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# Formulation and In vitro Evaluation of Peppermint Oil Based Nanoemulgel of Luliconazole for Topical Application

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## Abstract

Background: Luliconazole is an imidazole antifungal agent belonging to dichlorobenzene class of organic compounds and is an optically active R enantiomer. It is practically insoluble in water. The study aims to formulate and evaluate gellified nanoemulsion of luliconazole as a topical usage to intensify antifungal activity by improving dispersibility and permeation of luliconazole. Methods: Three pseudo ternary phase diagrams were fabricated, including surface active agent mixture (S mix) represented by between 80 as surfactant and ethanol as cosurfactant at proportions of (1:1, 2:1 and 3:1), peppermint oil as an oil phase and aqueous phase. Fourteen formulas were prepared by using aqueous titration method and were taken for the characterization of prepared nanoemulsions, and then 0.5% carbopol 934 incorporated into selected formulas to obtain nanoemulgel formulas (G-1 to G-6). These six nanoemulgels were introduced to specific evaluations. Results: G-2 was the optimal formula with oil: Smix (3:1): water (15:40:43.5) ratio containing 1% drug and 0.5% carbopol 934 characterised by fine droplet size (26.26), satisfactory polydispersity index PDI (0.30), excellent physical appearance, pH (6.1), which is within the range of the topical medication, accepted per cent of luliconazole content (98.13), encouraged viscosity (1131.72 mPa.sec) and higher releasing profile of the drug that improve therapeutic efficacy. The optimized formula subjected to further investigation that was given zeta potential (-10.48). Morphological studies demonstrated that the optimized formula (G-2) was nano-sized globules virtually shaped like a sphere; this suggests stability of intended nanoemulgel (G-2). According to FTIR studies, there was no interaction between luliconazole and the excipients used. Eventually, the luliconazole nanoemulgel (G-2) gave close antifungal bustle when contrasted to that of marketed Micazole® gel. Conclusions: The results advocate the future applying of developed nanoemulgels as vehicles for topical dosage form the transportation of luliconazole.

## Keywords

Nanoemulsion, Luliconazole, Nanoemulgel, Carbopol 934, Pseudo Ternary Phase Diagrams.

Luliconazole is a new drug and a wide spectrum antifungal of an imidazole applicant for the treatment of skin fungal infections. It is particularly inhibiting fungal cytochrome P 450 14-αdemethylase enzyme, which destroys the transformation of lanosterol to ergosterol, thus retards the cell wall creation of fungi. The drug potency is great opposed to filamentous species, recommended in eradicating of different interdigital fungal infections and dermatophytosis such

as *Tinea pedis, Tinea cruris* and *Tinea corporis* caused by the organisms *T. rubrum and E. floccosum.* [1]

The luliconazole solubility is very low and will be the problem that hampers the permeation of the luliconazole through the skin layers when topically applied to. On the other hand, traditional topical cream formulations be blessed with a number of obstacles of low permeation from the skin barrier layer as well as lower retention at the site of infection. [2]

To defeat these barriers, various nano-carrier approaches have been occupied for the industrial production of nanoparticles by dissimilar features for the management of skin disorders. Nano-carriers that have been advanced and completely tested for their *in vitro* dissolution properties of drug, these include nano capsules, solid lipid nanoparticles, micelles, nano-gels, nano spheres, nanoemulsions, micro-emulsions, liposomes and etc. The ability of these nano carriers to comfortably penetrate the skin layers and consequently, they can simply deposit under the skin. [3]

Nanoemulsion is optically transparent or translucent with the globule sizes range from one hundred to five hundred nm. It is composed of the oil, surfactant, co-surfactant and aqueous phase. [4]

Besides, its low viscosity causes less retention into the skin and makes it inappropriate for putting onto the human skin. [5]

This restriction is answered by appending gelling agents to made fit product for topical application, widely known as nanoemulgel. [6]

Almost all of the non-hydrophilic drugs cannot be integrated at once into the gel base because the solubility trouble that becomes noticeable throughout the drug release. Nanoemulsion based gel aid in the lipophilic drugs amalgamation into the oil phase and after that oil droplets are dispersed in an aqueous phase leading to oil in water (o/w) nanoemulsion then the prepared nanoemulsion can be merged into gelling agent to gain appealing delivery network that is nanoemulgel. [4]

Hence, the purpose of this project is to formulate and evaluate gellified nanoemulsion of luliconazole used topically, so, to enhance the therapeutic efficacy that is by enhancing the different pharmacokinetic criterion of the drug molecule such as solubility, permeability and thus local availability with non-irritant nature (fewer side effects).

# Materials and Methods

## Materials

Luliconazole was provided by Hyper-Chem LTD CO, China. Peppermint oil was gifted from Alpha Chemika, India. Methanol and ethanol from Haymankimia, United Kingdom. Tween 80 from Alpha Chemika, India. Carbopol 934 was purchased from Sigma Aldrich, USA. KH2 PO 4 from Central Drug House (p) limited, Delhi India. Na2 HPO4 and Triethanolamine from Thomas Baker (chemicals) Pvt, Ltd India.

# Methods

## Pseudo ternary phase diagram construction

According to the results from the saturated solubility study, the diagram of pseudo ternary phase was fabricated using aqueous titration method (low energy emulsification). Distilled water (DW) has been used as an aqueous medium, participated with varied Smix of tween 80 and ethanol in 1:1, 2:1, 3:1 ratios, on the basis of rising surfactant concentration at a constant level of co-surfactants. For drawing each phase diagram, chosen oil was peppermint oil (as oil phase) combine gradually with mixture of surfactant and cosurfactant (Smix) at accurate proportions for each phase diagram in unlike vials of glass in ratios of (9:1, 8:2, .....1:9) (w / w). A transparent and constant mixed by a vortex mixer for five minutes was subjected to each Smix. After that, each mixture was titrated slowly with mixing by a vortex at room temperature (without heating) with aqueous phase (DW) in a drop-wise fashion with observation of system clarity. [7]

The amount of DW at which the end point of the titration was detected (change from clear to turbid). Then, these points were utilised to detect the borders on the nanoemulsion zone that suit the the oils chosen. [8]

## Preparation of luliconazole loaded nanoemulsion

Different formulations of O/W nanoemulsion have been fabricated according to pseudo-ternary phase diagrams using the Smix mixture and oil concentrations by water titration method. The preparation of 5 gm of 1% luliconazole nanoemlsion has been formulated

through dissolving 50mg of luliconazole in (peppermint oil as desired quantity) using a vortex mixer for 10 minutes, afterwards the addition of the chosen S-mix in a constant proportion for oil loading drug until a clear solution was observed, after that, the aqueous phase (DW) was titrated drop wisely to form a clear (o/w) nano-emulsion. [9, 10]

## Globule size measurement

The average particle size for luliconazole nanoemulsion droplets was performed by Malvern (Germany) particle size analyser. Using the dynamic light scattering method, the globule size of samples was detected by scanning alterations in light scattering originated from Brownian motion (for particles). The samples to be tested were prepared by diluting small sample of nanoemulsion in double DW to avoid multiple scattering, then moving the diluted sample in the analyser piece for assessment. [11]

## Polydispersity index (PDI) measurement

The determination of PDI of nanoemulsion was done by (Malvern particle size analyzer) to inspect the homogeneity of droplet size. The ranges of PDI are from zero to one. [12]

## Selection of the drug formulas for preparation of nanoemulgel

The selection of luliconazole loaded o/w nanoemulsions subjected to nanoemulgel preparation depending on the particle size tested less than 100nm as well as all the remaining studies.

## Preparation of luliconazole nanoemulgel

The low viscosity of nanoemulsion render it is easily removed because of high content water and gives low skin retention when applied topically. A carbopol 934 was utilised to develop the luliconazole nanoemulsion gel. The first step was the preparation of aqueous dispersion for gelling agent (carbopol 934), the dispersion phase was left for one day (at 4 °C) to eradicate air bubbles and complete swelling achieved. Then, the aqueous dispersions of polymer (0.5% w/v) gently combined with selected luliconazole loaded nano emulsions (containing drug equivalent 1%) which was contained low amount of water while stirring constantly with a magnetic stirrer. During the preparation method, the pH of the obtained gel should be neutralized by increasingly adding few drops of triethanolamine to pH 6-6.5, leading to production a desired topical gel of luliconazole o/w nanoemulsion. [13, 14]

The compositions of prepared luliconazole nanoemulgels were manifested in table (1)

**Table (1):** Composition Of Luliconazole Nanoemulgels

| Carbopol 934 %w/w | DW %w/w | Smix ratio | Tween80:Ethanol %w/w | Oil %w/w | Drug %w/w | NEG no. |
|---|---|---|---|---|---|---|
| 0.5 | 23.5 | 2:1 | 60 | 15 | 1 | **G-1** |
| 0.5 | 43.5 | 3:1 | 40 | 15 | 1 | **G-2** |
| 0.5 | 33.5 | 3:1 | 50 | 15 | 1 | **G-3** |
| 0.5 | 33.5 | 2:1 | 45 | 20 | 1 | **G-4** |
| 0.5 | 33.5 | 3:1 | 45 | 20 | 1 | **G-5** |
| 0.5 | 23.5 | 3:1 | 55 | 20 | 1 | **G-6** |

## Characterization of the prepared luliconazole nanoemulgel Homogeneity of luliconazole loaded nanoemulgels

The organoleptic and homogeneity characteristics of the produced luliconazole loaded nanoemulsion gel were evaluated by visual investigations. This including appearance, colour, transparency, phase separation, homogeneity and consistency. [15]

## pH measurement

The pH values of luliconazole o/w nanoemulgels were measured using Digital pH meter at 25±0.5ºC, the pH of terminal topical nanoemulgels is significant for their harmony with the skin pH to prevent any irritation. The investigation was repeated in triplicate. [16]

## Viscosity measurement

The NDJ-5S digital viscometer with spindle no. (3) was used for measuring the rheological properties of prepared nanoemulgel formulations by inserting the spindle into cylinder containing gel and rotating at 6, 12,

30, and 60 rpm at room temperature. The test was achieved in three copies. [17]

### Drug Content Determination

The drug content of prepared emulgel was determined by dissolving one gram of the formulation in 100 ml methanol, suitable dilutions were done and then filtering using Millipore syringe filter (0.45 µm). Luliconazole concentration was calculated by reading the absorbance (at 296 nm) using UV-vi spectrophotometric analysis through linear equation of methanol calibration curve. [18]

### In vitro drug release study

The release of luliconazole from nano-emulgel was carried out using the dialysis bag method. The experiment was performed in a USP dissolution apparatus type II (paddle technique) with a 50-rpm rotation speed at temperature of 37 ± 0.5 °C was maintained of release medium (900 ml). Previously, the dialysis bags were soaked (for 24 hr) in dissolution media (phosphate buffer pH 7.4 with 2% Brij 35). Then, each dialysis bag contains 1 ml (approximately 1gm) of nanoemulgel (equals to 10 mg of drug), tightly sealed from both ends and submerged in a using medium comprising 900 ml phosphate buffer pH (7.4 with 2% Brij 35). The samples of 5 mL were withdrawn at predominant intervals and replaced with a new solution. Afterwards, the calculation of drug concentration in each sample at detected intervals using

UV-VIS spectrophotometric at its maximum wavelength (at 299 nm). [19, 20]

### Evaluation of the optimum formula

#### Zeta potential measurement

The nanoemulsion's zeta potential was detected by using Malvern zeta sizer. The final results were recorded at the time the samples were sited in dirt-free disposable zeta cells. [11]

### Fourier transforms infrared spectroscopy (FTIR)

To decide any probable interactions between the medication and the excipients used in the formulation. Samples (pure drug and the optimum formula) were tested using FTIR spectroscopy from 4000-400cm$^{-1}$. [21]

### Field Emission Scanning Electron Microscopy (FESEM)

The configuration and surface morphology of the optimised formulation of luliconazole nanoemulsion was determined by FESEM. The sample was fixed to an aluminium stub by using carbon tape then gold coating was done with ion sputter MC1000. [22]

### Atomic Force Microscopy (AFM)

The AFM is able to scan surfaces at specific circumstances and can estimate the droplet size of the nanoparticles precisely. Following drying the formula, AFM was used to examine the size and surface morphology of luliconazole nanoemulsion. [23]

### Determination of in vitro antifungal activity

*In vitro* antifungal activity of luliconazole of the optimised nano emulsion formulation (F-6) and drug loaded-nanoemulgel G-2 was performed exploiting *Candida albicans* as indicative fungi, embracing the cup plate method. Marketed miconazole gel (Micazole® gel) was taken as a reference standard. Suspension of *Candida albicans* was streaked on previously prepared "sabouraud dextrose "agar medium by dissolving 65gm agar in 1000 ml distilled water then pH was adjusted to 6.8 and sterilized by autoclaving. It was cooled down to 45-50°C then poured into sterile petridish. Wells were done in plate exploiting borer and the formulations were discharged into wells. These plates were incubated at 37°C for one day; the inhibition zone breadth limited each well was calculated exploiting a ruler. [24]

## Results and Discussion

### Development of pseudoternary phase diagram

As shown in figure 1, three phase diagrams were drawn individually at each S-mix ratio of (1:1, 2:1, 3:1) in order to distinguish the region of nanoemulsion with proper oil, S-mix and water ratios for the preparation of nanoemulsion. According to the resultant diagrams, by using different Smix ratios, different nanoemulsion regions were gained in each phase diagram, the larger shaded area demonstrates greater emulsification tendency of the system. [25]

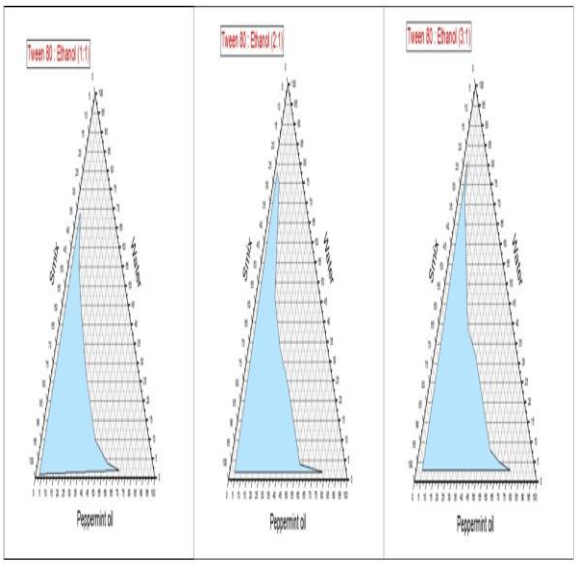



**Figure (1):** Pseudo ternary phase diagram of peppermint oil, Tween 80, ethanol and distilled water for different S-mix ratio.

The results revealed that almost the same area was noticed for all Smix ratios with a slight increase in nanoemulsion area with more concentrations of surfactant (tween 80) when compared with cosurfactant (ethanol). In consequence, drawing with Smix ratios (1:1, 2:1 and 3:1) exhibit the following descending order for areas 3:1> 2:1>1:1, wider nanoemulsion area at 3:1 Smix figures. The increasing in this area of nanoemulsion with Smix proportion would be ascribed to the enlargement in HLB scale of nanoemulsion state originated from addition of hydrophilic tween 80 with HLB 15 [26] leading to enhanced hydrophilicity of dispersion system with better micelle configuration, increased solubilizing capacity of nanoemulsion by the penetration of co-surfactant into holes between surfactants and then good aqueous miscibility. Furthermore, high stable nanoemulsions were manufactured with the increase in surfactant tween 80 concentration which diminishes the interfacial tension and as a result of upraise the o/w interface fluidity resulting in increased entropy of nano system. [27, 28]

## Preparation of luliconazole loaded o/w nanoemulsion formulations

Fourteen formulations of luliconazole o/w nano-emulsion were prepared with (Smix ratio and weight percent used of Smix, water, oil and drug for each nanoemulsion).

The formulas taken for luliconazole o/w nanoemulsions formulation from each phase diagram contained minimum concentrations of peppermint oil (15% and 20% weight percent) were sufficient to totally solubilise the desired dose of drug (1% weight percent) according to results of saturated solubility study.

## Characterization of the prepared luliconazole nanoemulsion

### Particle size measurement

The droplet size outcomes as summarized in table (2) were ranged from 26.2 nm for F-6 to 327.8 nm for F-10. That means all prepared nano emulsion formulation possess droplets size in nano scale. In general, the larger surface area resulted from the small size globules which in turn promotes the drug penetration and permeation through skin layers as well as dissolution and finally absorption at site of action.

## Poly dispersity index (PDI) assay

As shown in table (2), all prepared luliconazole formulation, PDI results were from (0.16 to

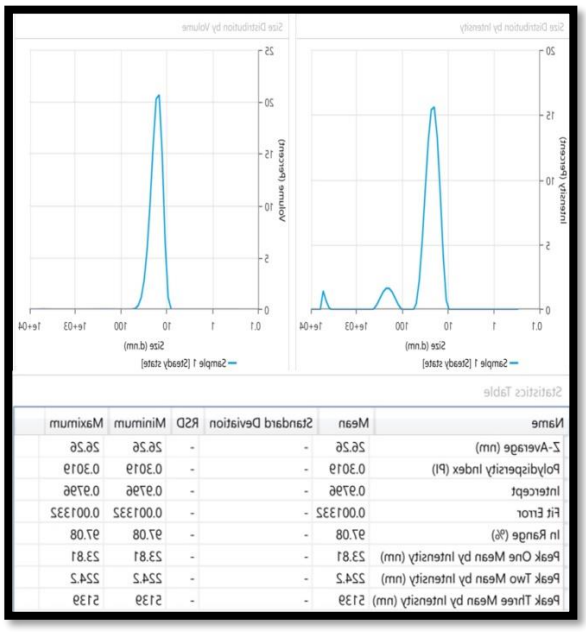



**Figure 2:** Globule size measurement and PDI measurement of luliconazole nanoemulsion F-6

0.66) which manifested those luliconazole nanoemulsion formulations possessed a wide uniform and limited size distribution.

**Table (2):** Particle Size Measurement &Polydispersity Index (PDI) of Nanoemulsions.

| Formula no. | Particle size | PDI |
|---|---|---|
| F-1 | 141.8 | 0.39 |
| F-2 | 180.5 | 0.29 |
| F-3 | 135.4 | 0.40 |
| F-4 | 102.2 | 0.66 |
| F-5 | 29.2 | 0.17 |
| F-6 | 26.2 | 0.30 |
| F-7 | 40.6 | 0.28 |
| F-8 | 204.5 | 0.33 |
| F-9 | 266.7 | 0.18 |
| F-10 | 327.8 | 0.23 |
| F-11 | 36.2 | 0.38 |
| F-12 | 173.8 | 0.46 |
| F-13 | 54.6 | 0.16 |
| F-14 | 27.4 | 0.36 |

## Preparation of luliconazole o/w nanoemulsion based gel

As it is known, the low viscosity of nanoemulsion is one of the common drawbacks that limits direct nano-emulsion using upon topical application, therefore the selected formulations should be prepared as luliconazole nanoemulgel by using carbopol 934.

Firstly, the gel base 0.5% Carbopol 934 (w/v) was constructed with entirely swollen in the aqueous phase. Then, nanoemulsion slowly mixed with the constructed gelling agent. Leading to agglomeration were not occurred due to the entire distension of the polymer. Inversely, if the polymer was added directly to the nanoemulsion system throughout the preparation of nanoemulgel, finally the disadvantages summarised as (1) the formulation came to be white and turbid which attributed to the dehydration of known constituents (surfactant and co-surfactant) causing the separation of polymers from their typical hydrated state and (2) the fabrication of mini-agglomerates due to insufficient swelling of carbopol 934 as polymer resulting in the formation of non-homogeneous gel containing nano emulsion.[14]

## Characterization of luliconazole nanoemulgel

## Homogeneity of luliconazole loaded o/w nanoemulsion based gel

All of the advanced formulations had an elegant appearance of bright yellow colour with a characteristic odour of peppermint oil and clear homogenous gel with no rough particles or aggregates feels upon thumb pressing, as well as appropriate consistency with no disconnecting particles or phase separation observed upon visual careful examination. [29]

## The results of the pH measurement

The pH values of the prepared luliconazole o/w nanoemulsion based gel formulation is illustrated in table (3), which lies in the pH range of the skin (4.5-6.5), proposing that topical administration at the skin surface is well suited without causing side effects like irritation. [30]

## Drug content

Drug content of the prepared luliconazole nanoemulgel formulation was in a range of (96.22-104.20) as manifested in table (3). All these results were established within the standard outline range.

**Table (3):** The physical appearance, pH and the percent of drug content in luliconazole nanoemulgels

| Nanoemulgel code | Colour | Clearness | Homogeneity | Phase separation | PH | %Drug Content |
|---|---|---|---|---|---|---|
| G-1 | Yellow | Transparent | Excellent | None | 6.2±0.26 | 98.05±0.23 |
| G-2 | Yellow | Transparent | Excellent | None | 6.1±0.24 | 98.13±0.05 |

| | | | | | | |
|---|---|---|---|---|---|---|
| G-3 | Yellow | Transparent | Excellent | None | 6.5±0.05 | 96.22±0.68 |
| G-4 | Yellow | Transparent | Excellent | None | 6.3±0.27 | 98.11±0.67 |
| G-5 | Yellow | Transparent | Excellent | None | 6.4±0.29 | 104.2±0.68 |
| G-6 | Yellow | Transparent | Excellent | None | 6.4±0.25 | 98.09±0.45 |

## Viscosity measurements

The gel usefulness of luliconazole o/w nanoemulsion is managed by its rheological behavior, which controls the developed gel consistency, spreadability, flowability, and drug release from nanoemulsion gel.

As detailed in table (4), the prepared luliconazole o/w nanoemulgel manifests non-newtonian pseudoplastic behavior with shear-thinning viscosity profile suggesting the evolution of colloid network structure because of polymer (carbopol) chain bonding lining up itself in the river of shear and when shear rate increases cause decreasing viscosity, that means hindering free flow and the gel design permits satisfactory spreadability and skin sticking of the developed product on the infected region.[31]

It is concluded that increase the surfactant concentration (tween 80) contributes, the viscosity increases and donates the composition of pseudoplastic system and this is due to the increment surfactant tween 80 molecules concentration leading to the formation temporary and weaker intermolecular links between tween 80 molecules (not covalent in nature) and hence cutted in by low shear rates. The partial break takes place immediately upon simple thrill.[32]

This pseudoplastic behavior of luliconazole o/w nanoemulsion gel is the preferred behavior in pharmaceutical formulation by keeping the gel components in homogenous distribution because of low ability to move of dispersed phase due to high apparent viscosity at low shear. In contrast, upon shear stress included, the luliconazol nanoemulsion gel would display decreased viscosity and free flowing that allow easy of spreading upon topical application.[33]

**Table (4):** Viscosity data of the prepared nanoemulgels

| Formula no. | Velocity (rpm) | Viscosity (mpa.s) mean±SD(n=3) | Formula no. | Velocity (rpm) | Viscosity (mpa.s) mean±SD(n=3) |
|---|---|---|---|---|---|
| G-1 | 6 | 1225.31±0.34 | G-4 | 6 | 1240.79±1.06 |
| | 12 | 618.41±0.46 | | 12 | 569.44±0.55 |
| | 30 | 361.09±0.21 | | 30 | 436.33±0.55 |
| | 60 | 271.38±0.38 | | 60 | 292.40±0.43 |
| G-2 | 6 | 1131.72±0.34 | G-5 | 6 | 1330.72±0.27 |
| | 12 | 614.11±0.38 | | 12 | 555.45±0.46 |
| | 30 | 309.20±0.97 | | 30 | 412.73±0.33 |
| | 60 | 222.91±0.93 | | 60 | 302.98±0.91 |
| G-3 | 6 | 1207.30±0.26 | G-6 | 6 | 1364.78±0.68 |
| | 12 | 600.27±0.51 | | 12 | 706.38±0.44 |
| | 30 | 292.50±0.66 | | 30 | 460.13±0.54 |
| | 60 | 223.43±0.44 | | 60 | 327.24±0.32 |

## In vitro release study

All preparations show complete release at the end of 6 hrs. Regardless of the variation in the percent of the components between the prepared nanoemulgels, all nano emulsion based gel showed greater amount of drug released than that of pure luliconazol dispersion (as control) in dissolved medium and they have dissimilar release profile ($f2$<50) as illustrated in figure (3). Therefore, a greater surface curvature allowed more diffusion of drug in solubilized form at the droplet interface and consequently, leading to a rapid drug releasing rate into dissolution solution.[34]

Besides, the plain luliconazol dispersion revealed roughly 22.9% released at the end of 6 hrs due to its very poor solubility.

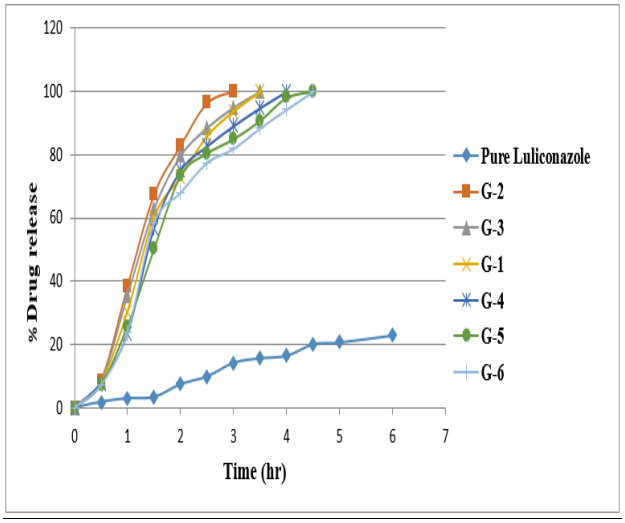



**Figure (3):** Dissolution profile of luliconazole nanoemulgels (pure luliconazole, G-1, G-2, G-3, G-4, G-5 and G-6) in 900ml PBS pH 7.4 with 2% Brij 35

As surfactant concentration increase, the luliconazole release decrease and thus it is concluded that the outline of drug release of the nanoemulgels follow the order: (G-2> G-3> G-1) and (G-4> G-5> G-6) as shown in figures (4) and (5). Also, this increasing of the surfactant will decrease the release of the drug may be due to an increase in the formula's viscosity.[21]

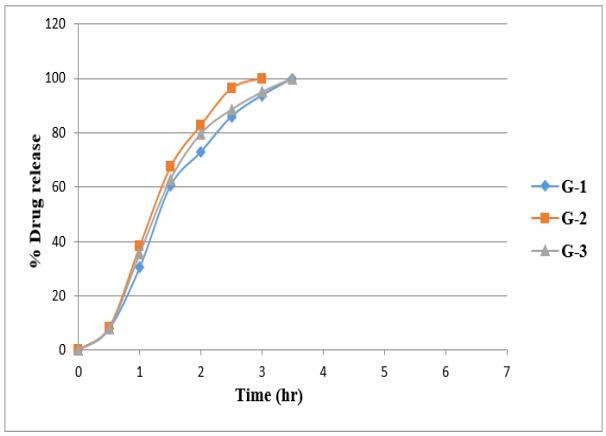



**Figure (4):** Dissolution profile of luliconazole nanoemulgel (G-1, G-2, and G-3) in 900ml PBS pH 7.4 with 2% Brij 35

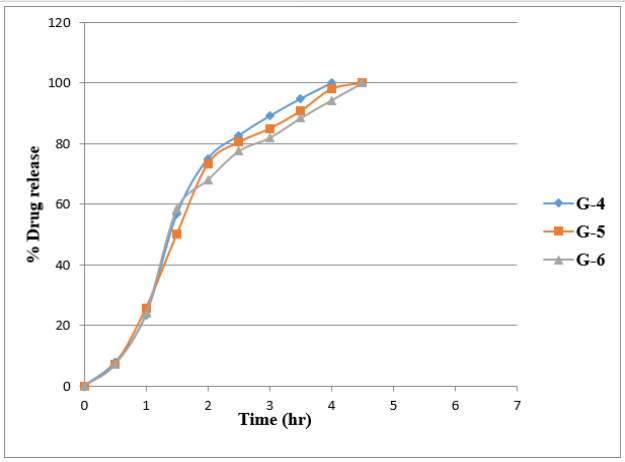
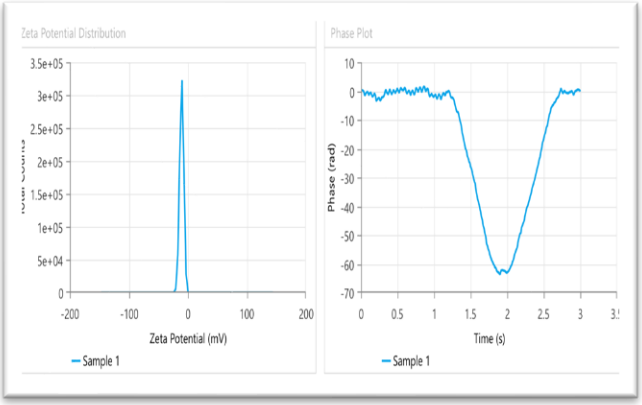



**Figure (5):** Dissolution profile of luliconazole nanoemulgel (G-4, G-5, and G-6) in 900ml PBS pH 7.4 with 2% Brij 35



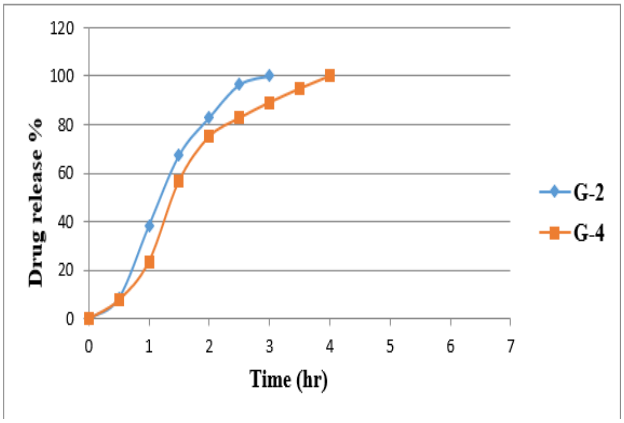




**Figure (6):** Dissolution profile of luliconazole nanoemulgel (G-2 and G-4) in 900ml PBS pH 7.4 with 2% Brij 35

The results revealed that the release of luliconazole was higher from formulae with less peppermint oil concentration (15%) than that from formulas with oil concentration (20%), this attributed to the luliconazole molecules be faced with retarding effect from high concentration peppermint oil that increase hydrophobicity of formulations in addition to increase a diffusional pathway for luliconazole molecules as showed in figures (6).[4]

## Selection of the optimum nanoemulgel formulation

According to the results acquired from the above gel evaluation investigation of the ready nanoemulgels, G-2 was harvest as the optimum formula, hence it is characterised by fine droplet size (26.26), acceptable PDI (0.30), excellent physical appearance, pH (6.1), which is within the range of the topical medication, accepted percent of drug content (98.13), encouraged viscosity (1131.72 mPa.sec) and suitable elevated releasing of the medication from the formula that improve therapeutic efficacy. The optimized formulas subjected to further investigation.

## Measurement of the Zeta Potential

### Evaluation of the optimized luliconazole nanoemulgel formula

Zeta potential evaluations help in gauging colloidal dispersion, shelf-life and stability. Zeta potential result illustrated in figure (7). The zeta potential of G-2 was -10.48. The entire value of zeta potential was fairly low; this would be giving ground for the composition of nanoemulgels.[35]

Tween 80 is a non-ionic surfactant that does not donate any charge to the system, and hence, it lowers an electrostatic stabilization mechanism and depends on higher steric stabilization, where particles are coated with hydrophilic excipients to physically hamper aggregation.[36]

The steric stabilization provided by tween 80 (surfactant) occurs when the adsorbed layers of large molecules of nonionic surfactant shift the plane of shear for long distance from droplets surface.[37]

**Figure (7):** Zeta potential values of luliconazole nanoemulgel G-2

## Compatibility study by Fourier transforms infrared spectroscopy (FTIR)

The FTIR spectra of luliconazole and optimum nanoemulgel formula G-2 are presented in figures (8-9).

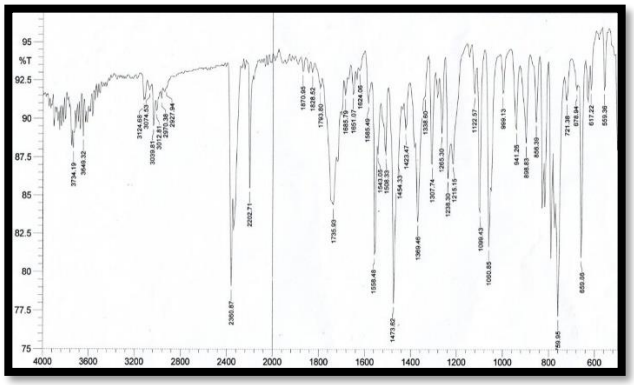


**Figure (8):** FTIR spectra of luliconazole

Luliconazole powder's IR spectrum displayed a characteristic major peak observed were as 2360.87cm$^{-1}$ (C≡N Stretch), 1735.93cm$^{-1}$ (C=C Alkene Stretch), 1651.07cm$^{-1}$ (C=N Stretch), 1558.48cm$^{-1}$ (C=CAromatic Stretch) and 1099.43cm$^{-1}$ (C-Cl Stretch). The characteristic peaks of luliconazole within the range or very close to the characteristic peaks of standard value proving drug as luliconazole.[38, 20]

Furthermore, the FTIR spectra of the selected nanoemulgel formula (G-2) were confirmed the chemical stability of drug structure and the non-appearance of any change that would occur during the preparation of nano emulsification and gelation process.

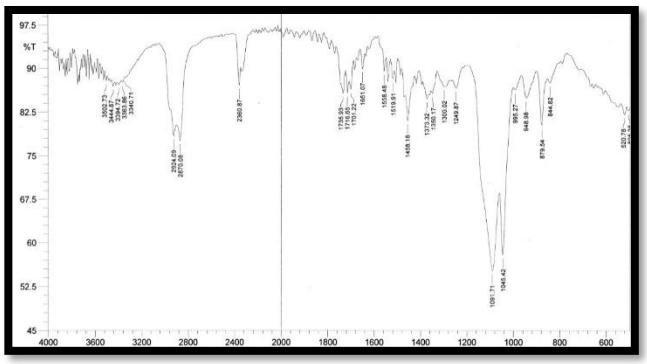


**Figure (9):** The selected formula (G-2) FTIR spectrum

## Field emission scanning electron microscopy (FE-SEM)

FE-SEM is the inspection by microscope which can support the globule size of ideal formula F-6. The microscopy would examine the nano sized particles of

F-6 formulation as it clear from figure (10). The average range is 56.06- 197.12 nm with round shape.

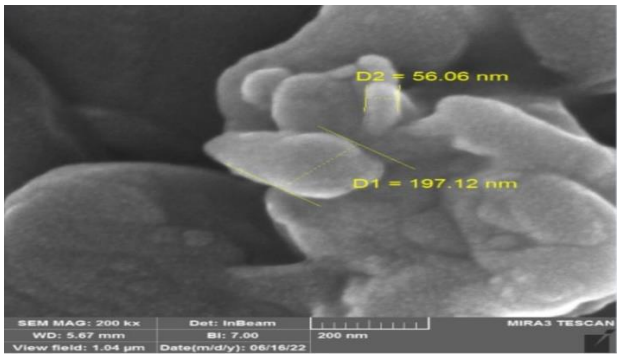



**Figure (10):** FE-SEM of optimum F-6 formulation

## Atomic force microscopy (AFM) analysis

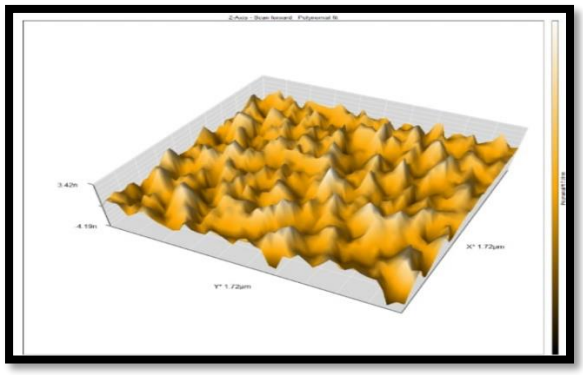


**Figure (11):** The AFM image of optimum formula F-6

The end result of this test suggests that the morphology of the droplets of F-6 was nano-sized closed to ball-shape and had a flat surface, that confirms the stability of F-6 as displayed in figure (11).

## In vitro antifungal activity

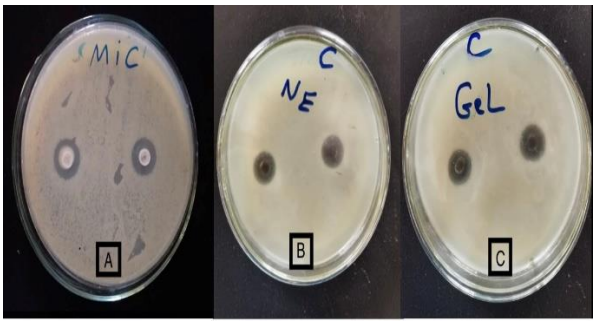



**Figure (12):** Photographs of the zone of inhibition against *C. albicans*: (A) Micazole® gel (B) drug loaded nanoemulsion formula F-6 (C) drug loaded-nanoemulgel G-2

The antifungal activity evaluation results of luliconazole from drug loaded nanoemulsion formula F-6 and drug loaded-nanoemulgel G-2 compared with Micazole® gel are shown in figure (12). The results obtained for all tested samples were adequate in terms of antifungal activity represented as the mean of inhibition zone, which were 14.5 mm and 15 mm for nano emulsion F-6 and nanoemulgel G-2 respectively, while the marketed (Micazole® gel) produced a 15 mm inhibition zone. The alike mean diameters of inhibition zone revealed by optimum nanoemulgel G-2 and the commercial gel might be determined by similar antifungal activity from these semisolid systems.

# Conclusion

In the light of the current study, it is concluded that luliconazole containing nanoemulsion fabrication for solubility boost was successfully prepared by the aqueous phase titration and then made nanoemulgel.

The in-vitro diffusion study of the prepared luliconazole nanoemulgel formulations was higher as compared to pure drug, which indicate it is having higher solubility, permeability and availability at site of action.

The prepared nanoemulgel of G-2 containing peppermint oil, tween 80 and ethanol reveals the better release profile than others and give the same antifungal activity in comparison with the commercial product (Micazole® gel)

The above result concluded that prepared nanoemulgel can be effective for the topical application in the eradication of fungal diseases with fewer side effects and advanced for further study *in vivo* test to evaluate the clinical achievement of the prepared medical formulations.

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