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Natural Oil Nanoemulsion-Based Gel Vehicle for Enhancing Antifungal Effect of Topical Luliconazole

DOI: https://doi.org/10.32007/jfacmedbagdad.6512058.

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J Fac Med Baghdad
2023; Vol.65, No. 1
Received: Jan, 2023
Accepted: Mar., 2023
Published: April 2023

Abstract:

Background: Luliconazole, a newer class of imidazole anti-fungal agent, is very effective against several species of fungi, especially dermatophytes. It has very low aqueous solubility acting as barrier for topical delivery and limiting its dermal availability.

Aim of the study: This study aimed to formulate luliconazole oil/water nanoemulsion by the aqueous titration method.

Methods: Solubility study resulted in selecting peppermint oil, tween 80 and transcutol p as oil phase, surfactant and cosurfactant respectively, although pseudoternary phase diagram construct nanoemulsion area for picking formulations. Fifteen o/w nanoemulsion formulations prepared and characterised for droplet size, polydispersity index, pH values, percent transmittance, luliconazole content. Among formulations, eight preparations introduced to enhance the viscosity of prepared nanoemulsion by combining 0.5% carbopol 934 as gelling agent.

Results: The selected preparations demonstrated homogeneous nanoemulgels with pH values appropriate for skin application and accepted luliconazole content. Viscosity results manifested non-newtonian pseudo plastic behavior with shear-thinning viscosity profile. *In vitro* release studies revealed dissimilar release profile ($f2<50$) than that of pure luliconazole dispersion. The results revealed that the formula NG-1 with oil: Smix(2:1):water (15:40:43.5) ratio containing 1% drug and 0.5% carbopol 934 was the optimised formula with excellent spreadability.

Conclusion: The study concluded that nanoemulsion-based gel is contemplated an encouraging and proceed technique for the topical preparation and upgrade solubility, dissolution rate and permeability of insufficient water-soluble drugs across the skin.

Keywords: Carbopol 934, Luliconazole, Nanoemulsion, Pseudoternary phase diagram, Surfactant

Introduction:

Luliconazole a topical imidazole antifungal drug ((−)- (E)-[(4R)4-(2,4-dichlorophenyl)-1,3dithiolan-2ylidene(1H-imidazol-1-yl) acetonitrile that possesses broad spectrum antifungal activity. It is very effective against *Trichophyton rubrum* and *Epidermophyton floccosum*, specifically *tinea pedis*, *cruris*, and *corporis*. Luliconazole acts by impeding the enzyme lanosterol demethylase which is needed for biosynthesis of ergosterol, a major component of the fungus cell membrane (1).

It is classified according to biopharmaceutical classification system (BCS) as class II drug with low solubility and high permeability (2).

It has very low aqueous solubility restricting its dermal availability and acting as barricade for topical delivery. Traditional and marketed formulations have low skin permeation and shorter retention of drug at the dermal infection site (1). Many nanotechnology-based techniques were used to decrease particle size leading to increase solubility and enhance dissolution (3). One of these common promising implements for topical drug application is the (lipid-based dispersion nanoemulsion), due to preferred attributes of the nanoemulsion (NE) structure that establishes magnified solubility of hydrophobic medications, better physical stability and refine skin drug activity by penetration and permeation boost things of its constituents through skin layers, greater drug loading amplitude and lower limit or negligible skin irritation propensity. Nanoemulsion is a translucent heterogenous system consisting of 2 immiscible liquids (water and oil) with nano metric diameters ranging from 20 to 200nm. It is stabilised by an interfacial layer of mixture of surfactant/cosurfactant or specifically Smix, formatting isotropic system that hold the drug molecules in solubilised form within the oil phase droplets (4).

To increase the viscosity of NE, it should be incorporated with carbopol 934 as gelling agent to

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get interesting topical delivery system which is named nanoemulgel (NG) (5).

The purpose of this work was to formulate and evaluate a luliconazole nanoemulsion (LNE) as topical use, which will improve solubility, permeation and keep away from skin irritation. Then, to made nanoemulgel coupled with suitable viscosity as a favourable medicinal product to enhance patient's compliance.

Material and Methods:

Luliconazole was purchased from Hyper-Chem LTD CO, China. Peppermint oil was purchased from Alpha Chemika, India. Transcutol p and triacetin purchased from Hyper-Chem LTD CO, China. Carbopol 934 was purchased from Sigma Aldrich, USA. Tween 80 and tween 20 obtained from Alpha Chemika, India. Methanol and ethanol obtained from Haymankimia, United Kingdom. Glycerol and labrasol obtained from Sigma Aldrich, USA. KH2 PO4 obtained from Central Drug House (p) limited, Delhi India. Na2 HPO4 and Triethanolamine obtained from Thomas Baker (chemicals) Pvt, Ltd India.

Solubility study of luliconazole : Excess amount of luliconazole powder was mixed (by Vortex mixer) with exactly 5mL of oils, surface active agents and co-solvents each separately in firmly stoppered 10mL vials and vortex for 5 min, then shaken in water bath shaker for 72h at 25±0.5˚C. After reaching an equilibrium, centrifugation of each mixture at 3500rpm for 20min was done to separate the excess of insoluble drug, and then suitably dilute the supernatant with methanol after been filtered using a 0.45µm syringe filters (to remove the remaining solid particles) and, finally, quantifying the solubilised amount of luliconazole (as mg/ml) spectrophotometrically using UV-Visible spectrophotometer at luliconazole **λ** max using methanol as blank (4).

Construction of pseudo ternary phase diagram

Depending on outcomes from the saturated solubility study, the diagram of pseudo ternary phase (PTP) was made-up using aqueous titration method (low energy emulsification). Distilled water (DW) has been used as an aqueous phase, assorted with different mixtures of surfactant (tween 80) and co-surfactant (transcutol p) in 1:1 ,2:1, 3:1 ratios, on the basis of rising surfactant concentration at constant level of co-surfactants. For drawing each phase diagram, chosen oil (peppermint oil) was combined gradually with mixtures of surfactant and cosurfactant (S-mix) at accurate ratios for each phase diagram in different vials of glass in ratios of (9:1, 8:2, 7:3, 6:4, 5:5, 4:6, 3:7, 2:8, 1:9) (w / w). A transparent and steady mix by a vortex mixer for five minutes was subjected to each surfactant and co-surfactant mixture (S-mix). After that, each mixture was titrated slowly with mixing by a vortex at room temperature (without heating) with aqueous phase of DW in a drop-wise fashion with observation of system clarity. The amount of water at which the end point of the titration was detected (change from clear to turbid). Then, these points were utilised to specify the borders on the NE region that suit the value of oils chosen (6).

Preparation of luliconazole loaded nanoemulsion

Different formulations of O/W NE have been made-up according to pseudo-ternary phase diagrams using the S-mix mixture and oil concentrations by water titration method. The preparation of 5gm of 1% LNE has been formulated through dissolving 50mg of luliconazole in (peppermint oil as desired quantity) employing a vortex mixer for 10minutes. 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) NE (7).

Characterization of the prepared luliconazole nanoemulsions

Thermodynamic stability study

There were three tests included in this study

1) Centrifugation test

All of the prepared formulations were centrifuged for thirty minutes at 3500rpm, and destability issues (precipitation, phase separation, creaming, and cracking) were observed. The selected stable NEs were moved for the heating-cooling cycle (4).

2) Heating- cooling cycle (H/C cycle)

Six cycles of the H/C cycle were applied to examine the stability of NEs between the refrigerator degrees of temperature (4°C) and (45°C) with storage at each temperature not lower than two days. Then, the formulation that passed these six cycles of temperatures was introduced to freeze-thaw cycle (8).

3) Freezing - thawing (FT) test

Three FT cycles (between -21 and +25°C) were performed with storage at each temperature for at least two days. The formula that overcome all these thermodynamic stress tests was transported to further characterisation study (6).

Globule size measurement: The average particle size for LNE droplets was performed by Malvern (Germany) particle size analyser. Using the dynamic light scattering method, the droplet size of samples was detected by scanning fluctuations in light scattering originated from Brownian motion for particles (9).

Polydispersity index (PDI) measurement: The determination of PDI of NE was done by (Malvern particle size analyser) to inspect the homogeneity of droplet size. The ranges of PDI are from (zero to one) (10).

pH measurement: The pH measurement is crucial for compatibility of a topical formulation with the pH of the skin to avert its irritation. The pH values of luliconazole loaded o/w NEs were examined by using digital pH meter. The measurement done in triplicate to avoid error (4).

Light Transmittance Study (T%): This study was performed to estimate NE transparency (optical clarity). The T% was studied for all prepared NEs by

using UV- vis spectrophotometer at 650nm regarding DW as a standard blank (6).

Drug content: The content of luliconazole in each prepared formula was performed by using UV/Vis spectrophotometric analysis, in which weighting approximately 1g of each drug loading NE formula containing (10mg) luliconazole and dissolved in 100ml methanol and mixed in magnetic stirrer for 30 minutes to break the vesicles. This solution was filtered and suitably diluted with methanol and estimated spectrophotometrically at 296nm using methanol as blank. The contents of luliconazole were calculated by using the calibration curve equation of drug in methanol (11).

Selection of the drug formulas for preparation of nanoemulgel: The selection of luliconazole loaded o/w NEs subjected to NG preparation depending on the particle size tested less than 100nm as well as all the remaining studies. Eight formulations were chosen among fifteen LNE formulations to develop a luliconazole o/w NE based gel.

Preparation of luliconazole nanoemulgel: The low viscosity of NE made it is easily removable because of high water content and resulted in low skin retention when applied topically. A carbopol 934 was utilised to develop the luliconazole NE gel. First step was 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 contain small volume of water while stirring constantly with a magnetic stirrer. Finally, the pH of the obtained gel was neutralised by increasingly adding few drops of triethanolamine to pH 6-6.5, producing a desired topical gel of LNE (12, 13).

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

Table (1): Composition of Nanoemulgels

Nano Emulgel No.	Drug %w/w	Oil%w/w	Tween80: Transcutol p %w/w	Smix ratio	DW.%w/w	Carbopol 934 %w/w
NG-1	1	15	40	2:1	43.5	0.5
NG-2	1	15	60	2:1	23.5	0.5
NG-3	1	15	40	3:1	43.5	0.5
NG-4	1	15	50	3:1	33.5	0.5
NG-5	1	20	45	2:1	33.5	0.5
NG-6	1	20	55	2:1	23.5	0.5
NG-7	1	20	45	3:1	33.5	0.5
NG-8	1	20	55	3:1	23.5	0.5

Characterization of the prepared luliconazole nanoemulgel

Homogeneity of luliconazole-loaded nanoemulsion gel

The organoleptic and homogeneity characteristics of the produced luliconazole loaded NE gel were evaluated by visual investigations. This included appearance, color, transparency, phase separation, homogeneity and consistency (14).

pH measurement

The pH values of luliconazole o/w NE were measured using digital pH meter at 25±0.5ºC, the pH of topical formulations is significant for their affinity with the skin pH to prevent any irritation. The examination was repeated in triplicate (15).

Drug Content Determination

The drug content of prepared NG was determined by dissolving one gram of the formulation in 100ml methanol, suitable dilutions were done and then filtering using millipore syringe filter (0.45µm). Drug content was calculated by reading the absorbance (at 296nm) using UV-vi spectrophotometric analysis through linear equation of methanol calibration curve (16).

Viscosity measurement

The NDJ-5S digital viscometer with spindle (No.3) was used for measuring the rheological properties of prepared NG formulations by inserting the spindle into cylinder containing gel and rotating at 6, 12, 30, and 60rpm at room temperature. The test was done in triplicate (8).

***In vitro* drug release study**

The release of luliconazole from NG was carried out using the dialysis bag procedure. The experiment was performed in a USP dissolution apparatus type II (paddle technique) with a 50rpm rotation speed at specific constant temperature was maintained of release medium (900ml). Previously, the dialysis bags (Molecular cut off 8000 – 14000 Da) were soaked (for 24hr) in dissolution media. Then, each dialysis bag contains 1ml (approximately 1gm) of nanoemulgel (equals to 10mg of drug), tightly sealed from both ends and submerged in using medium. Afterwards, calculation of drug concentration was done in each sample at detected intervals using UV-VIS spectrophotometric at its maximum wavelength (at 299nm) (1, 17).

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 located in new disposable zeta cell and then put the sample to be assessed (9).

Transmission Electron Microscope (TEM)

Internal structure, surface morphology and approximate particle size of LNE were studied by TEM with 100kV accelerating voltage. The

procedure involves employing one drop of sample on carbon coated copper grid, then left till dry to form thin film. After that, the sample was introduced to the instrument for examining (18).

Spreadability of luliconazole loaded o/w nanoemulsion gel

The spreading ability was performed by putting 0.5g of the prepared luliconazole o/w NG within a previously marked circle of 1cm diameter on a glass slide, then a second glass slide with same dimensions was placed over the first slide and thereby sandwiching the gel between upper and lower slides. Afterward, a 50g weight was permitted to set over the upper slide for 5min, so that the NG between the two slides would squeeze and spread by the force of applied weight, then the increase in spreading diameter was calculated. The investigation was carried out in a triplicate (19).

Results

Saturation solubility of luliconazole

According to saturated solubility data obtained as showed in table (2), the peppermint oil as oil phase, tween 80 as surfactant and transcutol p as co-surfactant were chosen for the NE system preparation.

Table (2): Solubility study of luliconazole in various oils, surfactants and co-surfactants

Oil	Solubility (mg/ml)	Surfactant	Solubility (mg/ml)	Co-Surfactant	Solubility (mg/ml)
Oleic acid	61.5	Tween 20	84.2	Ethanol	48.5
Triacetin	44.9	Tween 80	107.5	Transcutol P	166.4
Peppermint	82.2	Cremophor EL	102.7	P.G.	29.3
Castor	36.3	Triton-X100	95.4	Glycerol	8.5
Olive	9.1	Labrasol	62.5		
IPM	8.7				

Development of pseudo-ternary phase diagram

Three PTP diagrams were drawn individually at each S-mix ratio of (1:1, 2:1, 3:1) in order to distinguish the region of NE with proper oil, S-mix and water ratios for preparation of NE as shown in figure (1).

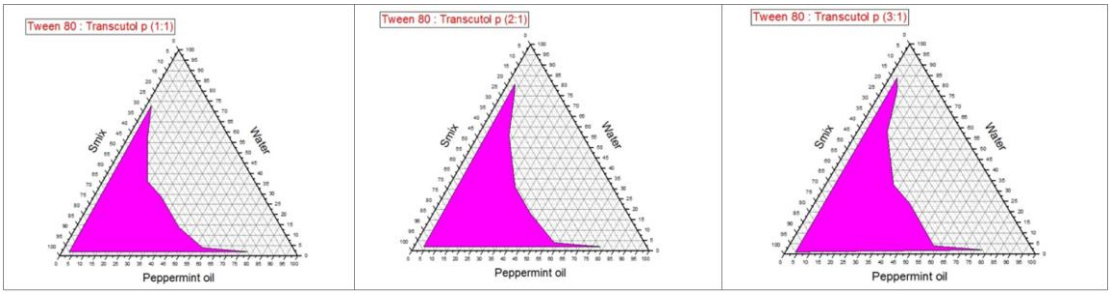


Figure (1): Pseudo-ternary phase diagram of peppermint oil, tween 80, transcutol p and distilled water for different S-mix ratios.

Preparation of luliconazole loaded o/w nanoemulsion formulations: Depending on the results got from PTP diagrams, a large possible number of formulae within this region can be obtained in each diagram. Fifteen formulations of LNE were prepared from all phase diagrams. As mentioned in table (1) that was illustrated composition of prepared o/w NEs. The formulas taken for LNE 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 above results of saturated solubility study.

Characterization of the prepared luliconazole nanoemulsions

Thermodynamic stability tests for luliconazole o/w nanoemulsions: According to the results of the thermodynamic stability study, the fourteen formulas from NE1 to NE14 had very good physical consistency and thermodynamic stability and only NE15 would be excluded from these studies due to the failure in thermodynamic stability study.

Globule size measurement: The globule size outcomes, as summarised in table (3), ranged from 33.6 nm for NE3 to 465.9 nm for NE1. That means that all prepared NE formulation possess droplets size in the nano scale.

Polydispersity index (PDI) measurement: As shown in table (3), all prepared luliconazole formulation except those for NE2 PDI results were from (0.15 to 0.57) which demonstrated that LNE products possessed a substantial homogeneous and limited size distribution. The NE2 should be excluded from further studies due to high value reading (1.28) for PDI.

pH measurement: The pH values resulted from digital pH meter were presented in table (3); the results disclosed a pH range of (5.7– 6.4) for the prepared formulations of luliconazole o/w NE.

Transmittance percent (%T) measurement : The percent transmittance is a measurement for

transparency of the system due to isotropic mixtures of o/w NEs. The results ranged from 93.55 % – 98.88 % as shown in table (3), which are near to 100 % transmittance.

Drug content estimation results: All measured values for prepared LNE formulae concur with official range of British Pharmacopeia range (95%-110%) as illustrated in table (3).

Table (3): Particle Size Measurement, Polydispersity Index, pH Value, Percentage of Transmittance and Drug Content Percent of the Nanoemulsions

Formula No.	Particle size	PDI	pH value	% Transmittance	% Drug content
NE1	465.9	0.40	5.8±0.21	93.55±0.58	104.20±0.33
NE2	447.4	1.28	XXX	XXX	XXX
NE3	33.6	0.45	6.4±0.25	97.11±0.64	97.88±0.32
NE4	126.2	0.18	6.3±0.24	95.78±0.32	97.18±0.25
NE5	34.8	0.18	6.3±0.15	98.88±0.76	96.44±0.27
NE6	89.3	0.49	5.7±0.16	94.39±0.63	95.70±0.33
NE7	69.8	0.57	5.9±0.18	98.31±0.76	101.60±0.38
NE8	288	0.31	6.4±0.25	95.41±0.39	104.7±0.32
NE9	236.9	0.21	6.0±0.21	96.77±0.64	98.57±0.26
NE10	206.3	0.28	6.3±0.11	95.68±0.53	97.09±0.17
NE11	58.3	0.48	6.2±0.56	98.14±0.64	98.88±0.23
NE12	74.4	0.57	6.1±0.15	96.28±0.14	96.07±0.73
NE13	99.2	0.54	5.9±0.10	98.77±0.47	98.34 0.30
NE14	82.8	0.15	6.3±0.18	97.91±0.73	97.92±0.24

Selection of the formulas for nanoemulgel preparation: From all prepared fifteen formulations, only eight formulas demonstrated ultra-fine droplet size below 100nm as well as they were described by low polydispersity index, accepted pH values, good percent of transmittance and high drug content. These eight formulas were (NE3, NE5, NE6, NE7, NE11, NE12, NE13 and NE14) which were selected to prepare LNE.

Preparation of luliconazole o/w nanoemulsion based gel: The prepared gel with potency of 10mg luliconazole in each 1g of the prepared (o/w) NE based gel, and by employing 0.5% Carbopol 934 (w/v) as a gelling agent.

Characterization of luliconazole nanoemulgel

Homogeneity of luliconazole loaded o/w nanoemulsion based gel

All of the advanced information of gel had an excellent homogeneity and transparent clarity as detailed in table (4).

The results of the pH measurement

The pH values of the prepared luliconazole o/w NG formulations were illustrated in table (4), these values ranged from 5.9 to 6.5.

Drug content

Drug content results of the prepared luliconazole NG formulation were manifested in table (4) and ranged from (96.22-102.34).

Table (4): The physical appearance, pH and the percent of drug content in luliconazole nanoemulgel formulations

Formula code	Clarity	Homogeneity	pH	(%) Drug content
NG-1	Transparent	Excellent	6.5±0.21	102.34±0.77
NG-2	Transparent	Excellent	6.3±0.28	97.87±0.34
NG-3	Transparent	Excellent	5.9±0.14	96.22±0.51
NG-4	Transparent	Excellent	6.0±0.61	99.01±0.79
NG-5	Transparent	Excellent	6.2±0.25	101.91±0.43
NG-6	Transparent	Excellent	6.3±0.24	98.77±0.44
NG-7	Transparent	Excellent	6.1±0.05	96.90±0.33
NG-8	Transparent	Excellent	6.4±0.28	99.05±0.53

Viscosity measurements: The rheogram for the prepared luliconazole o/w NE gel was created by graphing the applied shear rate (6, 12, 30 and 60rpm) on the X-axis opposed to the corresponding viscosity data represented at each shear rotating speed on the Y-axis, as shown in figure (2).

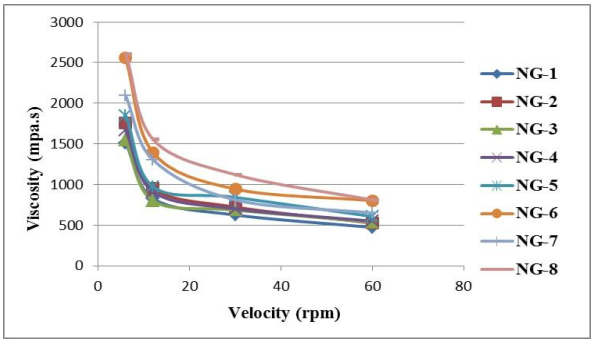
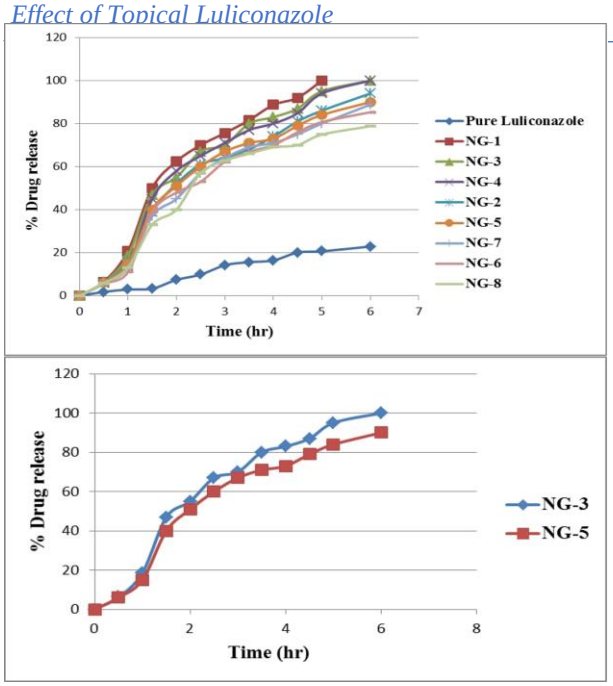


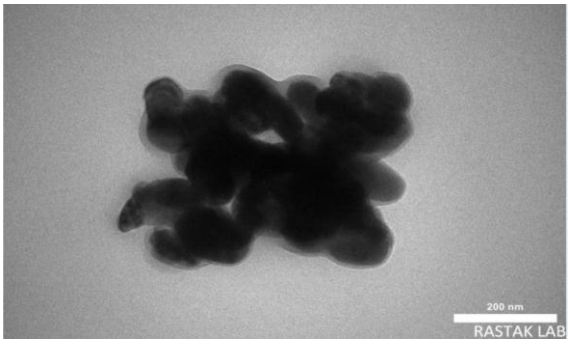
Figure (2): Viscosity results of prepared luliconazole nanoemulgel.



***In vitro* release study of selected luliconazole o/w nanoemulgels**

All nanoemulsion-based gel showed dissimilar release profile ($f2$<50) higher than that of pure luliconazole dispersion (as control) in dissolved medium as illustrated in figures (3). Besides, the plain luliconazole dispersion revealed roughly 22.9% released at the end of 6 hrs due to its very poor solubility. Also, the results revealed that the release of luliconazole was higher from formulae with less peppermint oil content, as showed in figures (3).

Figure (3): Release profile of eight selected formulas in comparison with pure luliconazole dispersion and the effect of oil concentration shown dissolution profile of NG-3 and NG-5.



Selection of the optimum nanoemulgel formulation: The results revealed that NG-1 might be nominated as an optimum formulation due to their appropriate pH value, drug content, release profile, and viscosity for topical administration. The optimized formulas are subjected to further investigation.

Zeta potential measurement of optimum nanoemulgel: The entire value of zeta potential was fairly low; this would give a ground for the composition of NGs. The zeta potential of NG-1 was -9.07mV.

Spreadability of optimised nanoemulsion based gel: The spreadability of luliconazole o/w NE gel NG-1was found to be 3.18 ± 0.03cm.

Transmission Electron Microscope: The morphology of LNE formula (NE3) showed smooth, spherical droplets with no evidence of particle aggregation or cluster formation as shown in figure (4).

Figure (4): TEM photomicrographs of luliconazole o/w nanoemulsion NE3 formulation.

Discussion

Saturation solubility of luliconazole: NE components must be selected according to maximum drug solubility in addition to optimal miscibility with each other to give a stable formulation in which the drug is in a solubilized form with high loading capacity (4).

Development of pseudo ternary phase diagram: The pink-coloured area in each diagram includes clear NE area, whereas the free (non-coloured) area represents emulsion (turbid) zone and the larger shaded area demonstrates greater emulsification tendency of the system (20). In this drawn NE area, spontaneous dispersion of NE can occur through kindly continuous mixing or stirring of components. This would be due to the movement of surfactant molecules (tween 80) toward the surface of NE droplets, leading to interfacial free energy to be reduced and formation of mechanical barrier that prevents aggregation or coalescence (21). Co-surfactant transcutol p can modify the effect of tween 80 as surfactant in NE system by (a) its presence with tween 80 in one dispersion system that would be regulating the surfactant solubility in aqueous solutions by effecting the hydrophobic extent, or by (b) Co-surfactant enhances NE system solubility by their placing into gap rooms between the tween 80 molecules and therefore participate in the lessening of interfacial tension and increase fluidity, or (c) because of hydrophobic nature of co-surfactant (transcutol p and ethanol) with low HLB value that improves luliconazole dispersibility in the nano system (4).

Characterization of the prepared luliconazole nanoemulsions

Thermodynamic stability tests for luliconazole o/w nanoemulsions

The results as mentioned above which can be attributed to continual Brownian motion of the small-sized droplets indicating longer shelf life (22).

Globule size measurement: All these alterations in NE constituents influence globule size by affecting the curvature of the interfacial film by altering its fluidity and flexibility. As shown in the results section, when surfactant (tween 80) concentration increases and also increases in S-mix ratio of surfactant to co-surfactant (transcutol p), a decrease in globule size of the prepared o/w NEs was established. As result, smaller droplet sizes were achieved when using S-mix ratios of 3:1 and 2:1.

This inverse relationship between a surfactant and globule size could be attributed to (a) the greater HLB value and therefore, hydrophilicity of surfactant which results into reduction in surface curvature of the peppermint oil interface which is available with justly high solubility and thus leading to droplet size reduction and/or (b) the localization of tween 80 surfactant molecules at the interface between oil and water which gives stabilisation effect of the peppermint oil droplets resulting in small-scale droplet size and higher stability (4).

Polydispersity index (PDI) measurement: The value of PDI is oppositely proportional to the physical stability and uniformity of droplet size distribution of LNE formulations (23).

pH measurement: The measured pH values were suitable for topical application because of the required pH in the topical formulation is in pH range of the human skin (4.5-6.5). pH should not be excessively acidic that initiates skin annoyance and not be high alkaline because it can cause dry and flaking skin (24).

Transmittance percent (%T) measurement : Available results strongly suggested that all tested luliconazole formulations transport light simply, and hence, optically clear and transparent. This transparency and high %T could be attributed to their nano-sized droplets (less than 25% of the wave length of visible light) (25).

Drug content estimation results: Indicate high content constancy and acceptable loading without any aggregation or degradation of the drug in the preparation method (26).

Characterization of luliconazole nanoemulgel

Homogeneity of luliconazole loaded o/w nanoemulsion based gel: The 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 (27).

The results of the pH measurement: The results revealed that the pH was within the 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 (28).

Drug content : The results reported negligible loss or disintegration of the drug during the incorporation of the gelling agent (0.5% carbopol) with the LNE during the production of LNE gel (29).

Viscosity measurements: The prepared luliconazole o/w NG manifests non-newtonian pseudo-plastic 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, causes decreasing viscosity (30, 31).

It is concluded that when increasing the surfactant concentration (tween 80), the viscosity increases. Tween 80 structure has large number of polyoxyethylene groups with hydrophilic nature (HLB = 15) which gave the tendency to soak up the aqueous phase. As a result, reduction of free water of the formulations has led to increasing the viscosity (32). The viscosity study results revealed that as increment in the oil concentration, as internal phase, viscosity increases between formulas. As a result, as the oil content was increasing from 15% w/w to 20% w/w, the viscosity of the formulations also increased (33).

***In vitro* release study of selected luliconazole o/w nanoemulgels:** The *in-vitro* dissolution study was conducted to compare drug releasing from eight successful formulations and pure luliconazole dispersion. Furthermore, the extent of drug release from formulations is determined by the dissolution rates of drug which is considered as a function of particle size and aqueous solubility. The high NG dissolution profile may be effected by the small scale particle size of the prepared products, hence, the exposed surface area increases to the dissolution medium and the elevated drug solubilization probability (6, 10). The release profile of all NG in the releasing medium reflects the effect of surfactant concentration on the luliconazole release in each Smix ratio at a constant concentration of peppermint oil. As surfactant tween 80 concentration increase, the luliconazole release decrease due to an increase in the formula's viscosity (8).

The release of luliconazole was higher from formulae with less peppermint oil concentration (15%) than that from formulas with oil concentration (20%), this may be attributed to that luliconazole molecules encounter retarding effect from high concentration peppermint oil that increase hydrophobicity of formulations in addition to increase diffusional pathway for luliconazole molecules, to reach dissolution medium after fleeting from dialysis bag as showed in figures (3) (5).

Zeta potential measurement of optimum nanoemulgel: The steric stabilisation provided by tween 80 (non-ionic surfactant that does not donate any charge to the system) occurs when the adsorbed layers of large molecules of non-ionic surfactant shift the plane of shear for long distance from the surface of the droplets (34).

Negative values of formulations indicate reduced aggregation of the globules in the continuous phase (35).

Spreadability of optimised nanoemulsion based gel: The spreadability of luliconazole product indicating effortless spreading gel by giving small shear with maximum slip and haul (30).

Conclusion

The characterization approaches and *in vitro* release studies of prepared luliconazole nanoemulsion based gel formulations exhibited notably improved solubility, increased dissolution rate, permeability and save with good spreadability for practical topical application. In future, an extensive comprehension

and studying is necessary in order to develop the topical luliconazole nanoemulgel in pharmaceutical applications by experiencing to *in vivo* test to evaluate the clinical performance of the formulated dosage form.

Authors' declaration:-

Conflicts of Interest: None.-

We hereby confirm that all the Figures and Tables in the manuscript are mine/ ours. Besides, the Figures and images, which are not mine /ours, have been given permission for re-publication attached with the manuscript.-

Author's contributions:

Ahmed M. Kmkm: Master students

Mowafaq M. Ghareeb: Supervisor

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How to Cite this Article
M. Kmkm A, M. Ghareeb M. Natural Oil Nanoemulsion-Based Gel Vehicle for Enhancing Antifungal Effect of Topical Luliconazole. JFacMedBagdad [Internet]. 2023 Apr. 27 [cited 2023 May 14];65(1):65-73. Available from:https://iqjmc.uobaghdad.edu.iq/index.php/19JFacMedBaghdad36/article/view/2058

هلام مبني على مستحلب نانوي لزيت طبيعي كوسيط لتحسين التاثير الموضعي ضد الفطريات لدواء اللوليكونازول

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خلفية البحث: لوليكونازول ، فئة جديدة من العامل المضاد للفطريات إيميدازول، وهو فعال للغاية ضد العديد من أنواع الفطريات، وبشكل كبير الفطريات الجلدية. لديه قابلية منخفضة للذوبان في الماء تعمل كحاجز للتوصيل الموضعي وتحد من توافرها عن طريق الجلد.
الاهداف: يهدف هذا البحث إلى صياغة مستحلب نانوي زيت/ماء لدواء اللوليكونازول بطريقة التسحيح المائي.
المواد وطرق العمل: دراسة الذوبانية ينتج عنها اختيار زيت النعناع، توين 80 و ترانسكيوتول بي كطور زيتي، خافض للتوتر السطحي ومواد مساعدة لخفض التوتر السطحي على التوالي ،وفي نفس السياق أن المخططات مثلثة الشكل تبني منطقة مستحلب نانوي للصيغ المنتقاة. تم تحضير خمسة عشر صيغة زيت/ماء مستحلب نانوي وتم تقييمها من حيث حجم القطرة ، مؤشر التشتت المتعدد، قيم الأس الهيدروجيني، نسبة النفاذية و محتوى اللوليكونازول. من بين الصيغ، يتم إدخال ثمانية تركيبات لتحسين لزوجة مستحلب النانو المحضر عن طريق المزج مع 0.5% كاربوبول 934كعامل يحدث التهلم.
النتائج: هذه التركيبات المختارة اظهرت هلامات مستحلبة نانوية متجانسة مع قيم الأس الهيدروجيني المناسبة للاستعمال الجلدي ومحتوى لوليكونازول مقبول. تظهر نتائج اللزوجة سلوكًا بلاستيكيًا زائفًا غير نيوتني مع مظهر لزوجة رقيق القص. تكشف دراسات الإطلاق في المختبر عن شكل تحرير غير متماثل ($f2$<50) من ذلك الخاص بمحلول تشتت لوليكونازول النقي. أظهرت النتائج أن الصيغة NG-1 بالزيت: Smix (2: 1): نسبة الماء (15: 40: 43.5) والتي تحتوي على 1% دواء و 0.5% كاربوبول 934 كانت التركيبة الأمثل مع قابلية انتشار ممتازة.
الاستنتاجات: خلصت الدراسة إلى أن الهلام القائم على مستحلب النانو يعتبر أسلوبًا مشجعًا ومتقدمًا للتحضير الموضعي ويحسن قابلية الذوبانيه وسرعة الانحلال ونفاذية الأدوية غير القابلة للذوبان في الماء عبر الجلد.
الكلمات المفتاحية: كاربوبول 934 ، مستحلب النانو، لوليكونازول، مخطط الطور المثلث الكاذب وخافظ التوتر السطحي