risk factors (Kong *et al.*, 2011). Nail discoloration, which can be white, black, yellow, or green and thickening are most typical sign of a fungal nail infection. Worsening of infection results in nail brittleness, breaking off in parts or fully falling away from the toe or finger (Mason *et al.*, 2006). The fungal kingdom is home to dermatophytes, *Candida* (yeast), and non-dermatophyte moulds, which are the pathogens responsible for onychomycosis. While yeast and moulds are more typically implicated in the subtropics (hot-humid climate) and tropics, fungi dermatophytes are causative for onychomycosis in warmer western countries (Kale and Deore, 2017). The first topical triazole created especially for the treatment of onychomycosis is efinaconazole. *In vitro*, it exhibits strong antifungal activity against the most prevalent onychomycosis pathogens and a broad spectrum of activity (Talegaonkar *et al.*, 2008).

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Mixture design would be selected when the goal is the optimization of proportion or ratio of components and when factors are components of a mixture, the total of all components is a constant and response is a function of components (Souto *et al.*, 2022). If 2 or 3 mixture components are involved and the ranges of all components are the same then use a simplex lattice mixture design. If 3 or 5 components are involved and the ranges of all components are the same then simplex centroid design could be used. If 2-4 components are involved and the ranges of all components are different then use constrained mixture design also known as D-Optimal design (Costa *et al.*, 2014). In the present study statistical optimization of efinaconazole nanoemulsion was done by D-Optimal mixture design. Optimized nanoemulsion based gel for ease of application and anti-fungal studies against *Candida albicans* and *Candida glabrata* were studied for its efficacy.

MATERIALS AND METHODS

Efinaconazole is gifted from Suven life sciences, from moly chem manufacturer oleic acid is procured. From local market Olive oil, castor oil, and sunflower oil is obtained. Capmul MCM and captex is gifted from 200 ABITEC corporation. Tween 40, Tween 20, Tween 60, Tween 80, Span 80, and Span 20 is gifted from Sisco research laboratories Pvt. Ltd., PEG 400, PEG 600 is obtained from S.D fine chemicals Ltd., Methanol is procured formed Alfa Aesar-A Johnson Matthey Company. Transcutol is gifted from research Lab Fine Chem Industries. Neem oil is gifted from the chemical company, BASF. From S.D. Fine Chemicals Ltd., Hydrochloric acid and from In-house, Distilled water.

Solubility studies

Drug in excess with excipients was added to 2 mL Eppendorf tubes. The tubes were shaken for 48-72 hr to achieve a homogenous slurry in an orbital shaker. Then subjected to centrifugation for 10 min at 4500 rpm. Supernatant sample using a syringe filter were collected, diluted with water and analyzed by UV spectrophotometer (Tata *et al.*, 1997).

Drug-excipient compatibility study Interactions by FTIR

Compatibility studies by FTIR spectrophotometer were studied by Potassium bromide (KBr) disks method and the IR spectrum was recorded from 4000cm^{-1} to 500 cm^{-1} in a scan time of 12 min. The resultant spectrum was compared for any spectra changes.

Optimization using D-Optimal mixture design

Phase Titration Method

Pseudo-ternary phase diagrams of different weight ratios of oil: emulgent (1:9 to 9:1) from which the better formulations ratios were selected based on that the design was applied (Liu *et al.*, 2008; Gursoy and Benita, 2004). Oil was heated at 60°C in a beaker on continuous stirring, add a mixture of emulgent and drug with gradual stirring slowly dropwise to above mixture. On attaining room temperature, water drop wise was added with continuous stirring for diluting the sample to the total content, it results in formation of nano emulsion (Porras *et al.*, 2005; Uskokovic and Drofenik *et al.*, 2005). D-Optimal Mixture Design with levels minimum 20,20,10 and maximum 80,60,40 was used to analyse and construct quadratic response surfaces. These three parameters' levels were determined from preliminary trials. Drug content, R1, and drug release in 8 hr, R2, were set as dependent factors (Aucouturier *et al.*, 2002). The formulations were prepared by phase titration method according to Table 1, which was generated by Design Expert Version 11 software. In Table 1, 16 runs representing oil phase (component 1), emulgent (component 2), water (component 3), responses (Drug content, drug release) were given by the D-optimal mixture design.

EVALUATION PARAMETERS OF NANO EMULSION

Thermodynamic stability studies

The diluted nanoemulsion formulations were centrifuged for 15 min at 3500 rpm, and observed for any phase separation visually. They were subjected to six heating and cooling (4°C and 45°C) cycles, for 48 hr (Kumar and Singh, 2012).

Visual observation and phase separations

The contents were taken in a beaker and gently swirled magnetically at 100 rpm at room temperature. Later after 24 hr, the generated emulsion was inspected for precipitation (Montilla *et al.*, 2002).

Robustness to dilution

The nano emulsion was diluted upto 1000 times with water and solution of 0.1N HCl. Any separation of phases and precipitation was checked over.

Dispersibility test

0.5 mL formulation dispersibility in 500 mL distilled water using paddle type dissolution apparatus at 50 rpm, 37°C (±0.5°C) temperature was assessed visually and graded.

Identification of type of emulsion

Emulsion type was identified by standard tests, conductivity test, dye test and dilution test.

Globule size dimensions

Determined using a microscopic approach using stage micrometre and eye piece micrometre. The size and frequency of 100 globules was measured.

Transmittance %

Measured by UV spectrophotometer at 261 nm. Percentage transmittance against distilled water as a blank.

pH

pH was measured by digital pH meter three times, and standard values were determined.

Viscosity

An LVD II+ pro Brookfield viscometer using spindle 61, RPM 60, torque 91.2% at room temperature was used to determine viscosity of the nanoemulsion formulation.

Determination of drug content

Formulation containing efinaconazole 1 mL was added to 100 mL PBS 7.4 with tween 20 (1.5%), 5 mL methanol and sonicated. The solution was filtered, diluted and estimated by UV spectrophotometry at 261 nm (Koroleva and Yurtov, 2012).

In vitro drug diffusion

Using dialysis membrane, diffusion studies were performed. 1 mL of sample was taken in dialysis membrane, 200 mL of phosphate buffer saline (pH 7.4) with tween 20 (1.5%) as dissolution medium at 37±1°C. Samples of 5 mL were withdrawn periodically and replaced with fresh media for every 1 hr till eight hours, to maintain sink condition. Drug content was analysed by UV- spectrophotometer at 261 nm (Kumar, 2014).

Drug kinetics

The data was analysed for zero order, first order, Higuchi release, Korsemeyer Peppas equation.

Statistical Analysis

Optimisation of D-Optimal mixture design, is done by establishing a mathematical relationship between factors and responses in the form of model by Design Expert Version 11 software. Model is selected by ANOVA with Lack of Fit and Goodness of Fit Statistics, where F value greater than 1 and p value less than 0.05 is preferred. Lack of Fit should be insignificant where p value should be greater than 0.1, the difference between precision value and adequate precision should be greater than 4 and difference between adjusted R^2 and Predicted R^2 should be greater than 0.2. Once model relating the factors and responses is selected, it is analysed by graphical indicators like Actual plot, Predicted plot, Contour plot and Response surface plot. Then design space is developed by Overlay plot and verified by minimum three confirmatory experimental runs.

Droplet size measurement

Poly Dispersibility Index (PDI), Mean globule size of the optimized nanoemulsion formulations were determined using zetasizer (Delsa Max analytics) at temperature of 25°C (Kumar and Singh, 2012; Jain *et al.*, 2013).

Zeta Potential (ZP)

Zeta potential for the optimized nanoemulsion formulation was determined using Zetasizer at 25°C.

Table 1: Formulation Table of nanoemulsion.

Run	FC	Component 1 A: oil	Component 2 B: emulgent	Component 3 C: water	Response 1 drug content %	Response 2 drug release %
1	NEP1	55.6821	34.001	10.3169	100.4±0.90	81.72±0.15
2	NEP2	40	20	40	98.6±0.83	54.36±0.22
3	NEP3	41.9277	33.4198	24.6525	100.2±0.76	80.41±0.16
4	NEP4	20	41.5	38.5	98.61±0.45	45.92±0.12
5	NEP5	41.9277	33.4198	24.6525	100.2±1.26	80.41±0.18
6	NEP6	54.0816	20	25.9184	98.6±1.03	54.78±0.24
7	NEP7	20	60	20	99.42±0.11	75.69±0.13
8	NEP8	20	60	20	99.42±0.14	75.69±0.15
9	NEP9	41.9277	33.4198	24.6525	100.2±0.18	80.41±0.84
10	NEP10	42.9711	47.0289	10	100.7±0.24	82.85±0.25
11	NEP11	67.4062	20	12.5938	96.54±0.34	59.52±0.36
12	NEP12	31.7723	32.2222	36.0055	96.11±0.40	45.23±0.15
13	NEP13	29.6215	46.2254	24.153	98.56±0.17	54.63±0.54
14	NEP14	31.6123	58.3877	10	100.6±0.20	76.24±0.56
15	NEP15	20	41.5	38.5	98.61±0.12	45.92±0.66
16	NEP16	67.4062	20	12.5938	96.54±0.23	59.52±0.78

Formulation of Efinaconazole nanoemulsion gel

Efinaconazole nanoemulsion gel was prepared by using Carbopol 971, triethanolamine and distilled water. The required quantity of Carbopol was dispersed in water, using three-blade stirrer, required quantity of ethanol, propyl paraben and triethanolamine added drop wise. Add efinaconazole nanoemulsion to another portion of ethanol, and this solution is added dropwise to the above mixture. The prepared gel was evaluated for clarity, homogeneity, extrudability, pH, spreadability and drug content (physico-chemical properties) as per standard procedures.

Ex vivo permeation studies

This study was conducted according to the IAEC approval no: GPRCP/IAEC-1/27/03/2023/PCE/AE-1. Male Wistar rats were sacrificed by cervical dislocation, abdominal hair was removed and observed for presence of cuts or wounds. Skin section was cleaned with water and studies were performed using diffusion cell (Sugishita *et al.*, 1981).

In vitro antifungal activity studies

Antifungal activity against test organism *Candida albicans, Candida glabrata* using Sterile Dextrose Agar plates was studied. 0.1 mL of the test organism was spread on agar plates, 5 mm diameter holes contained Efinaconazole (pure drug) 100 mg/mL, marketed product (luliconazole 1%) 1 mL, pure drug in gel form 1 mL, nanoemulsion gel 1 mL separately in each plate. The plates were kept at 4°C for 4 hr and incubated at 28°C for 48 hr. After the incubation, the inhibition zones were measured.

Stability

As per ICH guidelines, stability of optimized formulation (G2) stored in glass bottles in a humidity chamber at 40°C±2°C and 75%±5% RH for one-month period was studied. The samples were evaluated for drug content, physical appearance, and dissolution every week (Jaiswal *et al.*, 2015).

RESULTS

Solubility studies

Oil and emulgent in which drug is more soluble was selected to prepare nanoemulsion. Efinaconazole concentration in different oils, surfactants as emulgents were determined by UV spectroscopy (Karthikeyan *et al.*, 2012). Efinaconazole drug was more soluble in Oleic acid (3.261±0.02 mg/mL), castor oil (2.65±0.0 mg/mL), neem oil (1.195±0.07 mg/mL), Tween 80 (1.148±0.1 mg/mL) and Transcutol (5.1±0.01 mg/mL) as depicted in Figure 1, Aqueous solubility was found to be 0.016±0.02 mg/mL.

Compatibility studies by FTIR

IR peaks at region 3703.07 and 3749.36 shows OH stretching (hydroxyl group), 2939.31 and 2889 C-H stretching (Alkyl group), 1612.38 and 1616.24 C=O stretching (carbonyl), 1380 and 1350 C-F stretching (Fluro compound) characteristic of drug were present in excipient-drug (1:1) combination spectrum.

Evaluation parameters of nanoemulsion

Nanoemulsions according to Table 1 were prepared and evaluated for physico-chemical evaluations. Formulations NEP-(3,5,9) NEP-(4,15) NEP-(7,8) NEP-(11,16) are the repetitions. From Table 2, thermodynamic studies by heating and cooling cycle indicated NEP 6 and NEP 13 were unstable, NEP-(3,5,9), NEP-(7,8), NEP10, NEP 14 showed phase separation and on centrifugation NEP-(11,16) showed phase separation and NEP 12 precipitation. Dispersibility test, Conductivity test, dye test and dilution test indicated NEP 2, NEP (4,15), NEP 12, NEP 13 formulations are O/W type rest formulations W/O type. % Transmittance was in the range of 68.6% to 98.42%. Globule size by optical microscopy was in range of 54.3 µm to 162.9 µm. Viscosity of nanoemulsion was in range of 93.41±0.24 cps to 254.8±0.27 cps and pH 5.08 to 5.72.

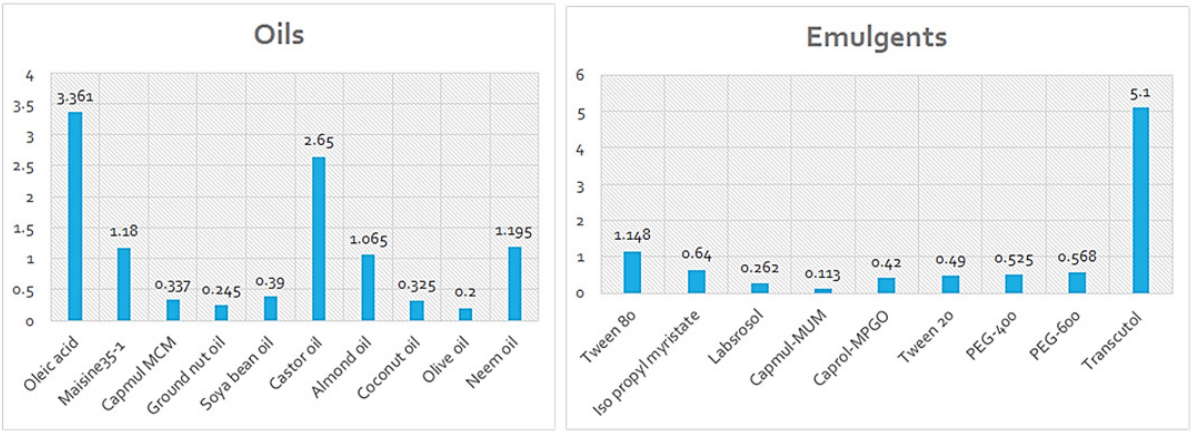

Figure 1: Solubility studies of efinacoazole in oils and emulgents.

Table 2: Evaluation parameters of Efinaconazole nano emulsion

FC	Thermodynamic studies			Dispersibility test#	% Transmittance	Globule size (µm)
	Heating and cooling cycle	Visual observation and phase separation	Centrifugation			
NEP 1	Stable	Stable	Stable	B(W/O)	98.42	90.5
NEP 2	Stable	Stable	Stable	A(O/W)	94.12	72.4
NEP 3,5,9	Stable	Phase separation	Stable	C(W/O)	68.56	162.9
NEP 4,15	Stable	Stable	Stable	A(O/W)	97.24	72.4
NEP 6	Precipitation	Stable	Stable	C(W/O)	91.41	181
NEP 7,8	Stable	Phase separation	Stable	D(W/O)	95.6	54.3
NEP 10	Stable	Phase separation	Stable	A(O/W)	97.84	126.7
NEP 11,16	Stable	Stable	Phase separation	D(W/O)	93.25	108.6
NEP 12	Stable	Stable	Precipitation	B(O/W)	92.76	54.3
NEP 13	Precipitation	Stable	Stable	A(O/W)	90.15	90.5
NEP 14	Stable	Phase separation	Stable	D(W/O)	93.21	72.4

Formulations: NEP-(3,5,9) NEP-(4,15) NEP-(7,8) NEP-(11,16) are the repetitions. #Dispersibility test: Grade A is for rapid-forming emulsion which is clear and transparent in appearance, B is for Rapid forming slightly less clear emulsion which has a bluish-white appearance, C is for bright white emulsion or greyish white emulsion with a slightly oily appearance that is slow to emulsify, D is for minimal emulsification with large oil droplets present on the surface.

In vitro diffusion studies

After performing diffusion studies the data depicts that percent drug release for NEP 1, NEP 3, NEP 5, NEP 9, NEP 10 shows highest drug release compare to other formulation as shown in Figure 2a up to 80% release within 8 hr. Formulation NEP 12, NEP 15 display less drug release during 8 hr, as shown in Figure 2b.

Model-dependent kinetics

All 16 formulations exhibit first order release and Korsemeyer Peppas with a "*n*-value" of greater than 0.6. As a result, all the formulations exhibit an anomalous transport mechanism.

Optimization: D-Optimal Mixture Design

For drug content (R1) response lack of fit was insignificant ($p>0.10$), F value was 5.26 which is greater than 1 for reduced quadratic model as shown in Table 3. Similarly, for % drug release (R2) response, F value was 6.33 and lack of fit was insignificant, which implies linear model is significant as there is only 1.2% chance of this large F value due to noise.

The difference between predicted R^2, adjusted R^2 should be less than 0.2 and Adequate precision which measures signal-to-noise ratio, greater than 4.0 is desirable. For R1 and R2 responses with reduced quadratic model and linear model the difference is less than 0.2 and ratio of 6.9021 and 7.192 respectively as depicted in Table 3.

From counterplots and response surface plots it was observed with increase in X1 (Oil phase), X2 (emulgent), the drug release percentage was increased, but increase in X3(water) there is decrease in drug release (R2). Increase in X1 (oil phase), X2 (emulgent) resulted more drug content and increase in X3 water, less drug content (R1) as shown in Figures 3A, 3B. Three dependent variables have mixed effects on drug content. The Contour and response surface plot shows that when emulgent concentration (B) is increased drug content increases. With increment in the quantity of emulgent the percentage drug release was also enhanced, with increased oil concentration, there was increased drug release.

Reduced quadratic equation for drug content = +0.8913A+0.9302B+0.9986C+0.004AAB

Linear equation for % drug release = +0.7688A+1.002B-0.0834C

The optimum variables according to the criterion of desirability were acquired by the numerical analysis. Linearity of Predicted vs. Actual responses as shown in Figure 3C for drug content R^2 is 0.9688 and for Drug Release percentage, R^2 is 0.985. From overlay plot (Figure 3D) for graphical optimization, an optimized

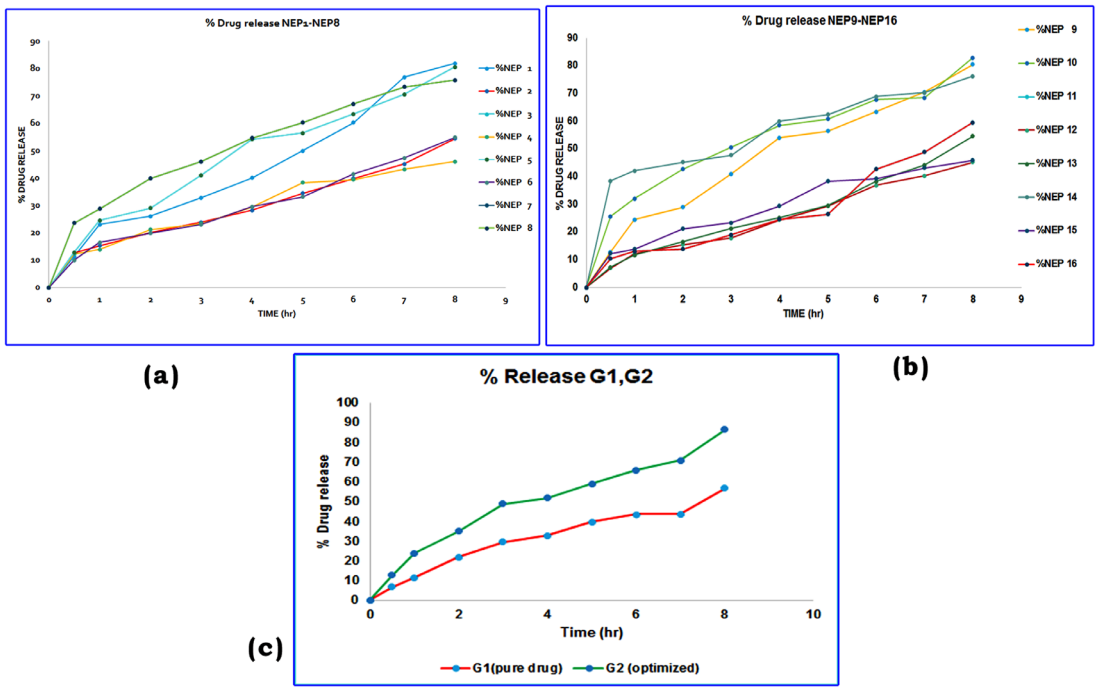

Figure 2: drug release graphs of (a) Percent Drug release for NEP1to NEP8, (b) Percent Drug release for NEP9 to NEP16, (c) Percent drug release of gel formulations.

Table 3: Fit summary for response percentage drug content (R1) and percentage drug release (R2).

Response	Drug content (R1)	Percentage drug release (R2)
Model	Quadratic	Linear
Sum of square	18.93	1558.73
Degree of freedom	3	2
F value	5.26	6.33
P value	0.0151	0.0120
Adjusted R^2	0.4601	0.4156
Predicted R^2	0.3084	0.2994
Adeq. Precision	6.9021	7.1927
Lack of fit	2.06	199.99
Pure error	0.0000	0.0000

formulation based on maximum drug release and drug content is produced with desirability co-efficient 0.951. Optimized nanoemulsion formulation (NEP-17) prepared by 31.29 mL oil, 58.70 mL emulgent, and 10.0 mL water, has shown rapid emulsion formation with transparent appearance, good thermodynamic stability, drug content R1, 101.7±0.12% and % drug release R2, 82.09±0.01% as shown in Table 4.

Zeta potential and globule size

The formulation has shown a Zeta-potential of -42.1 mv (Figure 4B). Nanoemulsion is confirmed by its size 229.7 nm (Figure 4A) and with poly dispersibility index of 0.284. As shown in Figure 4.

Evaluation of Gel

The optimized NEP-17 formulation was formulated into gel according to procedure in methodology and the gel was evaluated. Gel formulations (G1 (pure drug), G2 (nanoemulsion)) were evaluated for clarity (++), Homogeneity (++), Extrudability (++), pH (5.12±0.3, 5.38±0.4), Spreadability (33±0.32 gm.cm/sec, 41±0.21 gm.cm/sec) and drug content (98.34± 0.09%, 98.45±0.01%) respectively which were found to be ideal. There were no particles present in the gel and was uniform, with good spreadability, and the pH was found to be suitable for the application.

Ex vivo permeation studies

Nanoemulsion-based gel (G2) after evaluating physicochemical parameters showed *ex vivo* permeation of 86.42±0.14% and for Pure drug gel (G1) it was found to be 56.76±0.34% as depicted in Figure 2C. And different permeability parameters are reported as, steady state flux 0.896±1.67 to 0.8831±1.69µg/cm^2/hr, Lag time 0.2±0.4 to 0.16±0.3 hr, Permeability coefficient 4.48±0.93 to 4.41±0.87cm/hr.x10^{-3}, Enhancement ratio 1.286±0.36, Skin deposition 0.53±0.24 0.69±0.36 µg/cm^2. Model dependent kinetics shows that it follows first order kinetics (0.981) as it shows the R^2value higher than the zero order kinetics (0.9783) and shows higuchi release mechanism with the value of 0.9868 which is greater than Korsemeyer peppas.

Antifungal studies

Antifungal studies against *Candida glabrata* and *Candida albicans* were performed and results are shown in Figure 5. In Figure 5a,

Candida glabrata antifungal studies, it is observed in control (A) presence of white spots representing fungal growth, randomly spread in whole area. In pure drug solution form (B), white spots were seen at 10 mm from center, in gel form of pure drug (C), at 11 mm from center, in marketed (D) at 7 mm, whereas in efinaconazole nanoemulsion gel (E) at 13 mm from center.

Similarly in Figure 5b, *Candida albicans* antifungal studies, it is observed in control (A) presence of white spots representing fungal growth, randomly spread in whole area. In pure drug solution form (B), white spots were seen at 8 mm from center, in gel form of pure drug (C), at 10 mm from center, in marketed (D) at 15 mm, whereas in efinaconazole nanoemulsion gel (E) at 13 mm from center.

Stability study

Nanoemulsion-based gel G2, formulation in ambered colored bottle was tested. It passed through accelerated conditions as per ICH guidelines (40ºC±2ºC/75%±5% RH) for one month. Physical appearance, drug content, and *in vitro* drug release were evaluated every week. It was stable, with insignificant change in the physical appearance, % drug content and % drug release (Table 5).

DISCUSSION

Solubility of efinaconazole in different oils and emulgents were determined to select oil phase and emulgent in nano emulsion preparation by phase titration method. Among oils selected highest solubility was observed in oleic acid and among emulgents, transcutol followed by Tween 80. FTIR studies indicated drug excipient compatibility. Preliminary trials were performed with oleic acid, transcutol, water and oleic acid, Tween 80, water as nanoemulsion and evaluated for thermodynamic stability studies. Among the trials oleic acid, transcutol, water nanoemulsion was more stable. So, these were considered as independent factors in D-optimal design with varying ratios in a mixture. Nanoemulsions were prepared and evaluated for type of emulsion, thermodynamic stability, % transmittance, globule size, viscosity and pH. Except NEP 2, NEP (4,15), NEP 12, NEP 13 formulations all are W/O type, thermodynamically stable, clear transparent nanoemulsions with optimum globule size, viscosity and pH. *In vitro* diffusion studies were performed for 8 hr and formulation NEP 10 showed maximum drug release. All formulations followed first order and anomalous transport.

On optimization by D-optimal mixture design, it is observed the dependent variables, drug content followed reduced quadratic model and was dependent on each component oil, emulgent, water and interaction of oil and emulgent. Percent drug release followed linear model and had positive effect with oil and emulgent component, negative effect with water component. Predicted vs Actual responses showed linearity with R^2 nearly 1. With the criteria of maximum drug content and maximum drug release, design has given two solutions of desirability 0.951. The

Table 4: Evaluation of physio-chemical parameters for optimized formulation NEP 17.

Optimized Formulation Nep -17	
Parameters	**Inference**
Type of emulsion	W/O
PH	5.69
Drug content	100.7%
% drug release	82.19%
Zeta potential	- 42.1mv
Globule size	229.7nm
Viscosity (spindle 61 Rpm 60)	94.3 ±2.41 cps
Thermodynamic studies	Pass
Visual observation	Pass
Robustness to dilution	Pass

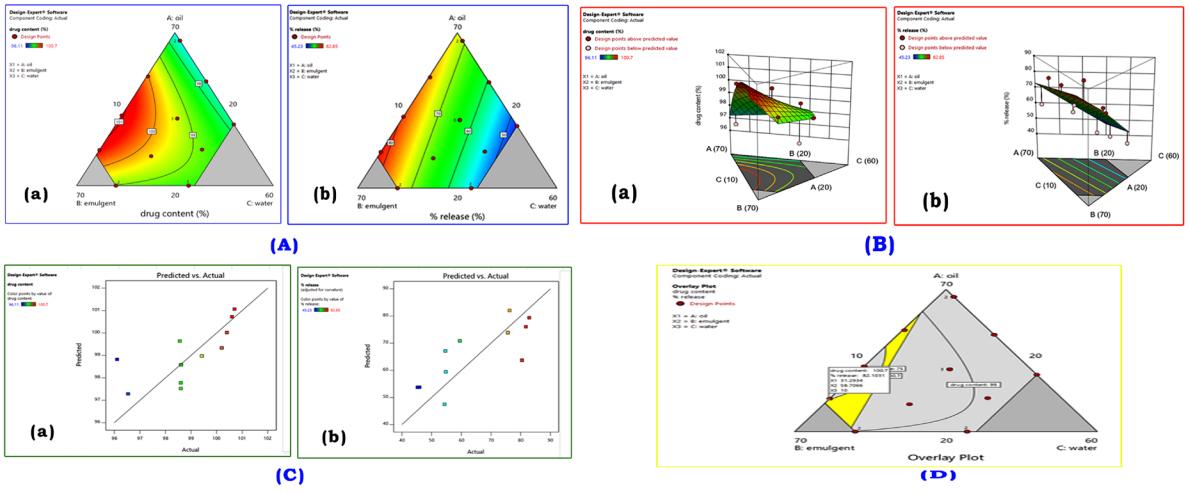

Figure 3: Optimisation by d-optimal mixture design for drug content and drug release, (A) Contour plot, (B) Response surface plot, (C) Predicted vs actual plot, (D) Overlay plot.

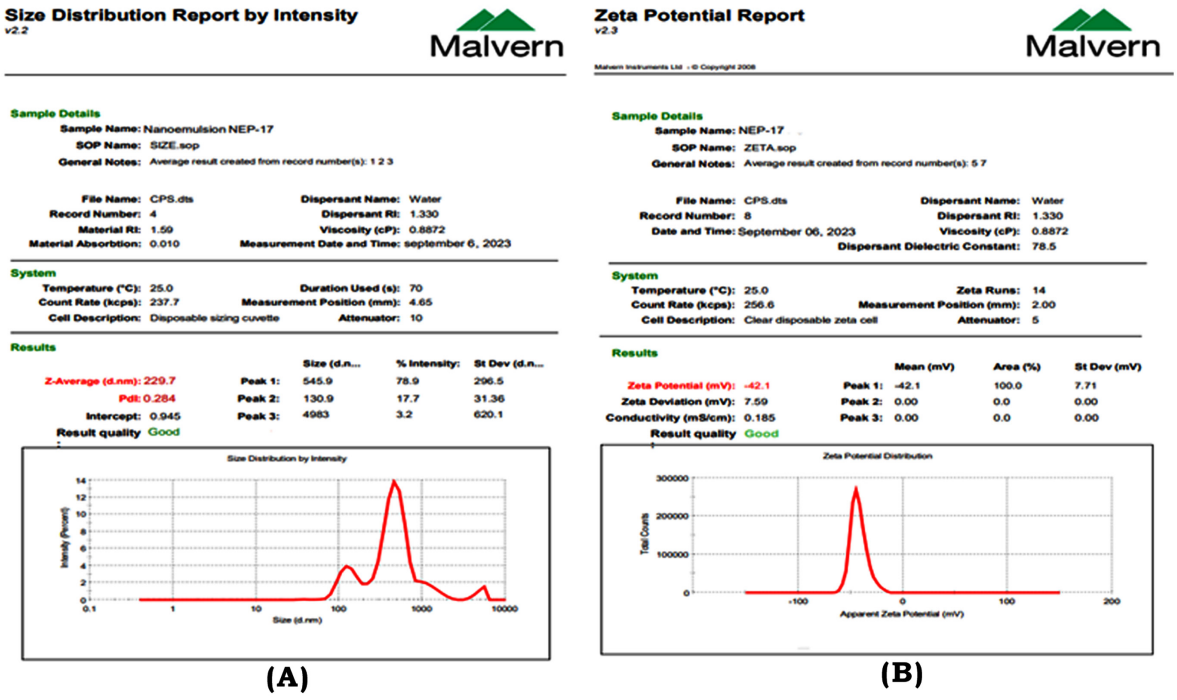

Figure 4: (A) Particle size and (B) Zeta potential of optimized nanoemulsion NEP 17.

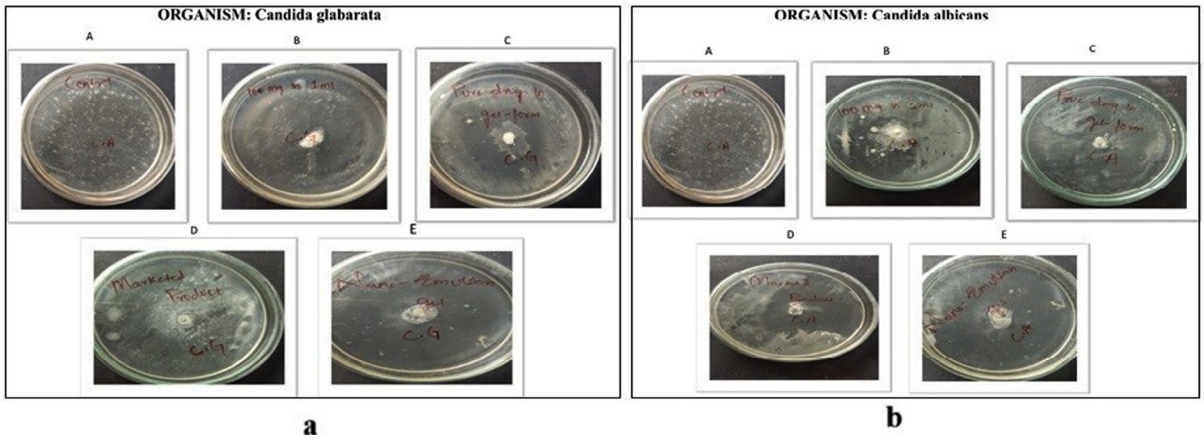

Figure 5: Antifungal studies against (a) *Candida glabrata* and (b) *Candida albicans*; A-Control; B-Pure Drug; C-Pure Drug in Gel form; D-Marketed preparation; E-Nanoemulsion gel.

Table 5: Stability studies for nanoemulsion-based gel G2.

Sl. No.	Parameter	Initial	1st week	2nd week	1 month
1.	Appearance	Clear gel	Clear gel	Clear gel	Clear gel
2.	Drug content	100.07±0.1	99.04±0.2	99.03±0.3	99.03±0.41
3.	% drug release	82.19±0.1	82.12±0.21	81.23±0.37	81.23±0.36

optimized formulation NEP 17 was prepared and evaluated for physico-chemical properties. The formulation has Zeta-potential of –42.1 mv, globule size 229.7 nm and poly dispersibility index of 0.284 indicating stability of formulation and uniformity of globules in nanosize.

Optimized nanoemulsion NEP 17 was formulated into gel for ease of application for onchomycosis. Gel was evaluated for physico-chemical properties, *ex vivo* permeation studies and compared with pure drug in gel form. Nanoemulsion gel had better permeability than pure drug gel.

Antifungal studies of G2 (nanoemulsion gel) against *Candida albicans* and *Candida glabrata* indicated antifungal activity similar to marketed Luliconazole preparation (LUZU). It was found to be efficient at very low dose, which was reduced to 10

times than the official dose where indication can be made that the solubility has been enhanced.

Stability studies of G2 showed no changes in drug content and % release. We conclude that the nanoemulsion-based gel was stable.

CONCLUSION

This study was aimed to enhance the solubility and bioavailability of efinaconazole. As the solubility of the drug is enhanced, efinaconazole 10% is efficacious, thus 10 times less than official dose could be administered. Further, determination of pharmacokinetics parameters and commercialization of optimized formulation would be beneficial.

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ABBREVIATIONS

BCS: Biopharmaceutical Classification System; **O/W:** Oil in Water; **W/O:** Water in Oil; **ANOVA:** Analysis of Variance; **FC:** Formulation Code; **cps:** Centipoise; **FTIR:** Fourier Transform Infrared Spectroscopy; **IR:** Infrared; **%:** Percent; **PEG:** Polyethylene Glycol; **UV:** Ultra Violet; **ICH:** International Conference on Harmonisation; **RH:** Relative Humidity; **NEP:** Nanoemulsion Formulation; **PDI:** Poly Dispersibility Index; **ZP:** Zeta Potential; **HCl:** Hydrochloric Acid; **PBS:** Phosphate Buffer Saline; **IAEC:** Institutional Animal Ethics Committee; **LUZU:** Marketed Luliconazole solution 1%; **RPM:** Revolutions Per Minute.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

AUTHOR CONTRIBUTIONS

Sagarla Usharani: Design of methodology, conducting research and investigation process.

Mohammed Mahboob Shareef: Conducting research and investigation process, Preparation of draft manuscript.

Ruhi Anjum: Preparation, creation and/or presentation of the published work, specifically writing the initial draft.

Vasavi Devireddy: Formal analysis of data.

Naseeb Basha Shaik: Reviewing and editing.

P.K. Lakshmi: Final editing the manuscript and citation.

Prasanthi Domaraju: Conceptualization, project administration, overall supervision, designing the final version to be published.

ETHICAL APPROVAL

Studies were conducted according to the IAEC approval no: GPRCP/IAEC-1/27/03/2023/PCE/AE-1.

SUMMARY

This study successfully optimized an efinaconazole nanoemulsion using a D-optimal mixture design, selecting a formulation (NEP-17) consisting of Oleic acid (31.29 mL), Transcutol (58.70 mL), and water (10.00 mL). The optimized nanoemulsion exhibited a globule size of 229.7 nm and a zeta potential of -42.1 mV, ensuring stability and effective drug delivery. When incorporated into a Carbopol gel (G2), the formulation demonstrated superior *ex vivo* permeation (86.42%) compared to the pure drug gel (56.76%) and showed antifungal efficacy against *Candida albicans* and *Candida glabrata* comparable to marketed products at a significantly lower dose.

REFERENCES

Aucouturier, J., Dupuis, L., Deville, S., Ascarateil, S., and Ganne, V. (2002). Montanide ISA 720 and 51: a new generation of water in oil emulsions as adjuvants for human vaccines. *Expert Rev Vaccines*, 1(1),111-118. https://doi.org/10.1586/14760584.1.1.111. PMID: 12908518.

Barry, B.W. (1991). Lipid-protein-partitioning theory of skin penetration enhancement. *J Control Release*, 15(3), 237-248. https://doi.org/10.1016/0168-3659(91)90115-T

Boonme, P., Junyaprasert, V.B., Suksawad, N., and Songkro, S. (2009). Microemulsions and nanoemulsions: novel vehicles for whitening cosmeceuticals. *J Biomed Nanotechnol*, 5(4), 373-383.

https://dx.doi.org/10.1166/jbn.2009.1046

Che Marzuki, N. H., Wahab, R. A., and Abdul Hamid, M. (2019). An overview of nanoemulsion: concepts of development and cosmeceutical applications. *Biotechnology and Biotechnological Equipment*, 33(1), 779–797. https://doi.org/10.1080/13102818.2019.1620124

Costa, I.C., Rodrigues, R.F., Almeida, F.B., Favacho, H.A., Falcao, D.Q., Ferreira, A.M., Vilhena, J.C.E., Florentino, A.C., Carvalho, J.C.T., and Fernandes, C.P. (2014). Development of jojoba oil (*Simmondsia chinensis* (Link) CK Schneid.) Based nanoemulsions. *Lat. Am. J. Pharm*, 33(3), 459-463.

Costa, P.R., and Santos, L. (2017). Delivery systems for cosmetics-from manufacturing to the skin of natural antioxidants. *Powder Technol*, 322(1), 402-416. https://dx.doi.org/10.1016/j.powtec.2017.07.086

Gursoy, R. N., and Benita, S. (2004). Self-emulsifying drug delivery systems (SEDDS) for improved oral delivery of lipophilic drugs. *Biomed Pharmacother*, 58 (3), 173-182. https://doi.org/10.1016/j.biopha.2004.02.001.

Jain, K., Kumar, R. S., Sood, S., and Gowthamarajan, K. (2013). Enhanced oral bioavailability of atorvastatin via oil-in-water nanoemulsion using aqueous titration method. *Journal of Pharmaceutical Sciences and Research*, 5(1),18-25.

Jaiswal, M., Dudhe, R., and Sharma, P. K. (2015). Nanoemulsion: an advanced mode of drug delivery system. *Biotech*, 5(2), 123-127. https://doi.org/10.1007/s13205-014-0214-0.

Kale, S. N., and Deore, S. L. (2017). Emulsion micro emulsion and nano emulsion: a review. *Syst Rev Pharm*, 8 (1), 39-47. https://doi.org/10.5530/srp.2017.1.8

Karthikeyan, S., Jeeva, P. A., Jerobin, J., Mukherjee, A., and Chandrasekaran, N. (2012). Formulation and characterization of nanoemulsion coatings from *Azadirachta indica*. *Int. J ChemTech Res*, 4(4), 1566-1570.

Kong, M., Chen, X. G., Kweon, D. K., and Park, H.J. (2011). Investigations on skin permeation of hyaluronic acid based nanoemulsion as transdermal carrier. *Carbohydrate Polymers*, 86 (2), 837-843. https://dx.doi.org/10.1016/j.carbpol.2011.05.027

Koroleva, M., and Yurtov, E. V. (2012). Nanoemulsions: the properties, methods of preparation and promising applications. *Russian Chemical Reviews*, 81(1), 21-43. https://dx.doi.org/10.1070/RC2012v081n01ABEH004219.

Kumar, S. H., and Singh, V. (2012). Nanoemulsification-a novel targeted drug delivery tool. *Journal of Drug Delivery and Therapeutics*, 2 (4), 40-45. https://doi.org/10.22270/jddt.v2i4.185.

Kumar, S. (2014). Role of nano-emulsion in pharmaceutical sciences-a review. *AJRPSB*, 2(1), 1-15.

Liu, M., Gan, L., Pang, Y., Xu, Z., Hao, Z., and Chen, L. (2008). Synthesis of titania–silica aerogel-like microspheres by a water-in-oil emulsion method via ambient pressure

drying and their photocatalytic properties. *Colloids Surf A: Physicochem Eng Aspects*. 317 (1-3), 490–495. https://dx.doi.org/10.1016/j.colsurfa.2007.11.024.

Mason, T. G., Wilking, J. N., Meleson, K., Chang, C.B., and Graves, S.M. (2006). Nanoemulsions: formation, structure, and physical properties. *J Phys: Condens Matter*, 18 (41), R635-R666. https://dx.doi.org/10.1088/0953-8984/18/41/R01

Montilla, L. J. C., Herrera-Morales, P. E., Pandey, S, and Shah, D.O. (2002). Spontaneous emulsification: mechanisms, physicochemical aspects, modeling, and applications. *J Dispers Sci Technol*, 23 (1-3), 219-268. https://dx.doi.org/10.1080/01932690208984202

Porras, M., Martınez, A., Solans, C., Gonzalez, C., and Gutierrez, J.M. (2005). Ceramic particles obtained using W/O nano-emulsions as reaction media. *Colloids Surf A: Physicochem Eng Aspects*, 270271 (1),189–194. https://dx.doi.org/10.1016/j.colsurfa.2005.06.018

Souto, E. B., Cano, A., Martins-Gomes, C., Coutinho, T. E., Zielińska, A., and Silva, A. M. (2022) Microemulsions and Nanoemulsions in Skin Drug Delivery. *Bioengineering (Basel)*, 9(4):158. https://doi.org/10.3390/bioengineering9040158. PMID: 35447718.

Sugishita, E., Amagaya, S., and Ogihara, Y. (1981). Anti-inflammatory testing methods: Comparative evaluation of mice and rats. *Journal of Pharmacobio Dynamics*, 4(8), 565-575. https://doi.org/10.1248/bpb1978.4.565. PMID: 7299620.

Talegaonkar, S., Azeem, A., Ahmad, F. J., and Khar, R.K. (2008). Microemulsions: a novel approach to enhanced drug delivery. *Recent Pat Drug Deliv Formul*, 2(3), 238-257. https://doi.org/10.2174/187221108786241679. PMID: 19075911.

Tata, M., Banerjee, S., John, V. T., Waguespack, Y., and McPherson, G.L. (1997). Fluorescence quenching of cds nanocrystallites in AOT water-inoil microemulsions. *Colloids Surf A: Physicochem Eng Aspects*, 127, 39-46.

Uskokovic, V., and Drofenik, M. (2005). Synthesis of materials within reverse micelles. *Surf Rev Lett*, 12(2), 239-277. https://doi.org/10.1142/S0218625X05007001

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