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Research Article

Design, Development and Characterization of Nanoemulsion developed by High Pressure Homogenization (HPH) method Containing Antifungal Drug

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Abstract

Nanoemulsion is one the most attractive drug delivery for researchers to treat fungal diseases and getting interested in increasing the solubility of low soluble drugs. The objective of this research was to develop a topical nanoemulsion formulation of Posaconazole drug with the intention to enhance its aqueous solubility and local action. By employing High Pressure Homogenization (HPH) method, oil in water nanoemulsion was formulated with 0.2% cinnamon oil as lipid phase, 0.4% tween-80 and poloxamer-188 as surfactants, 0.2% transcutol as a co-surfactant. The physical characteristics of formulations were found to be stable after thermodynamic stability testing. *In-vitro* diffusion study for optimized nanoemulsion was performed using a dialysis bag membrane and cumulative % drug release was determined. Viscosity and percent drug content was observed to be 0.0593 cps and 90.21 ± 0.23% respectively. The optimized nanoemulsion formulation (F8) was evaluated to be transparent and thermodynamically stable, with -9.46 zeta potential, 78.79 nm particle size, 0.315 polydispersity index. The optimized Nanoemulsion was stable for 3 month in three different temperature conditions. The result from the release study was indicative of improved solubility of Posaconazole, which may serve to boost up the action of the drug for the treatment of fungal diseases.

Keywords: Posaconazole, Nanoemulsion, HPH method, Physical Characterization

INTRODUCTION

The rise of fungus capable of infecting humans is becoming a severe public health issue. Fungal disease like blastomycosis, histoplasmosis patients are seen to treat this disease by several medicament which are available in market like topical, oral but it possesss less oral bioavailability therefore it is less effective . Posaconazole is an triazole antifungal drug that inhibits cytochrome P450-Dependent Enzyme resulting in impairment of ergosterol synthesis in fungal cell Membranes. Fungal illnesses including candidiasis, mucormycosis (zygomycosis), aspergillosis, cryptococcosis, and pneumocystosis appear and disappear often.

Complicated pharmacokinetics, prominent medication interactions, and very major side effects make oral administration of voriconazole problematic. Furthermore, intravenous injection has been linked to heart rhythm difficulties. As a result, a topical delivery strategy is needed to overcome the limitations of voriconazole and improve its antifungal activity against cutaneous candidiasis. Sertaconazole (log P: 6.2) is a broad-spectrum third-generation imidazole derivative that treats tineas, candidiasis, and pityriasis versicolor. It's available in cream and solution forms, as well as vaginal tablets and suppositories. The very lipophilic drug's low penetration ability is the fundamental issue of traditional topical sertaconazole formulations.

On the other hand, bloodstream infections are mostly caused by candidemia. with a mortality rate of more than 30%, whilst Aspergillus can impact over 45% of susceptible hosts [1] Severe fungal infections mainly occur in immunosuppressed individuals. Immunosuppression as a risk factor highlights the important role of the immune system in controlling opportunistic fungal pathogens; it also suggests supporting the host immunefunctions or targeting interactions between the host immune system and fungi as alternative therapeutic strategies that could be combined with antifungal treatment.[2] To treat fungal infections, drugs including Posaconazole, Clotrimazole, Econazole, Miconazole, Ketoconazole, and Nystatin are utilised. Posaconazole, a new oral triazole derivative, is being developed to treat of invasive fungal infections. Posaconazole has superior action against practically all fungal infections when compared to the actions of other azoles. Several in vitro studies show that Posaconazole shows wide antifungal action against the majority of yeasts, filamentous fungi, and azole-resistant Candida species. Posaconazole, as a triazole antifungal drug, inhibits the cytochrome P-450 dependent enzyme sterol 14-demethylase in fungi by attaching to the heme cofactor present on the enzyme. This inhibits the formation of ergosterol, an integral part of cell membrane of fungi, which results in the build-up of methylated precursors of sterol. This inhibits fungal cell development and finally cell death.

It is classified as high permeability and low solubility in the Biopharmaceutical Classification System (BCS Class II). It has a molar mass of 700.8 g/mol, an elimination half-life of 15-35 h, a bioavailability of 90%, a melting point of 170-172°, a log P of

5.5, and >90% protein binding to albumin. It also shows first pass metabolism. It has 10 mg/ml solubility in DMSO. Posaconazole has a large volume of distribution 1774 l, implying significant distribution into extravascular areas and tissue penetration.

Posaconazole is rapidly absorbed, with a Tmax of 3 to 5 h on average. Despite the fact that the high-fat meal delayed the median time to peak concentration (Tmax) by 1 h dose dependent saturable absorptions, a high-fat meal only marginally elevated the Posaconazole AUC by 50%, compared to a 400% increase in comparable conditions for the suspension. Posaconazole can be used in dosages as high as 800 mg/d.[3]

Because of their particular structural and functional characteristics, advanced topical carriers solve biopharmaceutical difficulties associated with conventional drug delivery vehicles, such as poor retention and limited bioavailability. Solid-Lipid nanoparticles, Liposomes, Microemulsions, Microsponge, Niosomes, Nanogel, Micelles, Nanoemulsion, and other nano-carriers are often utilised in topical anti-fungal therapy.[(4)] When compared to non-structured oily or aqueous carriers, nano-emulsions have higher drug loading because the amphiphilic interface may be seen as an extra area for drug solubilization. In addition to boosting penetration, nanoemulsions have been characterised as increasing skin hydration because water is considered an enhancer.[5] Topical formulations based on nano-emulsions are frequently used to improve the therapeutic effectiveness and tolerance of locally applied antifungal medicines. Furthermore, their capacity to increase the solubility of less soluble drugs as well as protect them from enzymatic and chemical deterioration makes them a good topical carrier for antifungal agents.[4]

Posaconazole is a medication with a poor aqueous solubility, which reduces its antifungal action. As a result, the nanoemulsion containing Posaconazole was created utilising the HPH technique to improve solubility.

The aim of this research work is to formulate and evaluate the nanoemulsion containing Posaconazole. The prepared Nanoemulsion will lead to increase the solubility of Posaconazole. Thus, it will also increase its local action.

MATERIALS

The active ingredient Posaconazole was purchased from Sigma Aldrich, Mumbai, India. The excipients cinnamon oil, oleic acid, castor oil, tween 80 were purchased from S. D. Fine Chemicals (Mumbai, India). Poloxamer-188 (Pluronic F68), Transcutol HP were obtained from Hi media (Mumbai, India) and Gattefosse (Mumbai, India) respectively. The chemicals PG, PEG 400, Methanol, Dimethyl sulphoxide, Glycerin were procured from Merck Ltd. (Mumbai, India). Potassium di-hydrogen phosphate, disodium hydrogen phosphate, sodium hydroxide obtained from Loba Chemie Ltd. (Mumbai, India). Sodium bicarbonate was obtained from RFCL Ltd. (New Delhi, India).

METHODOLOGY

Characaterization of Posaconazole drug:

Melting point:

The capillary technique is used to find a drug's melting point. The medicine is loaded up to 3 mm of height by shutting the capillary tube at one end. The capillary is inserted into the digital melting point apparatus. Keep track of the temperature when the Posaconazole starts to melt. [6]

Fourier Transform Infra-red Spectroscopy (FTIR):

Posaconazole spectra were collected using an FTIR spectrometer-430 (Shimadzu 8400 S, Japan). Posaconazole was mixed in a ratio1:100 with potassium bromide of IR grade and compacted at 15 tonnes pressure in a motorised pellet press machine (Kimaya engineers, India). Following that, pellets were identified using an FTIR spectrophotometer.[(6)] Compared it with reference standard IR spectrums of Posaconazole.[7]

UV Spectroscopy:

According to European pharmacopoeia, 10 mg of Posaconazole was dissolve in 100 ml of methanol. From the above solution, 0.1 ml was taken and diluted to 10 mL with methanol before being analysed at 200-400 nm. Then, in 100 mL Dimethyl Sulphoxide (DMSO), 10 mg of Posaconazole was dissolved. A sample of 0.1 mL of the stock solution was diluted in DMSO up to 10 ml before being analysed at 200-400 nm.[(6)]Obtained spectrum of Posaconazole drug sample was compared to Posaconazole reference spectrum.[7]

Differential Scanning Calorimetry (DSC):

Samples of 1 mg of pure drug loaded spanlastics was placed in a standard aluminium pan and heated from 35^0 to 300^o at a constant heating rate of 10^o/min, under nitrogen with a purging rate of 20 ml/min using a DSC (Shimadzu, Kyoto, Japan, DSC–60). Any incompatibility (significant shift or disappearance/appearance of shows display result) was observed or evaluated in the thermograms.[8]

Solubility of oil phase surfactant and cosurfactant:

Solubility of Posaconazole in various different vehicles like oils (oleic acid, castor oil, cinnamon oil), surfactants (tween 80, pluronic-188) and cosurfactants (transcutol HP, PEG 400, propylene glycol and Glycerine) was determined. Posaconazole was added to the 2 ml of each of the 5 ml stoppered vials that were chosen. The initial 5 ml was then combined using a magnetic stirrer for a few min. After being shaken for 72 h in a mechanical bath shaker, the followed by centrifugation at 10 000 r/min for 10 min. Solubility was evaluated using a validated UV - visible spectrophotometer at 261 nm or 274 nm after the supernatant was withdrawn, filtered, and diluted with methanol.[9,10]

Preparation of Nanoemulsion by High Pressure Homogenizer (HPH):

A Posaconazole-containing Nanoemulsion was created using an Ultrasonic-HPH technique. The trial-and-error approach was used to develop the product. Dissolved the drug in cinnamon oil at 75^o with magnetic stirring for 30 min then oil phases were filtered through 0.45 µm membrane. Hydrophilic surfactants (Tween 80 and Poloxamer 188) were dispersed in a lipophilic cosurfactant of Transcutol HP then added in distilled water. Then, using a magnetic stirrer, add the oil phase dropwise in the water phase. High shear mixing (FJ-200, Shanghai Sample Model Factory, Shanghai, China) at 10000 r/min for 10 min with 40 W ultrasonication intensity, followed by 60 min with a ultrasonic processor of high intensity, produced a nanoemulsion (Ultra cell 750 W, Sonics materials Inc. USA). Then volume was adjusted with double distilled water to 100 ml and pH adjusted to 6.8 with 0.1M HCL. High pressure homogenization was applied in 500-700 MPa pressurefor 8cycles at 40^o. Nanoemulsion further used for characterization at room temperature.[(12),(8),(13)]Various batches used for the optimization (Table 1).

Table 1: Selection of Optimized Posaconazole Nanoemulsion

Sr. No.	Oil (%)	Conc. of Surfactant (%)	Conc. of Cosurfactant (%)	No. of HPH Cycle (500-700)	Particle Size (nm)	Zeta Potential	PDI	RefractiveIndex(± SD)
1	1	0.5	0.2	8	206.8	-40.5	0.456	1.69± 0.31
2	1	1.5	1	5	282.8	-40.2	0.561	1.81± 0.27
3	1	1.1	1	7	187.3	-29.8	0.405	1.98± 0.54
4	1	1.3	0.2	7	196.8	-31.2	0.405	1.66 ±0.41
5	0.2	0.1	1	5	212.5	-32.4	0.336	1.52± 0.25
6	0.2	0.2	0.1	8	236.9	-27.3	0.37	1.73± 0.39
7	0.2	0.3	0.2	8	285.8	-48.5	0.463	1.71± 0.44
8	**0.2**	**0.4**	**0.2**	**7**	**78.79**	**-9.46**	**0.315**	**1.35± 0.12**
9	0.3	0.5	0.3	7	288.7	-25.8	0.391	1.68± 0.29
10	0.4	0.1	0.4	8	337.6	-30.4	0.467	1.64± 0.33
11	0.2	1	0.2	9	214.1	-27.7	0.312	1.59± 0.18
12	0.2	2	0.3	8	256.4	-29.4	0.342	1.55± 0.31
13	0.2	3	0.4	9	348.2	-27.9	0.572	1.34± 0.16
14	0.2	4	0.2	8	276.4	-30.5	0.428	1.25± 0.36

Dispersion stability test:

Heating and cooling cycles: Between the refrigerator temperature 4º and 45º with storage at each temperature for a 48 h minimum period, six cycles were performed. Those formulations which were stable at these temperatures, were subjected to centrifugation test.[6]

Centrifugation:

For 30 min, the centrifugation of formulations was run at 3500 r/min. The freeze/thaw stress test was performed on formulations that did not exhibit any phase separation, creaming, or cracking.[6]

Freeze/thaw cycles:

For the test, three freeze/thaw cycles were performed. Temperatures ranging from -21° to +25° were tested, with each temperature being stored for a total of 48 h. The formulations which demonstrated no creaming, phase separation, phase inversion or coalescence were chosen for the kinetic destabilisation test in these tests.[6]

Evaluation of Nanoemulsion:

Particle size and polydispersity index and Globule size:

The droplet size of nano-emulsion, reported as hydrodynamic diameter (DH), was calculated at 25⁰using DLS with a Zetasizer Nano ZS (Malvern Instruments Ltd., Malvern, UK), and the size distribution was expressed as polydispersity index in parallel. Before the measurements, the sample was filtered by using 0.22 µm pore size filter so as to eliminate any contaminants. Each number gives the average of three runs with at least ten measurements.[10,14]

Zeta potential (ξ-potential):

The -potential of nanoemulsion droplets, which indicates the electric charge on the particle surface, was measured by micro electrophoretic method utilising the Zetasizer Nano ZS (Malvern Instruments Ltd., Malvern, UK). All measurements were obtained and analysed at 25⁰. Each result was calculated as the average of three successive runs of the instrument with at least 20 readings.[10]

Thermodynamic Stability of Nanoemulsion:

To tackle the issue of metastable and unstable formulations, nano-emulsions were exposed to time-dependent size (DH) and potential measurements at room temperature, as well as dispersion stability experiments that comprised heating - cooling cycles, centrifugation, and freeze–thaw cycles. For the turbidity test, the nano-emulsion that showed no creaming, phase separation, coalescence, or phase inversion.[10,15,16]

Dilution Test:

To generate a stable nanoemulsion, the proper surfactant blending is required for the creation of NE formulation. The nanoemulsion was diluted with double distilled water and examined visually for cracking, phase separation, and clarity/turbidity. The effect of dilutions on PDI and globule size is investigated in an HPH formulation. The formulation is diluted 1000 times into distilled water before being tested by Dynamic Light Scattering method.(13)

Determination of viscosity:

The flow curves of nanoemulsions were calculated using a Brookfield viscometer (model DV-II+, Brookfield, Labomat Essor Ensor, Saint-Denis, France). This approach is also used to compute the flow curves of novel emulsions. The shear rate was changed between 1 to 100 s^{-1}. After a 5 min rest period; all measurements were repeated in duplicate at 201º equilibration of emulsion samples at the same temperature in the viscometer device. The power law model [the shear stress (Pa), the shear rate (s^{-1}), the flow consistency index, K (Pa s^n)], and flow index were used to characterise the rheological behaviour of emulsion samples.[17]

Refractive Index Determination:

The refractive index (n) of a medium is calculated as the ratio of the reference medium's wave speed (c) to the medium's phase speed (vp), n= c/vp. The refractive index of a nanoemulsion may be determined using an Abbe's type

of refractometer at 250.5° by putting a drop of formulation on a slide and compared it with refractive index of water (1.333).If the refractive index of a nanoemulsion is same as that of water, then nanoemulsion is said to be transparent. (Tokyo, China: Ningbo Biocotek Scientific Instrument, Ltd.)[18]

Drug Content by using UV spectroscopy:

Posaconazole was isolated from NE formulations by dissolving 1 ml of NE in methanol. Posaconazole concentration in methanol extract was measured using a spectrophotometer (UV 1700, Shimadzu, Japan) at 261 nm and a dimethyl sulfoxide (DMSO) at 274 nm.[19]

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

An optimised nanoemulsion formulation was subjected to in vitro release experiments (Batch F8). Diffusion tests were performed using a dialysis membrane (DM-135, Hi-Media, Mumbai). In the diffusion medium, a soaking hydrated membrane was employed. In dialysis membrane sac (area approximately 1.4 cm^2) 1 ml of formulation was placed and was sealed on both ends. Then the dialysis membrane placed in a glass beaker which contains 25 ml diffusion medium of pH 7.4 buffer solution. The drug release investigation was conducted at 37°± 0.5° at periodic intervals, with a known volume of sample taken and replaced with an equal volume of fresh warm buffer solution after 30 min. The drug concentration was determined using an UV spectroscopy at 274 nm. Similar process was performed for the marketed formulation, and and drug concentration was evaluated using a UV-Vis spectroscopy set to 274 nm.[6]

Accelerated Stability study:

Formulation's stability was assessed by centrifugation at 3500 r/min for 30 min. The stability of an optimised batch (F8) was evaluated over 3 month at three distinct temperatures: refrigerating condition (28 ± 2°/ 75 ± 5% RH); room temperature (25 ± 2°/ 65 ± 5% RH); and high temperature (40 ± 2°/ 75 ± 5% RH) as per ICH recommendations. Visual inspection (clarity/turbidity, phase separation), pH, zeta potential, globule size, and polydispersity index were used to assess the nanoemulsion formulation at 0, 30, 60, and 90 d.[8]

RESULTS AND DISCUSSION

Posaconazole's melting point was shown by using the glass capillary method, and the observed melting point was 167°-169°, which was validated by the standard melting point of Posaconazole, which is 170°-172°.

FTIR spectra of Posaconazole and mixture of Posaconazole was taken by using the KBr disk method. Obtained IR spectrum of Posaconazole is given in figure 1. Obtained IR spectrum of Posaconazole and pluronic 188 mixture is given in (Fig. 2). From this study, we concluded that there is no any interaction found between drug and excipients used.

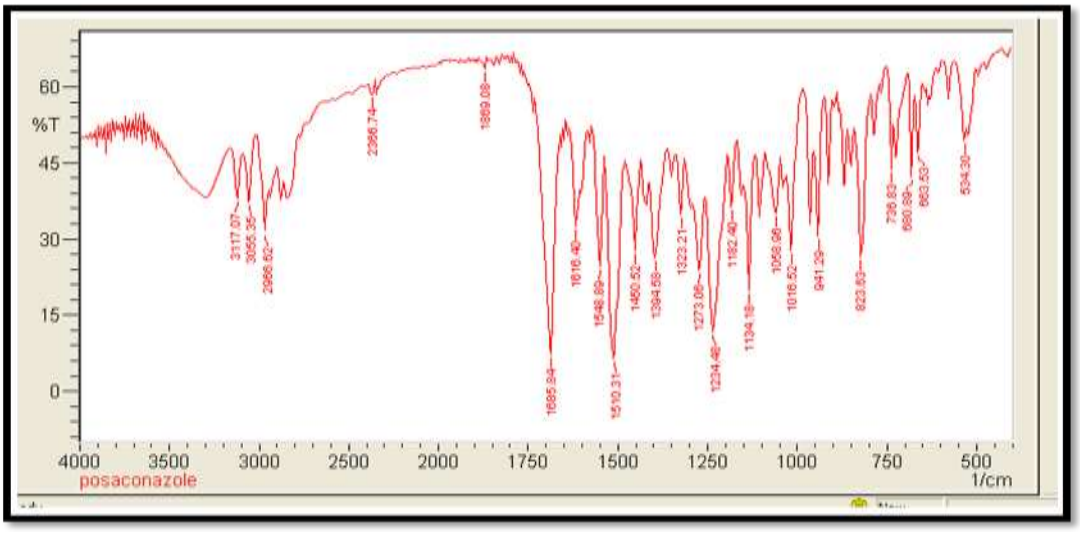

Figure 1: FTIR spectra of Posaconazole

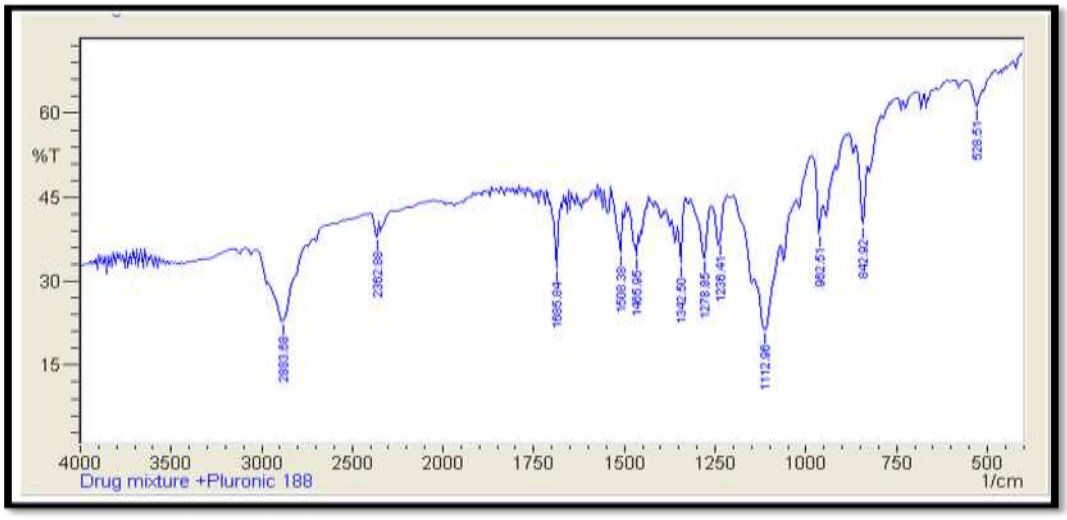

Figure 2: FTIR spectra of Posaconazole and pluronic 188 mixture

The solution of Posaconazole in methanol and DMSO was found to exhibit maximum absorption at 261 nm and 274 nm, respectively. The solution of Posaconazole in methanol was found to exhibit maximum absorption at 274nm after scanning on the UV-Vis spectrophotometer which was reported as λ max in the literature and thus the procured drug sample of Posaconazole complies with the reference spectra and produce drug sample Posaconazole complies with the reference spectra.

Melting point of Posaconazole was measured by using DSC (Mettler Toledo) was found to be 171˚C.

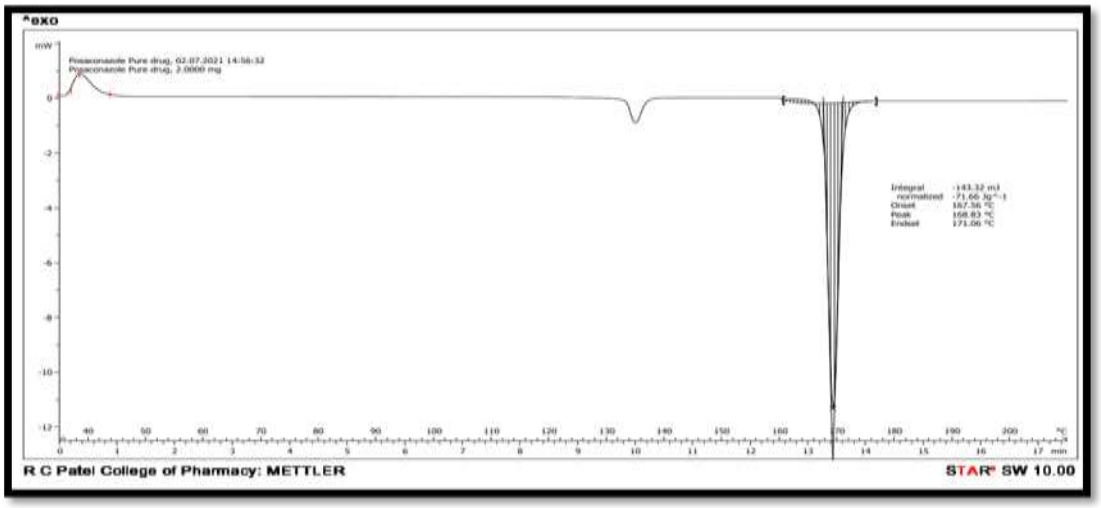

Figure 3: DSC Thermo gram of Posaconazole

The solubility of Posaconazole was found to be highest inoleicacid oil (0.83 ± 0.21 mg/mL) as compared to other oils presented in **(Fig.4.)** And the solubility of Posaconazole was found to be highest in cinnamon oil (0.83 ± 0.25 mg/mL) as compared to other oils presented in hence cinnamon oil was selected as the oil phase for the development of nanoemulsion formulation.

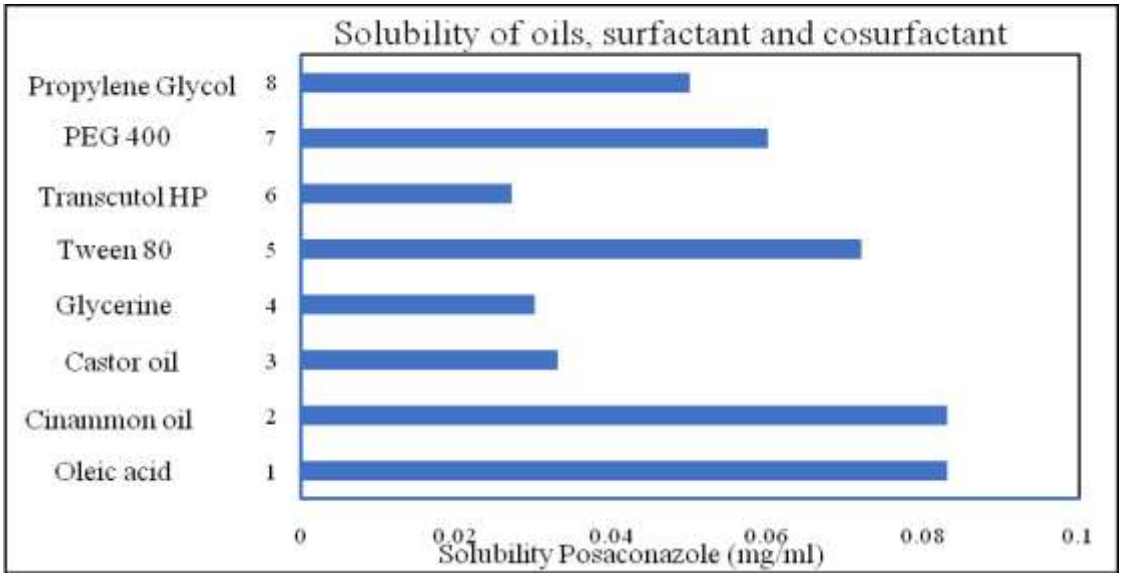

Figure 4: Solubility of Posaconazole in oils, surfactant and cosurfactant (mg/ml)

In preliminary trials total concentration of oil indicated that total concentration of oil is 1, 1.3, 1.5% then concentration of surfactant is 0.2,0.3,0.2 gm concentration of co-surfactant 0.1,02,0.5 % during preparation, main factor that affected the Globule size, Zeta-potential, and PDI of the Posaconazole Nanoemulsion. Total % Oil concentration (X_1), Surfactant concentration(X_2), HPH Cycle (X_3) as an independent variables and Globule size (Y_1), Zeta potential(Y_2) PDI, Zeta-potential (Y_3), are the dependent variables. When the concentration of independent variables changes. As Globule size, Zeta-potential, and PDI. Increase when Oil concentration increases and HPH Cycle increases then Globule size decreases. Thus, depending upon the result obtained the optimal range of independent variables concentration of total % oil 4-10 (%), HPH Pressure 500-700 (bar), and HPH Cycle7-15. Total 3 trials batches were conducted. That are shown in **(Table No.2,3)**

In independent variables are dependent on dependent variables.As per independent variablesconcentrations of oil is increases then dependent variables like PDI is increased.

As per concentration of surfactant is increased zeta potential is also increases.cycle of HPHthen PDI Increases the cycle as per the combination oil surfactant and cosurfactant HPH cycle increases.

Table 2: Preliminary experiments used in posaconazole NEs preparations

Sr.No	Batch (Mg)	Pressure & Cycles	% Oil Conc. (ml)	Surfactant conc. (gm.)	Co Surfactant conc. (gm.)	Globule Size (nm)	Zeta (mV)	PDI
1	10	500-700 Bar & 7	01	0.2	0.1	206	-30.46	0.315
2	20	500-700 Bar & 8	1.3	0.3	0.2	307	-15.6	0.480
3	15	500-700 Bar & 9	1.5	0.2	0.5	401	-32.5	0.456

Batch 3 shows good result having a minimum or maximum globule size and maximum zeta, PDI that indicate that the batch 3 containing % oil concentration, surfactant concentration, HPH Pressure and cycle was optimum. After Solubility it can give optimum concentration of % oil, surfactant and HPH cycle for preparation of nano emulsion. On that basis we apply designing of formulation.in an optimized formulationin (**F8**)optimized batch formulations.the trial batch of formulation of oil, surfactant and cosurfactant of drug is maximum concentrations ofdrugs.(n=3).

Table 3: Data of physicochemical characterization

Batch	% Oil (ml) (A)	Surfactant(ml) (B)	Cosurfactant (ml)	HPH cycle (rpm) (C)	Globule size (nm)	Zeta potential (mV)	PDI	viscosity (± SD)	Rf-value (± SD)
F1	01	0.5	0.2	8	206.8	-40.5	0.456	0.0387	1.69 ± 0.31
F2	01	1.5	1	5	282.8	-40.2	0.561	0.0407	1.81 ± 0.27
F3	01	1.1	1	5	187.3	-29.8	0.405	0.0427	1.98 ± 0.54
F4	01	1.3	0.2	7	196.8	-31.2	0.405	0.0432	1.66 ± 0.41
F5	0.2	0.5	1	7	212.5	-32.4	0.336	0.0493	1.52 ± 0.25
F6	0.2	0.2	0.1	8	236.9	-27.3	0.370	0.0497	1.73 ± 0.39
F7	0.2	0.3	0.2	8	285.8	-48.5	0.463	0.0504	1.71 ± 0.44
F8	**0.2**	**0.4**	**0.2**	**7**	**78.79**	**-9.46**	**0.315**	**0.0593**	**1.35 ± 0.12**
F9	0.3	0.5	0.3	7	288.7	-25.8	0.391	0.0598	1.68 ± 0.29
F10	0.4	0.1	0.4	8	337.6	-30.4	0.467	0.599	1.64 ± 0.33
F11	0.2	1	0.2	9	214.1	-27.7	0.312	0.0656	1.59 ± 0.18
F12	0.2	2	0.3	8	256.4	-29.4	0.342	0.0701	1.55 ± 0.31
F13	0.3	3	0.4	9	348.2	-27.9	0.572	0.0727	1.34 ± 0.16
F14	0.4	4	0.2	8	276.4	-30.5	0.428	0.0843	1.25 ± 0.36

From batch F1 to F14concentration of oil is 1% to 0.4 % respectively then concentration of surfactant is 0.5ml to 4ml and HPH cycle5-9 respectively. Concentration of oil was shows constant significant effect on that globule size is increases affect. Optimum concentration ofindependent variable shows significant effect on those dependent variables. In batch **F08**globule size was increased and zeta potential and PDI was decreased as compared to batch **F13**. In batch **F13**concentration of that independent variable shows significant effect on those dependent variables. The number of concentrationsis globule size of HPH cycle reduces the droplet size and zeta potential optimized concentration is dependent and independent variables determines.

In F1 batch concentration of oil(1%), surfactant(0.5%) and cosurfactant(0.2%), 8 HPH cycle then we got globule size206.8nm.F2 Batchconcentration of oil(1%), increases volume surfactant(1.5%), cosurfactant (1%), decrease HPH cycle is 5 then globule sizeis increases 282.8nm. In F3 batchoil (1%), of surfactant (1.1%),cosurfactant (1%) HPH cycle 5then globule size is decreases than F2 batch is 187.3 nm.surfactant concentration of batch F4 is (1.3%) cosurfactant (0.2%), Oil(1%) HPH Cycle is 7 globule size 196.8 nm.For F5 batch oil 0.2% surfactant concentrations (0.5%),and cosurfactant (1%)HPH cycle 7 globule size is 212nm as compare to F4 globule size decreases.F6concentrations oil (0.2%), surfactant(0.2%), cosurfactant (0.1%) is an HPH cycle 8 globule size 236.9 nm compare F5 batch to decreases globule size.F7 oil concentrations of (0.2%), surfactant (0.3%), and cosurfactant (0.2%) HPH cycle of globule size 285.8 nm compare to (F5,F6) decreases globule size.F7 oil concentration 0.2%,surfactant concentration 0.4%and cosurfactant 0.2% HPH cycle 7 globule size is 78.79 nm. Compare to F1to F7 batches increases globule size **F8** batch. Stress testing is mandatory to avoid the risk of metastable formulations. It was discovered that the optimised batch (F8) was stable. There was no evidence of phase separation, turbidity, creaming, or cracking. Nanoemulsions with thermodynamic stability have a longer shelf life than conventional emulsions with kinetic stability.

The mean globule sizes of the all fourteen formulations were found to be in the range (100 to 348) nm. The zeta potential of the all seventeen formulations was found to be in the range (-9 to -48.5). The polydispersity index (PDI) of the all seventeen formulations was found to be in the range (0.315 to 0.561) shown in table 4.

The given formulations were virtually observed. The improved formulation (Batch F 08) was transparent, with no signs of cracking or creaming. The mean globule sizes of the all fourteen formulations were observed in the range of 100 to 348 nm. All seventeen formulations' zeta potentials were determined to be in the range (-9 to -48.5). The polydispersity index (PDI) of the all seventeen formulations were observed in the range (0.315 to 0.561) given in the (Table 4). The concentration of oil, cosurfactant, surfactant were 0.2%, 0.4%, 0.2%, respectivelyof optimized batch F08.

Table 4: Mean globule size, zetapotential and polydispersity index.

Batch No.	Globule size	Zeta potential	PDI
F1	206.8	-40.5	0.456
F2	282.8	-40.2	0.561
F3	187.3	-29.8	0.405
F4	196.8	-31.2	0.405
F5	212.5	-32.4	0.336
F6	236.9	-27.3	0.370
F7	285.8	-48.5	0.463
F8	**78.79**	**-9.46**	**0.315**
F9	288.7	-25.8	0.391
F10	337.6	-30.4	0.467
F11	214.1	-27.7	0.312
F12	256.4	-29.4	0.342
F13	348.2	-27.9	0.572
F14	276.4	-30.5	0.428

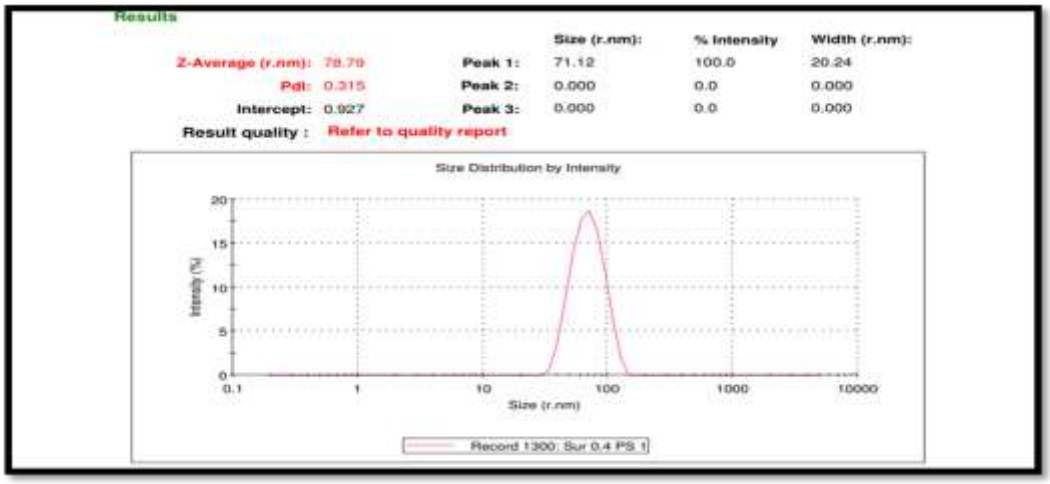

Figure 5: globule droplet size of nano formulation

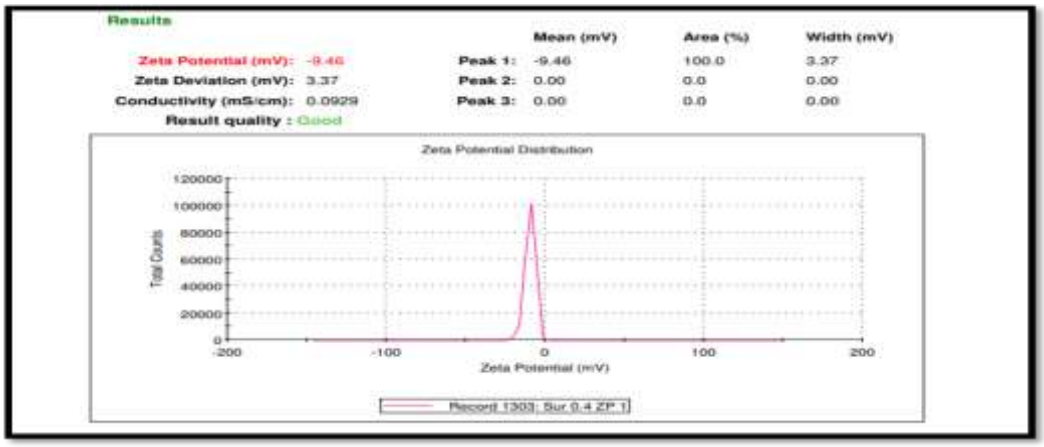

Figure 6: Zeta potential graph of optimized formulation (Batch08)

Nanoemulsion in optimized formulation (Batch F08) showed in 0.0593 cps. Non-newtonian value of viscosity flow is low. As shear stress decreases, shear strain increases (Table 4). Nanoemulsions exhibited pseudoplastic flow behaviour (Fig. 8).

Refractive Index of nanoformulation of Batch F08 was calculated to be 1.35 ± 0.12 (Table 1) which is equal to the Refractive Index of water (1.333). From this, we can conclude that the optimized nanoemulsion was transparent.

Drug content of optimized batch (F08) was found to be 90.21 ± 0.23%for posaconazole (mean ± SD, n=3). Optimum concentrations of oil, surfactant and cosurfactant is essential in maximum drug loading formulations to give Maximum drug content. Optimized batch (F08) had significant variables of drug content.

The release pattern of Posaconazole from optimized formulation (Batch F8) through a dialysis membrane at pH 7.4 was shown in (Fig. 8). The release pattern of optimized nanoemulsion appears to be fast release with negligible burst

effect. In F08 batch, percentage CDR Formulation of test product is equivalent to percentage CDR of marketed product. In F08 batch globule size was found to be 78.79 nm as the decreasing the globule size increases the percentage CDR according to time.

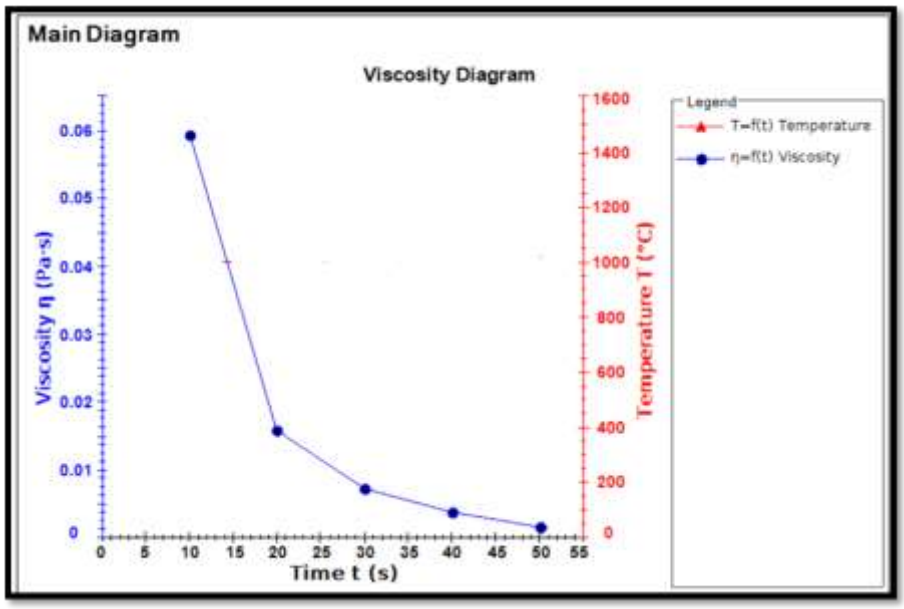

Figure 7: Viscosity study of optimized batch formulations (F8)

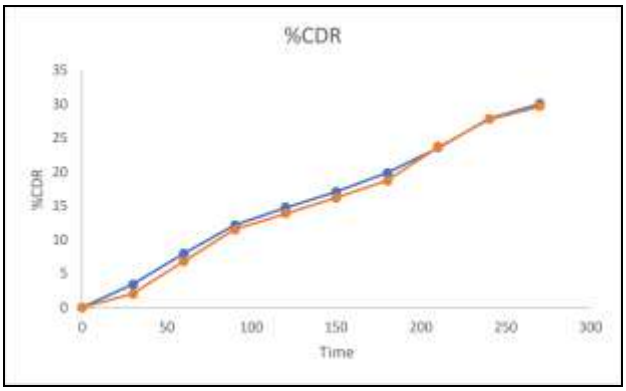

Figure 8: % Drug Release profile of Posaconazole loaded nanoemulsion

In ICH guideline of stability testing of Nanoemulsion optimized formulation was stored in room temperature and refrigerator conditions. Zeta potential, particle size and polydispersity index of optimized batch F08 was used to found stable. Stability study of zeta potential and particle size, polydispersity index of drug increased the activity up to 186 ± 0.59 nm and 196 ± 0.51 nm (Table 6).

Table 5: Stability Test of Zeta Potential, Particle Size And Polydispersity Index

Stability Parameters	Test Period			
	0 Months	**1 Months**	**2 Months**	**3 Months**
Phase Separation (PS)	No PS	No PS	No PS	No PS
pH	6.2 ± 0.16	6.9 ± 0.40	6.4 ± 0.16	6.6 ± 0.11
Globule Size (nm)	186 ± 0.59	196 ± 0.51	206 ± 0.38	212 ± 0.77
Zeta Potential (mV)	-15.02 ± 0.10	- 17.04± 0.10	-12.07± 0.17	-19.7 ± 0.17
Polydispersity Index (PDI)	0.394 ± 0.01	0.389 ± 0.04	0.374 ± 0.02	0.313 ± 0.03

DISCUSSION

Posaconazole is a triazole antifungal drug that inhibits cytochrome P450-Dependent Enzyme resulting in impairment of ergosterol synthesis in fungal cell Membranes. But it has low solubility in water, so that it shows less antifungal activity against *Candida albicans*. To overcome this problem, nanoemulsion is one the most attracted formulation by researcher to treat fungal disease and to increase water solubility of poor soluble drugs.

This study was undertaken to formulate, develop and optimize nanoemulsion formulations of Posaconazole to control the release characteristics of a poor water-soluble drug. The study revealed that nanoemulsions might be utilized for bioavailability improvement of drugs whose absorption is limited because of their solubility.Preparation of 14 batches (F01 to F14) with different ratios of oils, surfactants and cosurfactants. From this batches, F8 batch showed optimized results by analyzing the batches with parameters such as droplet size, PDI, zeta potential, viscosity, drug content, pH, *invitro* release of drug.

Posaconazole Nanoemulsion system with 0.2% Cinnamon oil as oil, 0.4% tween-80 and poloxamer-188 as surfactants, 0.2% transcutol HP as cosurfactant, and distilled water proved best for topical Posaconazole administration. This preparation showed maximum solubility in cinnamon oil, tween-80, poloxamer-188, transcutol HP up to 0.083 mg/ml, 0.072 mg/ml, 0.027 mg/ml.

Viscosity measurement of different batches with constant temperature showed different viscosities but for optimized batch (F08) it was raised to 0.0593 cps. Non-newtonian value of viscosity flow shows that as shear stress decreased, the shear strain increased. Nanoemulsion showed pseudoplastic flow behaviour.

In-vitro diffusion data showed, the release pattern of optimized nanoemulsion appears to be fast release with negligible burst effect showed highest diffusion coefficient when % CDR formulation of test product is equivalent to % CDR of marketed product,demonstrating its potential for enhancing permeation by topical route of Posaconazole.

The nanoemulsion system was stable at ambient conditions for 3 month with optimum pH, globule size, zeta potential, PDI and no phase separation was observed.

Based on the findings, it is possible to infer that NE mediated delivery is an economic approach for effective as well as safe localized delivery of Posaconazole against fungal infection.The nanoemulsion system is a potential technique for the topical distribution of Posaconazole for the enhancement of therapeutic effects.

CONCLUSION:

Nanoemulsion formulation was successfully prepared by using oily phase, surfactant and co-surfactant polymers by high pressure homogenization method. On the basis of result we can conclude that it was found to be helpful in near future for to treat fungal diseases.

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Conflict of Interest: None

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