Journal of Chemical Health Risks

www.jchr.org

JCHR (2025) 15(4), 2008-2019 | ISSN:2251-6727

Development and Characterization of Luliconazole and Chamomile Oil Loaded Nanoemulsion based Gel for Effective Treatment of Fungal Disease

Het Solanki[1,2], Punit B. Parejiya[2], Kavya Shah[3], Pranav C. Patel[4], Aqsa Sama[2], Divyang Dave[5]

[1]Research scholar, Kadi Sarva Vishwavidyalaya, Gandhinagar, Gujarat, India

[2] Department of Pharmaceutics, K.B. Institute of Pharmaceutical Education and Research, Kadi Sarva Vishwavidyalaya, Gandhinagar, Gujarat, India

[3] Research cell student, K.B. Institute of Pharmaceutical Education and Research, Kadi Sarva Vishwavidyalaya, Gandhinagar, Gujarat, India

[4] Department of Pharmacology and pharmacy practice, K.B. Institute of Pharmaceutical Education and Research, Kadi Sarva Vishwavidyalaya, Gandhinagar, Gujarat, India

[5] Principle, K.B. Institute of Pharmaceutical Education and Research, Kadi Sarva Vishwavidyalaya, Gandhinagar, Gujarat, India

(Received: 16 June 2025 Revised: 20 July 2025 Accepted: 04 August 2025)

KEYWORDS

Nanoemulsion, Luliconazole, Chamomile oil, Antifungal.

ABSTRACT:

Luliconazole (LNZ) is a selective, poorly water-soluble (0.000304mg/ml) azole effective against treatment of fungal infections when administered in doses. In the present Research work, to formulate nanoemulsion of luliconazole using chamomile oil for the treatment of fungal disease. Chamomile oil chosen as oil as it is known for its antifungal activity. The nanoemulsion system was optimized through D-optimal design and an equivalent amount of nanoemulsion is incorporated in Carbopol 934p based gel. Optimized nanoemulsion contained 33.13% Smix, 9.05% oil, 57.80% water which has smallest globule size of 140 nm and PDI was 0.367. Zeta potential was obtained as -14.6 mV. Viscosity of nanoemulsion and nanoemulsion based gel, 128 cP and 1380 cP respectively and pH of nanoemulsion was 7.25. All nanoemulsion were O/W type as per results of conductivity which is 84.2 µs/cm. For nanoemulsion based gel, ex vivo study revealed that drug release at 5 hours was 69.48%. and an in vitro study release 89.21 % drug release. The range of antifungal action was increased by combining chamomile oil with Carbopol 934p based gel of luliconazole Via its zone of inhibition. The antifungal activity of a gel based on nanoemulsion was compared to a marketed formulation. Observations led to conclude that the 0.4% concentration of NEBG had a greater zone of inhibition than other commercially available formulations. zone. Thus, 0.4% of NEBG was shown to be more efficacious than other commercially available formulations, and that too with a relatively modest dosage of luliconazole.

1. Introduction

It is not uncommon for superficial fungal infections to affect various body parts, such as the skin, cuticles, and mucous membranes. These infections are typically caused by fungi like Candida albicans, a type of yeast, and dermatophytes such as Trichophyton species. People often experience symptoms like itching, redness, scaling, and in some cases, pain or noticeable lesions, especially when the infection worsens. While these conditions usually aren't life-threatening, they can still be pretty uncomfortable and have a real impact on daily life.

However, taking LNZ orally has been linked to some side effects, including stomach issues like vomiting, bloating, and abdominal pain. It can also cause liver toxicity, which makes it less suitable for long-term use or for targeting localized infections. To get around these problems, alternative forms like gels, creams, and lotions have been developed. But these options aren't perfect—they often don't stay on the affected area long enough, dosing can be inconsistent which affects how well they work, and applying them in tricky spots, like between toes or in the groin, can be difficult. To tackle

Journal of Chemical Health Risks

www.jchr.org

JCHR (2025) 15(4), 2008-2019 | ISSN:2251-6727

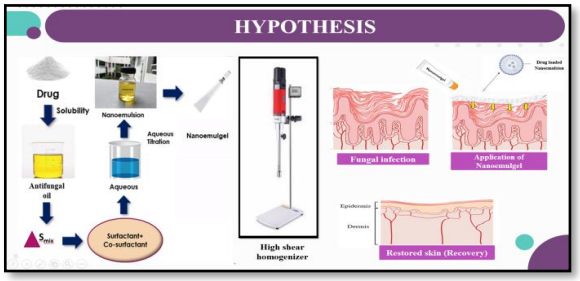
these challenges, scientists are exploring nanoemulsion-based in situ gels as a promising way to improve how LNZ is delivered.

Nanoemulsions are clear, stable mixtures made of oil, water, surfactants, and co-surfactants. They offer a lot of benefits, including: high bioavailability that improves drug absorption, better penetration through skin and mucous membranes, protection of the drug from oxidation, and versatility in carrying both water-loving (hydrophilic) and fat-loving (hydrophobic) drugs.

Using nanoemulsion-based in situ gels several clear advantages can be observed:

1. They extend how long the drug stays at the site of infection—resisting the natural mechanisms like mucociliary clearance in nasal applications or sweating and skin shedding in topical treatments—thus boosting therapeutic effects.
2. They enable more precise dosing, making sure the right amount of medication is delivered without wastage.
3. They enhance skin penetration, ensuring the drug reaches deep into the skin layers or nails where fungi tend to hide.

Adding chamomile oil into this nanoemulsion gel can provide extra antifungal effects because chamomile oil inhibits ergosterol synthesis, which disrupts fungal cell membranes. Combining chamomile oil with LNZ might create a synergistic effect, making the treatment more effective overall. Including carrageenan, a natural polymer, into the gel helps increase its viscosity. This means the gel can stay on the skin or mucous membranes longer, giving the drug more time to work. Also, it's gentle enough not to interfere with normal skin or mucosal function. Overall, nanoemulsion-based in situ gels look like a really promising step forward in managing superficial fungal infections [1-2][13][16].



2. Materials and Methods

2.1. Materials :

Luliconazole was procured from Redson Pharmaceuticals in Ahmedabad, India. The vehicle for the evaluation of Chamomile oil was prepared using the following chemicals: Tween 80, Tween 20, Span 80, Labrasol, PGE 400, Cremaphor RH 40 from BASF, Mumbai, India, Transcutol P from Abitech Corporation, Poloxamer 147, Poloxamer 188, Carrageenan, and meth

2.2 Methods:

Quantification of Luliconazole

We measured the absorbance of the luliconazole stock solution across a wavelength range of 100 to 700 nm using a UV spectrophotometer (Schimadzu UV-1800). To are a control, methanol was used. The maximum UV absorption of luliconazole was observed at 278 nm. To prepare the stock solution, exactly 10 mg of luliconazole was weighed and dissolved in 100 ml of methanol within a 100 ml volumetric flask. The solution was filtered through Whatman filter paper to ensure clarity. This resulted in a stock concentration of 10 µg/ml, which corresponded to a potency of 100 µg/ml. For the calibration curve, solutions with concentrations ranging from 2 to 32 µg/ml were made by transferring 0.2, 0.4, 0.6, 0.8, 1.0, 1.2, and 1.4 ml of the stock into 10 ml volumetric flasks and diluting with methanol up to the mark. Each splenty's absorbance at 278 nm was measured with the UV-Visible Spectrophotometer. A standard curve was then plotted over the entire range, and the best-fit line was calculated based on the average absorbance from three independent experiments.

2.2.1 Standard calibration curve prepared in 7.4 phosphate buffer solution (PBS):

To prepare a one- liter result, gather these constituents start by adding 800 mL of distilled water into a clean vessel. also, stir in 8 grams of NaCl, 200 milligrams of KCl, 1.44 grams of Na_2HPO_4, and 240 milligrams of KH_2PO_4 until everything is dissolved. For the stock result, dissolve 10 milligrams of Luliconazole in 100 mL of methanol this gives a attention of 10 µg/ mL, which we'll call Stock 1. Next, make up the total volume to 1000 mL. After that, take different quantities specifically 0.2, 0.4, 0.6, 0.8, 1.0, 1.2, 1.4, and 1.6 mL — of this stock result and add each into separate 10- mL volumetric steins. Mix them well with a phosphate buffer at pH 7.4 until you reach the asked attention. This results in results with attention of 2, 4, 6, 8, 10, 12, 14, and 16 µg/ mL. Eventually, measure the absorbance of each result at 278 nm using a UV-Visible Spectrophotometer to quantify the quantum of Luliconazole present.

Journal of Chemical Health Risks

www.jchr.org

JCHR (2025) 15(4), 2008-2019 | ISSN:2251-6727

2.2.2 Nanoemulsion Component Preliminary Screening:

The solubility of chamomile oil, Span 20, Span 80, Tween 20, Tween 80, labrasol, PEG 200, PEG 400, Transcutol HP, ethanol, propylene glycol, and isopropyl alcohol was assessed by dissolving a substantial quantity of LNZ in 2 ml of each solvent. The aqueous layers of the compounds were isolated by centrifugation of the mixtures at 3000 rpm for 15 minutes after a 24-hour period. Prior to determining the concentration of (LNZ) spectrophotometrically at 278 nm, the supernatant was diluted with methanol. Every experiment was performed thrice.

2.2.3 Construction of a pseudoternary phase diagram:

The nanoemulsion was prepared using the chosen oil (chamomile oil), surfactant (Tween 80), and co-surfactant (PEG 400), as established during solubility studies. The surfactant and co-surfactant were amalgamated in different mass ratios (1:1, 1:2, 2:1, 1:3, and 3:1). To guarantee comprehensive coverage of the optimal ratios, many combinations of oil and Smix (1:9, 2:8, 3:7, 4:6, 5:5, 6:4, 7:3, 8:2, 9:1) were used to create the pseudoternary phase diagram. The aqueous titration technique was used, whereby the aforementioned combination was augmented with double-distilled water, and the endpoint was ascertained by turbidity. The pseudo ternary phase diagram was constructed using the computed concentrations of the different components. The CHEMIX® School program was used to create the pseudo-ternary phase diagram. The stability of a particular system is represented by the nanoemulsion region in the phase diagram. This resulted in the identification of the Smix ratio that produces a more extensive nanoemulsion zone for further analysis[4].

2.2.4 Luliconazole-loaded nanoemulsion preparation:

Various component ratios were used to select luliconazole-loaded nanoemulsion phase diagrams. A magnetic stirrer dissolved luliconazole in chamomile oil to form a nanoemulsion. Tween 80 and PEG 400 are mixed. Water was added drop-by-drop to the oil phase. Finally, a high shear homogenizer (IKA T25 Digital ULTRA TURRAX, Germany) at 5000 rpm for 15 minutes reduced the nanoemulsion's size. As prepared, a digital ultrasonic cleaner sonicates the nanoemulsion for an hour to remove microbubbles. After sonication, the clear nanoemulsion was stabilized for 24 hours before analysis. (8).

2.2.5 Drug-loaded nanoemulsion characteristics (9):

Visual appearance:

The prepared luliconazole-loaded nanoemulsion was examined visually for color, appearance, and any indications of turbidity or phase separation after standing.

Percentage transmittance:

The homogeneity and optical transparency of the prepared nanoemulsion were assessed by measuring its transmittance. With distilled water serving as a blank, the nanoemulsion's percentage transmittance was determined using UV spectrophotometry at 271 nm. Measurements were made in triplicate at 25 ± 2°C.

Zeta potential:

The charge of the droplets is found by calculating their zeta potential. A typical nanoemulsion results in oil droplets with a negative charge because free fatty acids are present. At 25°C and a scattering angle of 173°, the zeta potential of the luliconazole-loaded nanoemulsion was determined using the light scattering technique. A Zeta meter was used for this purpose.

Distribution of globule size and polydispersity index (PDI):

A particle size analyzer (Horiba Scientific SF-100, Japan) was used to assess the globule size distribution and polydispersity index (PDI) of the luliconazole-loaded nanoemulsion via the Dynamic Light Scattering (DLS) method.

Conductivity of Nanoemulsion:

Using a conductivity meter, the nanoemulsion's electrical conductivity was determined. Electrical conductivity was used to determine the nanoemulsion system's phase system. The measurements were made three times.

Refractive index:

One drop of the nanoemulsion was placed on a slide and measured using an Abbe refractometer.

Measurements of pH:

Each formulation's pH was determined using a pH meter that had been calibrated using buffered solutions with pH values of 4 and 7. The pH was measured after 1 ml of the nanoemulsion was dissolved in 100 ml of distilled water. The measurements were made three times.

Rheological analysis:

Using the Brookfield viscometer (Brookfield DV-II+Pro, Brookfield Engineering Inc., USA), the viscosity of the prepared formulation was measured.

Journal of Chemical Health Risks

www.jchr.org

JCHR (2025) 15(4), 2008-2019 | ISSN:2251-6727

Nanoemulsion viscosities were measured using spindle number 63 at 100 rpm.

Determination of drug content:

1 ml of the nanoemulsion was dissolved in 10 ml methanol in a 10 ml volumetric flask. After adding methanol to the flask with a carefully measured 0.1 ml stock solution, Whatman filter paper was used to filter it. The produced solution was UV-spectrophotometer-tested at 271 nm. The drug concentration in the formulation was determined using luliconazole standard calibration curves in methanol. Measurements were done three times.

2.2.6. Optimization of Nanoemulsion:

D-optimal design:

The design of experiment (DOE) was used to optimize the nanoemulsion, and the D-Optimal design (mixture) was chosen. This design's features include multiple objective synchronous optimizations, high predictive accuracy, and simplicity. When experimental results rely solely on the ratios of the mixture's constituent parts, this kind of design is used to optimize the variables. Design factors are mixture components, and responses are functions of their proportions in this kind of response surface optimization technique. The components of the mixture cannot vary independently because their sum must equal 100%. To find main effects and interaction effects between the independent variables in an experiment, the D-optimal mixture design is commonly employed. (77). Expert in design The software used for the experimental design in the current studies was version 13 (State-Ease Inc., Minneapolis, USA). Different levels of the design constraints X1 (Smix), X2 (oil), and X3 (water) were taken. The component ranges for nanoemulsion optimization were as follows:

Thermodynamic stability study:

The formulation of the nanoemulsion was visually examined for phase separation after it was centrifuged for 10 minutes at 10,000 rpm. Three or four freeze-thaw cycles, which involved freezing at -4°C for 24 hours and then thawing at 40°C for 24 hours, were applied to the formulation that did not exhibit any phase separation following centrifugation. For five minutes, centrifugation was run at 3,000 rpm. Phase separation was then observed using the formulation. For additional research, only formulations that remained stable during phase separation were chosen. (78)

TEM (transmission electron microscopy): TEM was used to describe the formulation's nanostructure. On a copper grid coated with holey carbon, a single drop of roughly diluted nanoemulsion samples was directly applied. A single drop of phosphotungstic acid was added for negative staining. A filter was used to remove any unnecessary phosphotungstic acid from the sample, and it was then left to dry. Finally, a transmission electron microscope (TEM; TalosTM F200i) was used to analyze the formulation's size and shape.

2.2.7 Preparation of nanoemulsion based gel:

In order to achieve a smooth dispersion, the gel-forming agent Carbopol 934P was first agitated at about 150 rpm using a magnetic stirrer for two hours in 100 ml of luliconazole-loaded nanoemulsion. For fifteen minutes, it was left to stand in order to release any trapped air. By slowly stirring, the resulting viscous gel was neutralized with citric acid to a pH of 7. 1%, 1.5%, and 2% carbopol concentrations were used to create the gel formulation.

2.2.8 Nanoemulsion-based gel characterization (NEBG)
Visual examination:

Visual inspection was performed on the prepared luliconazole-loaded NEBGs to assess their appearance, color, homogeneity, consistency, grittiness, and phase separation.

Measurement of pH:
Using a digital pH meter, the pH of the gel based on nanoemulsion was determined. After dissolving 0.5g of NEBG in 50ml of distilled water to create a homogenous dispersion, the mixture was left for two hours. Each formulation's pH was measured at room temperature using triplicate measurements.

Viscosity:
A Brookfield viscometer (DV-II+Pro, Brookfield Engineering Inc., USA) measured formulation viscosity. With spindle number 63, nanoemulsion-based gel viscosities were tested. Three measurements were obtained.

Spreadability:

0.5 gm of gel was placed on a glass slide within a 1 cm circle to test the spreadability of NEBG. Put a second glass slide on top of it, then rest 500 grams

Journal of Chemical Health Risks

www.jchr.org

JCHR (2025) 15(4), 2008-2019 | ISSN:2251-6727

of weight on the top slide. The diameter increased as a result of gel spreading.

.

Spreadability is measured in term of mm^2. Equation to calculate is as followed (79):

$$s = d^2 \frac{\pi}{4}$$

Where,

S = spreading area of nanoemulsion based gel ($mm^{2)}$,

D = spreading area diameter(mm).

2.2.9 Uniformity of drug content:

To attain dose uniformity, the active ingredient must be distributed uniformly. By diluting 1 gram of the formulation in 10 ml of methanol, the drug content was ascertained. After properly diluting it, it was filtered. A UV/Visible double beam spectrophotometer was used to measure the prepared solution's absorbance at 271 nm. Next, the concentration of luliconazole was ascertained.

2.2.10 In vitro drug release:

A drug's release profile provides important information about how it behaves in vivo and forecasts how a delivery system might work[9]. The evaluation of luliconazole loaded with chamomile oil using a gel release profile based on nanoemulsion was done in vitro using a Franz diffusion cell. PBS 7.4:Methanol (60:40) was used to fill the receptor compartment. The donor compartment was filled with gels based on nanoemulsions that contained luliconazole loaded with chamomile oil. A 0.2µm cellulose membrane divided the donor and receptor compartments. The freshly made medium was refiled and a 1 ml sample was taken out at pre-arranged intervals. Using a UV Spectrophotometer, the amount of drug released was measured at 278 nm following the proper dilution with the mixture PBS 7.4: Methanol (60:40)[6].

2.2.11 Permeation and retention studies in ex vivo skin

Permeation analysis

Goat hide was acquired from the abattoir. The mucosal portion was excised in line with the diameter of the Franz diffusion cell. The Franz diffusion cell was used to investigate drug release from the improved luliconazole incorporated with chamomile oil in a nanoemulsion-based gel. PBS 7.4: The receptor compartment was filled with a methanol (60:40) solution. Goat skin delineated the donor compartment from the receptor chamber. One millilitre of the sample was extracted at predetermined intervals, and the freshly prepared medium was replenished. Following an appropriate dilution with the PBS 7.4: Methanol combination (60:40), the concentration of the released medication was quantified using a UV Spectrophotometer at 267 nm. Subsequently, the goat tissue was isolated and purified using a PBS 7.4: Methanol (60:40) solution, followed by water to eliminate any residual medicine. The tissue was then sectioned into minute bits. The sliced specimens were processed using a high shear homogenizer with a PBS 7.4: Methanol (60:40) solution. A high Tissue Homogenizer (Mac, Mumbai, India) was used to homogenize the goat tissue, and the resultant mixture was filtered. Methanol was used again to homogenize the tissue. Following the filtration of the whole material and centrifugation for 20 minutes at 5000 rpm, the supernatant was collected. Following the appropriate dilution of the obtained supernatant, its drug concentration was evaluated using a UV spectrophotometer. The quantity of medicine remaining on the tissue surface and diffused inside it was used to determine the mass balance.

2.2.12 Studies on antifungals:

The cup plate method of the agar diffusion test was used to accomplish this. The usual dosage of 400 mg of luliconazole was given along with the new formulations. Prior to being put on plates containing Candida albicans test organisms and potato dextroOSE agar, all of the previously mentioned preparations were sterilized. Agar plates were left in incubators set to 37°C for 24 hours after the solutions had solidified. The zone of inhibition (ZOI) of each plate was identified and contrasted with the control. Except for incubation, every step of the process was completed in a laminar airflow unit 16.

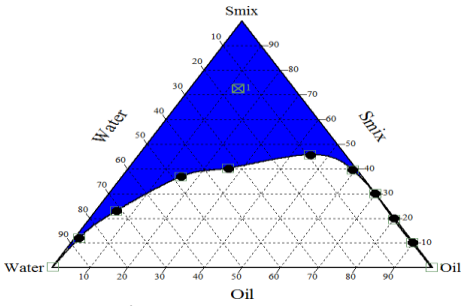
3. Results

3.1. Analytical method:

The range of luliconazole's absorbance in methanol is reported to be between 0.2-0.8 at concentrations between 100 and 350 g/mL. The calibration equation, y=0.0305x+0.0075 has R^2 value of 0.9991.

Journal of Chemical Health Risks
www.jchr.org
JCHR (2025) 15(4), 2008-2019 | ISSN:2251-6727

3.2 Screening of all components that constitute nanoemulsion:



Solubility of luliconazole in various oil, surfactant and co-surfactants was estimated for screening the component for nanoemulsion containing luliconazole. Solubility of luliconazole was highest in chamomile oil among all different oils. Luliconazole showed highest solubility in Tween-80. Among co-surfactant, PEG 400 had higher solubilizing power compared to other co-surfactants so it was selected as co-surfactant. Among surfactants, luliconazole showed higher solubility in Tween-80. As Tween-80 gave clear nanoemulsion so it was selected as surfactant.

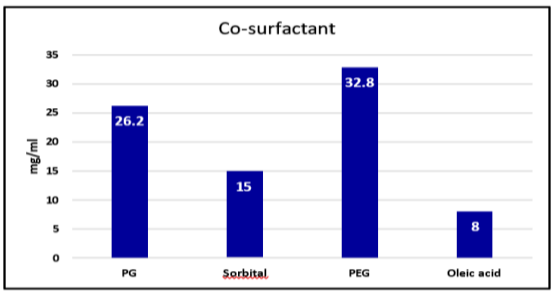


Figure-2: Screening of oil, surfactant and co-surfactant

3.3 Construction of pesudoternary phase diagrams:

By comparing the results of the present studies, compositions of 1:1 and 1:2 revealed that the number of phase diagrams with the highest share of the area of the nanoemulsion increased at the S/CoS weight ratio of 2:1. This is due to enhanced micelle formation as a result of an increase in the S/CoS ratio which enhances the solubilization ability of the nanoemulsion. Additionally, at a S/CoS weight ratio of 2:1, On the same wave length, the results revealed that Chamomile oil produced the largest nanoemulsion. enhanced micelle formation as a result of an increase in the S/CoS ratio which enhances the solubilization ability of the nanoemulsion. Additionally, at a S/CoS weight ratio of 2:1, On the same wave length, the results revealed that Chamomile oil produced the largest nanoemulsion.

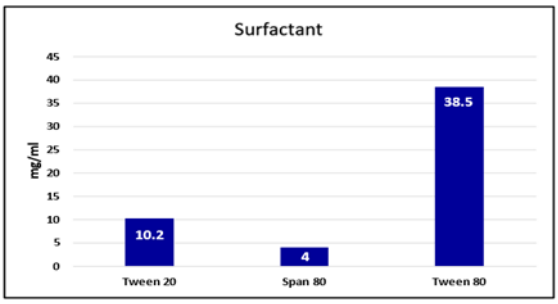


Figure - 3: Pseudoternary Phase Diagram of 1:1 Smix ratio

The pseudo ternary phase diagrams of the Smix (Tween 80: PEG 400) ratio 1:1, 1:2, 1.3,

2:1. and 3:1 of nanoemulsion was constructed. The nanoemulsion area was represented by the dark colored region in the phase diagrams. As 2:1 has larger nanoemulsion area it was selected for further optimization.

Figure-4: Batches of Nanoemulsion

Journal of Chemical Health Risks

www.jchr.org

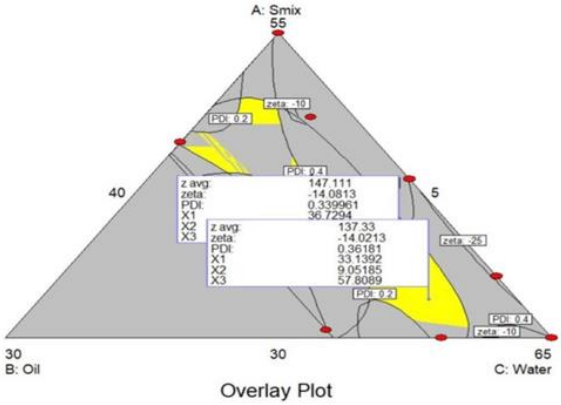
JCHR (2025) 15(4), 2008-2019 | ISSN:2251-6727

3.4 Optimization of nanoemulsion:

Table-1:Results of D-optimal design for nanoemulsion

Sr. no	Batch no.	Smix	Oil	Water	Globule size	Zeta Potential	PDI
1	H1	30	5.004	64.996	27.1	-20.4	0.405
2	H2	35.042	5	59.958	17.5	-27.3	0.215
3	H3	36.448	9.306	54.246	140.4	-14.6	0.315
4	H4	38.68	13.669	47.651	286	-16	0.495
5	H5	43.101	9.429	47.471	148.6	-12.4	0.41
6	H6	46.043	13.957	40	127	-14.7	0.359
7	H7	54.996	5.004	40	44.8	-17.1	0.425
8	H8	48.078	6.981	44.94	110.1	-10.5	0.52
9	H9	30.652	15	54.348	206.1	-15.7	0.409
10	H10	43.003	5	51.997	22.9	-23.9	0.394
11	H11	30	10.046	59.954	140.1	-13.9	0.239

An overlay plot represents a region within a design space where all factors are concentrated within their effective or desired ranges. This means that any combination of factor ranges chosen within this region will yield



Overlay Plot

Figure-5:Overlay plot of luliconazole nanoemulsions

Table-2: Composition of checkpoint batches

Checkpoint Batch	X1 (%Smix)	X2 (%Oil)	X3 (%Water)
CP1	36.72	8.61	54.65
CP2	33.13	9.05	57.8

Table-3:Characterization of optimized nanoemulsion

Physicochemical characteristics	Observation of chamomile oil based Nanoemulsion
% Transmittance	97.4 ± 0.52 %.
Conductivity	84.2 ± 0.25 µs/cm
pH	7.25 ± 0.18
Refractive index	1.39 ± 0.09
Viscosity	128 ± 2.34 Cp
Drug content	97.8 ± 0.54%
Type of nanoemulsion	O/W

formulations that deliver the desired outcome while maintaining robustness. In essence, it's a visual representation that helps identify the optimal parameter ranges for achieving desired outcomes in a formulation process.

A flag batch was generated and formulation was prepared and further evaluation was done.

Validation of checkpoint batch

In order to assess the reliability of the equations that describes the influence of the factors on the responses check point batch were formulated. Composition of check point batch is shown in table 2 and 3.

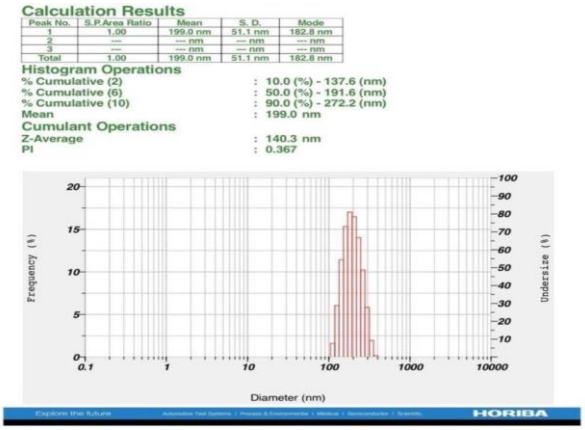
Journal of Chemical Health Risks

www.jchr.org

JCHR (2025) 15(4), 2008-2019 | ISSN:2251-6727

Globule size distribution and Zeta potential

There's a clear link between the size of the globules and how much surfactant is used. Sometimes, when you increase the surfactant concentration, you'll notice the droplets tend to have a smaller average size. This can be explained by how the surfactant molecules stabilize the oil droplets by gathering at the oil-water interface.



also, the size of these globules can influence how effectively a drug permeates through a system. Essentially, the smaller the globules, the greater the interfacial area available, which can enhance drug permeation.

Globule size (nm) of formulation was found to be **140.3 nm**, **PDI** as **0.367** and **-14.6 mV as Zeta potential**.

Figure-6: Globule size distribution and PDI

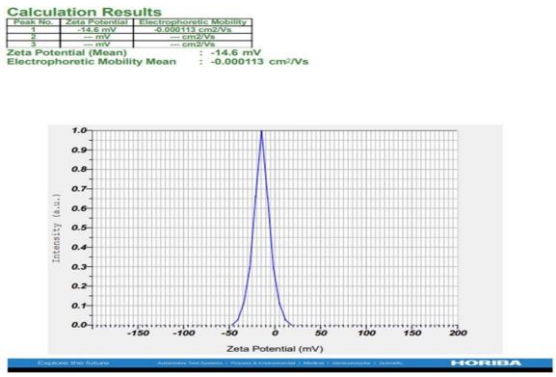


Figure-7: Zeta potential

3.5 Characterization of CTM Nanoemulsion System:

The optimised nanoemulsion, which had a PDI of 0.458 and the smallest globule size of 208 nm, contained 70% Smix, 5% oil, and 20% water. The measured zeta potential was -0.9 mV. Nanoemulsion and in-situ gel were discovered to have viscosities of 33 cP and 181 cP, respectively, and a pH of 6.71. According to the results of conductivity, which is 0.343 ms/cm, all nanoemulsions were of the o/w type. [14]

3.5.1 Transmission electron microscopy (TEM)

The prepared formulation of nanoemulsion was evaluated for globule size and aggregation. The globule of optimized luliconazole loaded nanoemulsion appeared to be almost round in shape, distributed uniformly and do not showed aggregation in transmittance electron microscope. Image of formulation is as such and magnified, showed in below figure.

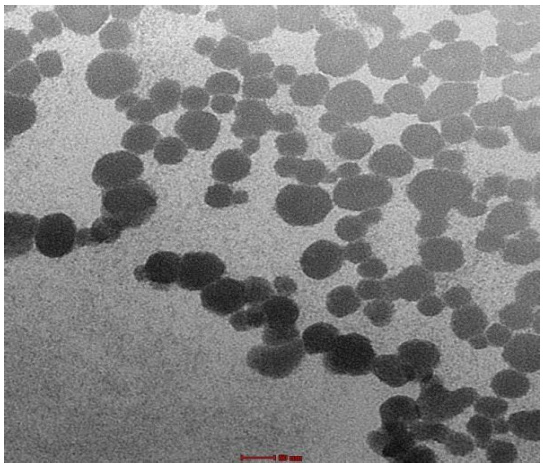


Figure-8:TEM images of nanoemulsion

3.6 *In vitro* drug release study

The maximum drug release of 90.0% from luliconazole loaded nanoemulsion based gel was achieved within 4 hours while in case of nanoemulsion 89.21% of drug release was achieved.[10]

Table-4: Result of In vitro drug release study

Time (min)	Nanoemulsion based gel (%CPR)	Nanoemulsion (%CPR)
30	24.5	25.62

Journal of Chemical Health Risks
www.jchr.org
JCHR (2025) 15(4), 2008-2019 | ISSN:2251-6727

60	38.1	49.33
90	50.06	66.96
120	61.54	82.51
150	74.6	89.21
180	80.91	
210	86.01	
240	90.06	

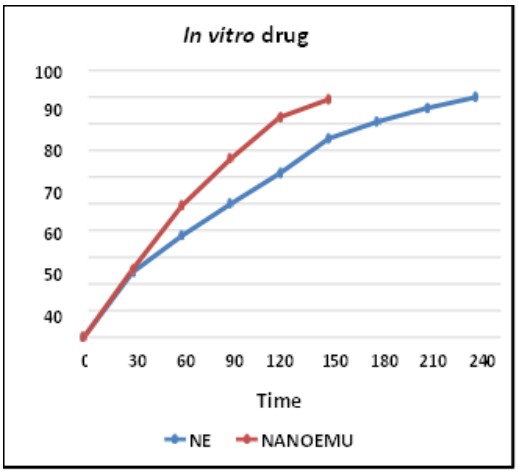


Figure-9: *In vitro* drug release study

3.7 *Ex-vivo* drug permeation study

Table-5: Result of ex vivo permeation study

Time (minute)	%CPR
30	8.69
60	11.98
90	15.45
120	18.53
150	26.3
180	29.28
210	41.61
240	45.5
270	52.12
300	65.48

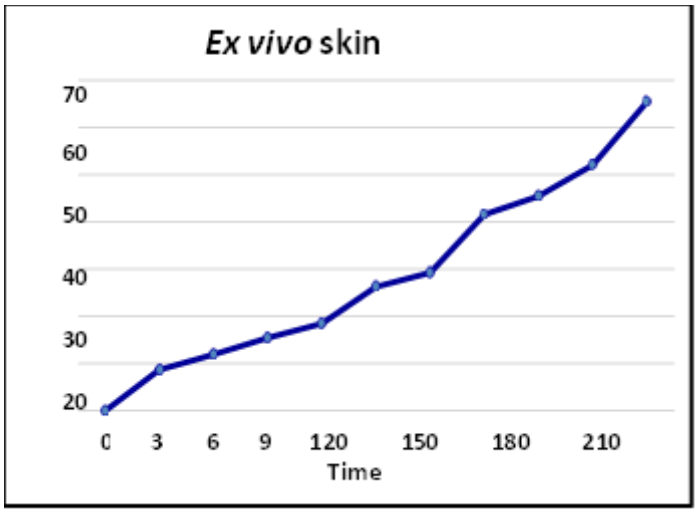


***Figure-10: Ex vivo* skin permeation study**

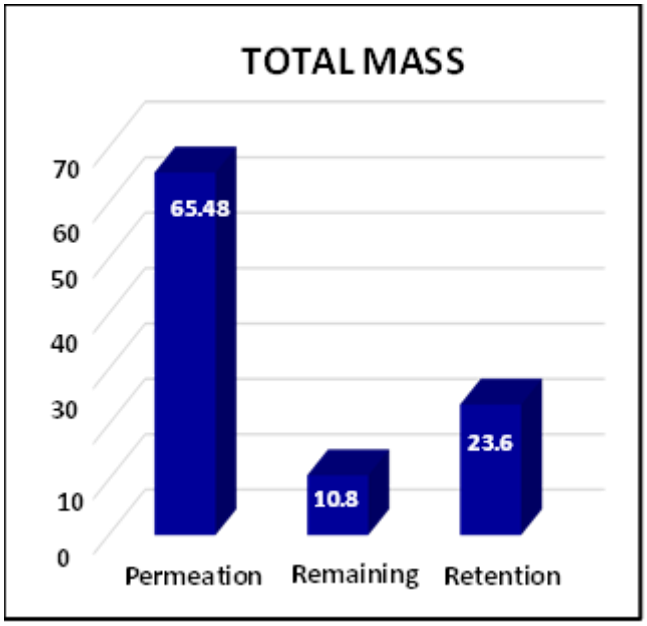


Figure-11:Mass Balance of Nanoemulsion based gel

Ex vivo skin permeation study on goat buccal mucosa was carried out for nanoemulsion based gel. Table 49 shows the %CPR of NEBG formulations. The permeation

Journal of Chemical Health Risks
www.jchr.org
JCHR (2025) 15(4), 2008-2019 | ISSN:2251-6727

profile of NEBG formulations through goat buccal mucosa is shown in figure 57. After 5 hours, the NEBG exhibited a permeation of 65.48%. Additionally, the retention of luliconazole within the skin was 23.6% for the NEBG. These results shows that the NEBG formulation enhances both the permeation and retention of luliconazole .

The ex vivo skin permeation investigation of NEBG demonstrated a satisfactory permeation profile through goat skin. The prepared nanoemulsion may function as a drug reservoir, facilitating the release of the drug from the internal phase to the exterior phase and enhancing permeability by modifying or compromising the tight junctions of the mucosal epithelium. The NEBG demonstrated enhanced penetration owing to its inherent properties that promote adhesion to mucosa, hence prolonging contact duration and facilitating improved diffusion. [8]

4. Result of Comparative Antifungal studies

Figure12:Comparative Antifungal Study (SET 1)

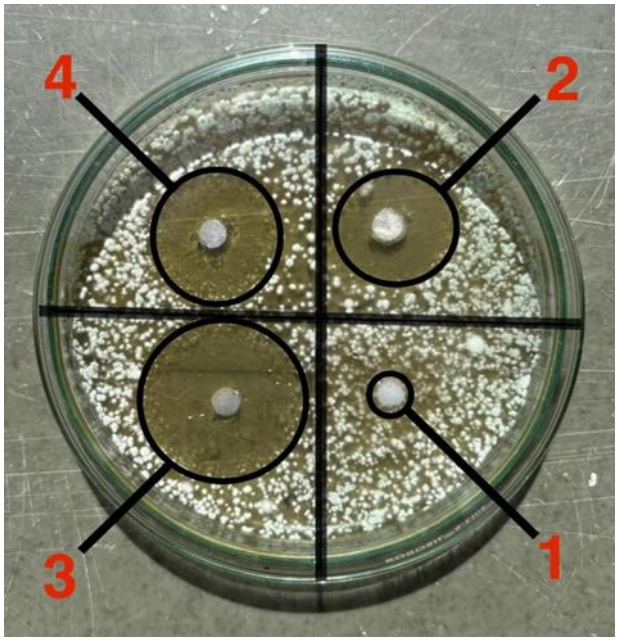


Table-6:Comparative Antifungal Study (SET 1)

Sr. No	Formulation	ZOI (mm) Diameter SET 1	ZOI (mm) Diameter SET 2	ZOI (mm) Diameter SET 3	MEAN	SD
1	Blank nanoemulsion	5	4	8	8	1.699673
2	Drug loaded nanoemulsion	20	24	26	26	2.494438
3	Drug loaded nanoemulsion based gel	28	25	32	32	2.867442
4	Marketed formulation	25	22	20	25	2.054805

Antifungal activity of luliconazole loaded nanoemulsion gel was compared with different prepared formulation and marketed formulation through its zone of inhibition. The antifungal activity based on zone of inhibition of chamomile oil comprising nanoemulsion based gel was found to be more effective than other prepared formulation as well as marketed formulation. Based on observations, it was found that zone of inhibition of 0.4% of NEBG was higher than marketed formulations.

5. Conclusion:

Luliconazole an antifungal agent has very poor aqueous solubility which limits the amount that is to be formulated gel (0.4%) for oral mucosal drug delivery. Tween 80 and PEG 400 were used as surfactant and co-surfactant respectively. Carbopol 934P used as gelling polymer. The pseudo ternary phase diagram studies of different Smix ratio concluded that 2:1 gives better nanoemulsion region. Composition of nanoemulsion was optimized using D-optimal design in which globule size, Poly dispersity index and zeta potential were taken as responses. Nanoemulsion BATCH CP2 was taken as optimized batch as it covered all aspects of responses and it was procured in overlay plot.

Optimized nanoemulsion contained 33.13% Smix, 9.05% oil, 57.80% water. Which has smallest globule size of 140 nm and PDI was 0.367. Zeta potential was obtained as -14.7 mV. Viscosity of nanoemulsion and nanoemulsion based gel 128 cP and 1380 cP respectively and pH of nanoemulsion was 7.25 and nanoemulsion based gel is 5.82. All nanoemulsion were O/W type as per results of conductivity which is 84.2 µs/cm. Optimized nanoemulsion was subjected to thermodynamic stability studies in which no phase separation was observed, conforming that nanoemulsion was stable.

In-vitro permeation study showed that the

Journal of Chemical Health Risks

www.jchr.org

JCHR (2025) 15(4), 2008-2019 | ISSN:2251-6727

nanoemulsion without gelling agent showed faster release of drug compared to nanoemulsion based gel. Slower release of drug in gel may be due to the gelling polymer[5].

Ex-vivo release was also conducted for Nanoemulsion based gel. At 5 hours 65.48% drug release was observed. Combination of Chamomile oil and luliconazole containing nanoemulsion based gel was increase the spectrum of antifungal activity. Antifungal activity of nanoemulsion based gel was compared with marketed formulation through its zone of inhibition. Based on observations, it was found that zone of inhibition of 0.4% of NEBG was higher than other marketed formulations. 0.4% of NEBG was hence proved to be more effective then other marketed formulation, that too with relatively lower dose of luliconazole . Thus, the chances of drug resistance shell be minimal[15].

References

(1) Piérard, G. E.; Hermanns-Lê, T.; Delvenne, P.; Piérard-Franchimont, C. Miconazole, a Pharmacological Barrier to Skin Fungal Infections. *Taylor & FrancisGE Piérard, T Hermanns-Lê, P Delvenne, C Piérard-FranchimontExpert opinion on pharmacotherapy, 2012•Taylor & Francis* 2012, *13* (8), 1187–1194. https://doi.org/10.1517/14656566.2012.687047.

(2) Puškárová, A.; Bučková, M.; Kraková, L.; reports, D. P.-S.; 2017, undefined. The Antibacterial and Antifungal Activity of Six Essential Oils and Their Cyto/Genotoxicity to Human HEL 12469 Cells. *nature.comA Puškárová, M Bučková, L Kraková, D Pangallo, K KozicsScientific reports, 2017•nature.com*. https://doi.org/10.1038/s41598-017-08673-9.

(3) JR Baker - US Patent App. 13/096, 836; 2011, undefined. Methods of Using Nanoemulsion Compositions Having Anti-Inflammatory Activity. *Google Patents*.

(4) MF Roca - US Patent 11, 540,981; 2023, undefined. Nanoemulsion Formulation with Improved Stability and Cell Penetration. *Google Patents* 2023.

(5) Kaplan, A.; Cetin, M.; … D. O.-J. of D. D.; 2019, undefined. Formulation and in Vitro Evaluation of Topical Nanoemulsion and Nanoemulsion-Based Gels Containing Daidzein. *Elsevier*.

(6) Gokhale, J.; Mahajan, H.; Pharmacotherapy, S. S.-B. &; 2019, undefined. Quercetin Loaded Nanoemulsion-Based Gel for Rheumatoid Arthritis: In Vivo and in Vitro Studies. *ElsevierJP Gokhale, HS Mahajan, SJ SuranaBiomedicine & Pharmacotherapy, 2019•Elsevier*.

(7) Sienkiewicz, M.; Głowacka, A.; Poznańska-Kurowska, K.; Kaszuba, A.; Urbaniak, A.; Kowalczyk, E.; Głowacka MUL, A. The Effect of Clary Sage Oil on Staphylococci Responsible for Wound Infections. *termedia.plM Sienkiewicz, A Głowacka, K Poznańska-Kurowska, A Kaszuba, A Urbaniak, E KowalczykAdvances in Dermatology and Allergology/Postępy Dermatologii i Alergologii, 2015•termedia.pl* 2015, *1*, 21–26. https://doi.org/10.5114/pdia.2014.40957.

(8) Mohammed, S.; Falih, J.; Falih, S. M. J.; Abd, A.; Abd, A.; Al-Ali, A.; Al-Ali, A. A. A. Determination of Ex Vivo Chemical and in Vivo Biological Antioxidant Activities of Clary Sage Essential Oil. *journals.ekb.egS Falih, A Al-AliJournal of Bioscience and Applied Research, 2024•journals.ekb.eg* 2024, *10* (3), 2356–9182. https://doi.org/10.21608/jbaar.2024.259473.1030.

(9) Blaskó, Á.; Gazdag, Z.; Gróf, P.; Máté, G.; Sárosi, S.; Krisch, J.; Vágvölgyi, C.; Makszin, L.; Pesti, M. Effects of Clary Sage Oil and Its Main Components, Linalool and Linalyl Acetate, on the Plasma Membrane of Candida Albicans: An in Vivo EPR Study. *SpringerÁ Blaskó, Z Gazdag, P Gróf, G Máté, S Sárosi, J Krisch, C Vágvölgyi, L Makszin, M PestiApoptosis, 2017•Springer* 2017, *22* (2), 175–187. https://doi.org/10.1007/S10495-016-1321-7.

(10) Nafee, N.; Ismail, F.; Boraie, N.; of, L. M.-I. journal; 2003, undefined. Mucoadhesive Buccal Patches of Miconazole Nitrate: In Vitro/in Vivo Performance and Effect of Ageing. *ElsevierNA Nafee, FA Ismail, NA Boraie, LM MortadaInternational journal of pharmaceutics, 2003•Elsevier*.

Journal of Chemical Health Risks

www.jchr.org

JCHR (2025) 15(4), 2008-2019 | ISSN:2251-6727

(11) Ahmed, T.; Mahmoud, M.; … A. S.-… J. of D.; 2011, undefined. Formulation, Evaluation and Optimization of Miconazole Nitrate Tablet Prepared by Foam Granulation Technique. *search.proquest.comTA Ahmed, MF Mahmoud, AM Samy, AA Badawi, KE GabrInternational Journal of Drug Delivery, 2011•search.proquest.com*.

(12) Sadique, A.; Khalid, S.; Asghar, S.; … M. I.-L. A. J.; 2018, undefined. Miconazole Nitrate Microemulsion: Preparation, Characterization and Evaluation for Enhancement of Antifungal Activity. *researchgate.netA Sadique, SH Khalid, S Asghar, M Irfan, M QadirLat Am J Pharm, 2018•researchgate.net*.

(13) Yen, C. C.; Chen, Y. C.; Wu, M. T.; Wang, C. C.; Wu, Y. T. Nanoemulsion as a Strategy for Improving the Oral Bioavailability and Anti-Inflammatory Activity of Andrographolide. *Int J Nanomedicine* 2018, *13*, 669–680. https://doi.org/10.2147/IJN.S154824.

(14) Sun, H.; Liu, K.; Liu, W.; Wang, W.; Guo, C.; Tang, B.; Gu, J.; Zhang, J.; Li, H.; Mao, X.; Zou, Q.; Zeng, H. Development and Characterization of a Novel Nanoemulsion Drug-Delivery System for Potential Application in Oral Delivery of Protein Drugs. *Int J Nanomedicine* 2012, *7*, 5529–5543. https://doi.org/10.2147/IJN.S36071;PAGE:STRING:ARTICLE/CHAPTER.

(15) Khani, S.; Keyhanfar, F.; Amani, A. Design and Evaluation of Oral Nanoemulsion Drug Delivery System of Mebudipine. *Drug Deliv* 2016, *23* (6), 2035–2043. https://doi.org/10.3109/10717544.2015.1088597.

(16) ABaroni, A.; Buommino, E.; De Gregorio, V.; Ruocco, E.; Ruocco, V.; Wolf, R. Structure and Function of the Epidermis Related to Barrier Properties. *Clin Dermatol* 2012, *30* (3), 257–262. https://doi.org/10.1016/J.CLINDERMATOL.2011.08.007.

(17) Farooq, U.; Rasul, A.; Zafarullah, M.; Abbas, G.; Rasool, M.; Ali, F.; Ahmed, S.; Javaid, Z.; Abid, Z.; Riaz, H.; Mahmood Arshad, R. K.; Maryam, S.; Amna, N.; Asif, K. Nanoemulsions as Novel Nanocarrieres for Drug Delivery across the Skin: In-Vitro, in-Vivo Evaluation of Miconazole Nanoemulsions for Treatment of Candidiasis Albicans. *Des Monomers Polym* 2021, *24* (1), 240–258. https://doi.org/10.1080/15685551.2021.1965724;JOURNAL:JOURNAL:TDMP20;WGROUP:STRING:PUBLICATION.