"FORMULATION AND EVALUATION OF VORICONAZOLE LOADED NANOEMULGEL FOR THE TREATMENT OF ONYCHOMYCOSIS"

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Abstract:

Aim: Voriconazole has been a more potent antifungal activity but this has some grade of medical application due to low solubility issue. The goal of the current work was to development of Voriconazole nanoemulgel, to improve aqueous solubility of drugs across the nail used for the treatment of onychomycosis.

Method: Aqueous titration method was use to prepare voriconazole nanoemulgel by using olive as oil, Tween 80, and PEG 400 as used as a surfactant and co-surfactant respectively. Its homogenized under high pressure to minimize the particle size of emulsion. Vesicle size and drug contain were optimized by 2 level, 2 factor, Simple Lattice design and the optimized batch. Nanoemulgel was prepared by using, the gelling agent is Carbopol 934. Thioglycolic acid is used as a nail penetration enhancer.

Result: Drug polymer compatibility study show that drug and excipient are compatible with each other. A nanoemulgel study of in vitro drug release revealed improved drug release, i.e. that is 68% in 8th hrs. Thus more than 90% of drug release will happen in 24th hrs. which shows good release. Zone of inhibition of optimized batch was found to be 9.8mm.

Conclusion: The designed system is used for the deliver the poor aqueous soluble drug. The Obtained nanoemulgel of Voriconazole is effective in treatment of Onychomycosis.

Keywords: Nanoemulsion, Nanoemulgel, Onychomycosis, Voriconazole.

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1. Introduction

Onychomycosis is a nail fungal infection; It's also called tinea ungium [1]. All worldwide 12-14 % of populations are infected by the disease 10% occur in the adult population, 20% in-between age 60, and 50% above age 70 [2]. Approximately 90% of toenail and 75% of fingernail shows a nail that becomes thickening, Parting of the nail from the nail bed, white or yellow Nail staining, etc. [3]. The causing agent of nail fungal infection is mainly by dermatophytes, non-dermatophytes, and yeast. In that 80% of tinea ungium is caused by dermatophytes, like Trichophyton rubrum, trichophyton mentagrophytes and Epidermophyton floccosum [4-7].

A Various types of antifungal drugs are used in the treatment either oral or topical route. But oral antifungal drugs show side effects; hepatotoxicity, drug interaction, abdominal pain, rashes, etc. Topical therapy gives fewer side effect and also deliver the drug on a specific site. Controlling onychomycosis has shown one or more omissions like low cure rates from amorolfine nail lacquer, ciclopirox nail lacquer, nail paint, efinaconazole solution, tavaborole solution [8-12]. Voriconazole show the 52% success rate in the primary therapy. Voriconazole is a triazole antifungal showing fungi static action in contradiction of fungal pathogens. Similar to other triazoles, voriconazole inhibits the demethylation of lanosterol as a component of the ergosterol production route in yeast and other fungi by binding to 14-alpha sterol demethylase, commonly known as CYP51. Ergosterol deficiency impairs fungal cell membrane functioning and prevents fungus cell proliferation. [13-14].

The nail is composed of the 80-90 layers of the keratinized cell. Its glowing construction contains extremely specialized epithelial cells. Due to the bond between the keratin fiber and surrounding area (disulfide, Hydrogen, electrostatic, acid-base bond) drugs show poor diffusion. New strategies need of to develop for this overcoming the problem [15].

Nanoparticles show the better penetration as well as prolonged retention of the drug on the nail. It's helped to avoided harmful surgical, and parental treatment. Nanoemulsion or ultrafine emulsion (droplet size 20- 200 nm) it helped in delivered the active pharmaceutical ingredient (API) because of nanoemulsion are highly kinetic stable [16]. It contains two immiscible liquids (oil, water) can homogenized to form a single phase liquid i.e. Emulsion, it's a thermodynamically unstable it should be stable by using of the emulsifying agent (Surfactant or co-surfactant) [17]. In nanoemulsion addition of gelling agent or gel it convert into the nanoemulgel. In this way, drug containing nanoemulgel and aqueous gel base system is a convenient way to attaining better stability and sustained release of drug. Irritation and side effect are least likely when nanoemulgel is applied [18].

The goal of our research work is a by using Thioglycollic acid as a penetration enhancer, we formulated a topical nanoemulgel of voriconazole that had desirable characteristics and enhanced transungual permeability. For their safe and effective topical usage, the optimized nanoemulgels were tested for ex vivo antifungal properties, and stability.

2.Materials

Voriconazole received as gift sample from MSN laboratories, Isopropyl Myristate, Olive oil, Polyethylene Glycol 400, Polyethylene Glycol 600, Methyl Paraben, Propyl Paraben, Carbopol 934, Carbopol 940, HPMC K-100, Sodium CMC (purchased from Ana Lab Fine Chemicals, Mumbai) Tween 80, Transcutol, Thioglycollic Acid, Triethanolamine (Loba Chime Pvt. Ltd. Boiser, Palghar).

3. Methods

a. Formulation and Development of Nanoemulsion

Due to voriconazole's maximum solubility (5.64 mg/ml) in olive oil was chosen as the oil phase compared with the other experimental oils. Since Tween 80 and PEG 400 demonstrated the highest levels of drug solubility (4.27 mg/ml and 4.73 mg/ml, respectively), they were employed for the surfactant and co-surfactant, respectively. Tween 80: PEG 400 was blended in a 2:1 ratio with a co-surfactant (S: cos) [19, 20]. Drop by drop, using a micropipette, water was added to the oil and Smix (S: cos) to create the crude emulsion (Aqueous titration technique). These is nanoemulsion is optimized by using the DOE software and stress stability study is explained below. Take a required quantity of oil, Smix, water, API from the optimized batch of the DOE software. The aqueous phase of the nanoemulsion was created by dissolving PEG 400 in filtered water, while the oil phase was created by dissolving Tween 80 in olive oil. Methanol was used to dissolve the medication (1% w/v), and then the methyl and propyl parabens were added. The oil phase was then coupled with this solution. The oily and aqueous phases were

both heated to 70 to 80 °C before the oily phase was added, and the two phases were then allowed to cool to ambient temperature while being stirred magnetically for 15 minutes at 100 rpm. The crude nanoemulsion, later passed on high pressure homogenizer for 3 hours at 5000 rpm to get the uniform sized droplets and done the stress stability study.

b. Preparation of Gelling Agent

The gel was created by dissolving carbopol 934, carbopol 940, sodium CMC, and HPMC k-100 in hot water and agitating them constantly with a magnetic stirrer at a moderate speed to achieve uniform solubility. To bring the pH of the gel to a neutral level, a few drops of triethanolamine were added. The concentration of the sodium CMC was chosen as the gelling agent varies and creates different batches.

c. Incorporation of nanoemulsion into gel nanoemulgel

Optimized batch of Emulsion and gel was mixed 1:1 and added the nail penetration enhancer (Thioglycolic acid) and mixed well [19].

4.Preformulation Study

Preformulation is well-defined as a study of the physical and chemical properties of a pharmacological ingredient, both alone and in combination with excipients. [20] The first step developing logical dose forms for a medicine is to conduct Preformulation testing. The goal of pre-formulation testing is to gather data that will aid in the development of stable and bioavailable dosage forms mass produced.

4.1 Organoleptic Properties

The organoleptic characteristics such as appearance, colour, odour was tested in visually in experiments.

4.2 Determination of Melting Point

The melting point of the API was measured by using the open capillary tube technique. For measuring the M.P. one side of the capillary tube was sealed and small quantity of the drug was inserted from another open side by spatula. The filled capillary was inserted into melting point apparatus and reading was calculated. This procedure was performed in triplicate and mean of three observations is considered as a melting point.

4.3 Solubility Study

Different type of organic solvent and water are used for the checked the solubility of Voriconazole. 10mg of drug was taken in a three test tubes and recommended volume of respected solvent was added. The test tubes were shaken and observed for clarity of a solution. [21]

4.4 FT-IR Spectroscopic Determination

The API and potassium bromide disk were prepared manually by KBR press method. Potassium bromide was used as a blank for screening. The scanning range was 400-4000cm. About 1mg of drug was triturated with 1mg of potassium bromide with help of motor and pestle and it the pressed into the pallet manually (1:1). Shimadzu iris 400 was used to obtain IR spectra of the prepared disc of Voriconazole. This procedure was performed in triplicate and mean of three observations is considered as an ideal spectrum. This spectrum was compared with the literature.

4.5 Estimation of Maximum Absorbance Wavelength

10mg dissolve in 10 ml methanol and makeup volume up to 100 ml in a volumetric flask using methanol (stock solution). From the stock solution removed 1ml and make volume up to 10 ml by methanol (10µg/ml) solution. From this 0.2ml,0.4ml ,0.6ml,0.8ml ,1 ml solutions removed and makeup volume up to 10 ml and get the concentration of 2µg/ml, 4µg/ml, 6µg/ml, 8ug/ml, 10ug/ml respectively. UV spectrum of drug solutions was recorded in the wavelength range 400-200nm and maximum absorbance was measured at 254 nm in UV spectrophotometer using methanol as blank. Using the data obtained, a calibration plot of voriconazole was plotted.

Same procedure followed for the calibration curve of Voriconazole in phosphate buffer pH 7.4.

4.6 Drug Polymer Compatibility Study

A compatibility study was carried out with excipients and drug. API and excipients were mixed in specific quantity and placed in sealed vials for week at 40°C ± 2°/75%±5% RH and 25°C± 2°/75% ±5% RH. The samples were examined physically for any change in following parameters. FT-IR spectroscopy was used in the compatibility between drug and polymer. About 1mg of drug and 1mg of polymer was triturated with 1mg of potassium bromide with help of motor and pestle and it the pressed into the pallet manually. The FT-IR spectra of drugs with polymers were compared with the standard FT-IR spectrum of pure drug. [22]

4.7 Screening of Excipients

Solubility of voriconazole in various oils, surfactants and co- surfactants were determined by using shake flask method. An excess amount of voriconazole (approximately 100 mg) was added to 2ml of the oil, surfactant, co- surfactant. The mixture was mixed using magnetic stirrer to get uniform solution. The solutions were centrifuged at 3000 rpm for 10 minutes to separate the supernatant. Aliqouts of supernatant were taken, filtered through whatmann filter paper, filtrate was diluted with ethanol and drug was measured by measuring absorbance at 254 nm using uv spectroscopy. [23]

4.8 Differential Scanning Calorimetry (DSC)

DSC is a thermoanalytical technique. It's mainly used for those compound doesn't contain any nitro group.

5.1. Evaluation of Nanoemulsion

5.1.1 Physical Examination

In appearance of prepared Voriconazole emulsion were observed visually such as colour, homogeneity, consistency, Phase separation etc. Vesicle size of formulation are measured by Pixel Pro Software and drug contain measured at 254nm by using UV spectroscopy.

5.1.2 Stress Stability Study

Samples are placed in the heating and cooling cycle at temperatures between 4 and 45 °C and kept there for 48 hours. In centrifugation, the material was rotated down for 30 minutes at 3500 rpm. In the Freeze-Thaw Cycle, the sample was held at temperatures between -21 and +25 °C for 48 hours while physical instability was observed. [24]

5.1.3 pH

The pH of topical medications dosage forms is crucial because if it differs from the skin's natural pH condition, it may irritate it. The pH of the formulation was measured using a calibrated pH meter at 25°C.

5.1.4 Percentage Transmittance

Transmittance was observed by using UV Spectrophotometer (Shimadzu, Japan) at 254 nm. One milliliter of nanoemulsion formulation was taken in a test tube and ethanoic dilution was analyzed at 254 nm.

5.1.5 Droplet Size and Poly Dispersity

Mean particle size is a crucial indicator for comprehending or predicting how the nanoemulsion will behave. Based on the idea of photons correlations spectroscopy, which examines variations in light scattering brought on by the motion of particles, it was discovered using Zetasizer.

5.1. 6 Zeta Potential

Using a Zetasizer, the potential for zeta of the diluted tiny emulsion was calculated. The zeta potential and surface charge of emulsion droplets are measured. The size of the zeta potential indicates the formulation's potential stability.

5.1.7 Stability Studies of Nanoemulsion

The ICH recommendations, prepared nanoemulsion (approximate 15 ml) was put in glass vials and left for stability experiments at the following temperatures and relative humidity conditions for a total of two months: 25°C/60°RH, 40°RH/65°RH, and 60°RH/75°RH. Samples were taken after 15 days and examined for physical appearance, pH, phase separation, and drug content. [25-26]

5.2 Evaluation of Nanoemulgel

5.2.1 Physical Examination

In appearance of prepared nanoemulgel were observed visually colour, homogeneity, consistency, Phase separation etc. The pH of 1 % aqueous solutions nanoemulgel were measured by a calibrated pH meter at 25 °C.

5.2.2 Viscosity

The viscosity was used to determine the rheological behavior of the formulation. Viscosity was determined using Brookfield viscometer LMDV-60 at room temperature with a CPE 01 spindle at 30 rpm.

5.2.3 Drug Content

For determination of drug content 1mg of nanoemulgel was diluted upto 10ml with pH 7.4 phosphate buffer. drug content was measured by using UV at 254nm.

Eq. No.1: Drug Content = Theoretical yield / Practical yield *100

5.2.4 Spreadability

Spreadability was assessed by utilizing a wooden block as a tool, the 'Slip' and 'Drag' technique was used by nanoemulgel. A circle of 1 cm in diameter, already drawn between two uniform slides set on the wooden block, where one glass slide was secured while another was tied to a weight, was filled with extra the nanoemulgel (about 1 g). For five minutes, a one-kilogram weight was placed on top of both slides to produce a uniform layer of

nanoemulgel. The amount of time needed for the top slide to move after weight application was measured., and spread ability was calculated by using the following formula, [27]

Eq. No.2: S = M *L/T

where , S Spreadability
M Weight applied on upper slide
L Length of spreaded emulgel (cm)
T Time taken (sec)

5.2.5 In -Vitro Diffusion Study

Franz diffusion cell technique was used for the 8-hour in vitro diffusion research of nanoemulgel. A phosphate buffer solution (pH 7.4) and ethanol (3:1) solvent system were used to activate a cellophane dialysis membrane for roughly an hour. For testing, 1g of nanoemulgel is used. The entire assembly was maintained at 37°F (1°C) with a magnetic stirrer spinning at 100 rpm. Then, at predefined intervals, aliquots of drug samples containing roughly 1 ml were removed from the receptor compartment and promptly replaced with brand-new solvent systems. To evaluate the amount of drugs present, the samples were first diluted, then filtered, and their absorbance was determined at 254 nm using a UV spectrophotometer. [28]

5.2.6 Transungual Permeation Study

Remove connective and cartilaginous tissues from the goat horns before immersing them in distilled water for 24 hours. A chunk of the hooves' bottom portion was excised, about 1 mm thick. The hoof membrane was carefully positioned above the 15-ml Franz diffusion cell. The membrane's surface was coated with around 1 g of nanoemulgel.

5.2.7 Antifungal Activity

The antifungal activity of API and drug-loaded nanoemulgel against Candida albicans was assessed using the disc diffusion technique. Voriconazole solution and drug-loaded nanoemulgel stock solutions were made in DMSO and filtered water, respectively. In petri plates having Sabouraud dextrose agar (SDA) medium, C. albicans strains were grown. The discs containing the medication, nanoemulgel, and DMSO are inserted onto the plate using sterile forceps. To estimate a significant drop in colony sizes, a total of three sets of petri plates were left to incubate at 28°C, and zone dimensions were measured after 48 hours. [29]

6. Results and Discussion

6.1 Preformulation Study

6.1.1 Organoleptic Properties, Melting Point Determination

The sample was identified by its appearance, colour, odour, melting point a which is shown in

Table 1: Organoleptic Characteristics, Melting Point

Characteristics	Observation
Appearance	Crystalline powder or slightly hygroscopic
Colour	White
Odour	Odorless
pH	4.9
Melting Point (°C)	232

6.1.2 Solubility

Voriconazole dissolve in various solvent and solubility mentioned in table no. 2

Table 2: Solubility of Drug

Solvent	Observed Solubility
acetone	freely soluble
methylene chloride	freely soluble
ethanol	soluble
methanol	soluble
DMSO	soluble
water	insoluble

6.1.3 IR Spectra of Drugs

Infrared spectroscopy was performed for identification of compound. The IR spectrum is shown in Figure No.1 and interpretation of IR spectra is shown in Table no.3

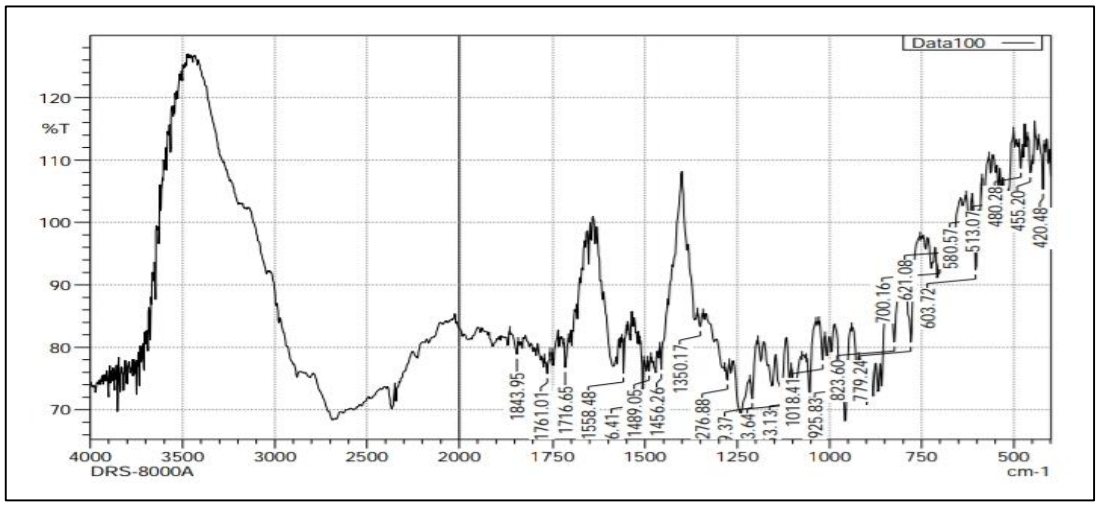


Figure 1: IR Spectra of Drugs

Table 3: Interpretation of IR Spectra of Voriconazole

Wave number (cm-1)	Observed group
3203	OH Stretching
2969	C–H alkyl Stretching
1716	C=O Stretching
1558	C–C arom Stretching
1456	N═N and C–N triazole Stretching
1276	C–H arom bending
1209	C–O–C asymmetric Stretching
823	C–H benzene and triazole bending
580	C-F Stretching

From the obtained data IR data, it is observed that the voriconazole drug obtained from the company as gift sample shows characteristic peak which is same as that of pure voriconazole drug.

6.1.4 Calibration Curve of Voriconazole

Calibration curve of Voriconazole was put by plotting absorbance V/s concentration. The λmax Voriconazole was found to be 254nm.The absorbance values are given in table 8.5 Standard calibration curve of Voriconazole followed Beer Lambert law range between 2-10 µg/ml as shown in fig 2,3. The equation of line was found to be y = 0.0723x + 0.0013 with correlation coefficient R^2 = 0.9895 for Calibration plot of Voriconazole in Methanol also y = 0.0757x + 0.0028 with correlation coefficient R^2 = 0.9965 for Calibration plot of Voriconazole in phosphate buffer 7.4.

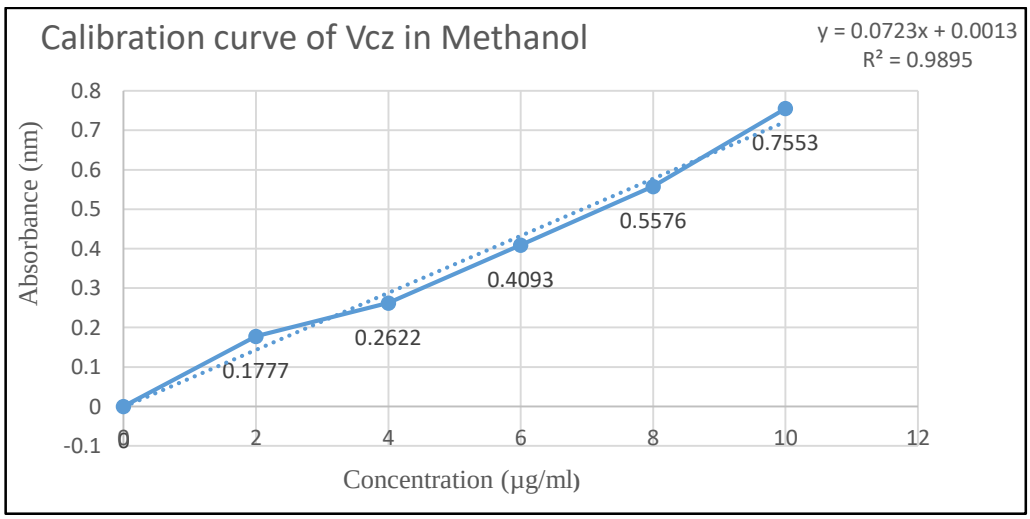


Figure 2: UV Calibration Plot of Voriconazole in Methanol at 254 nm

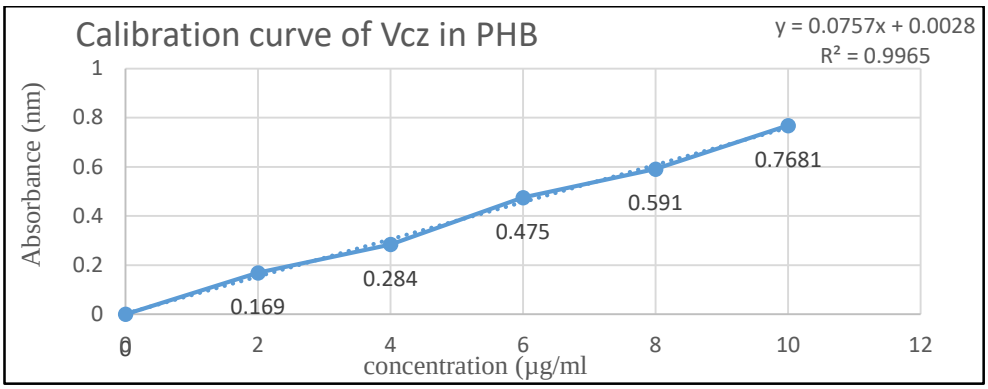


Figure 3: UV Calibration Plot of Voriconazole in Phosphate Buffer 7.4 At 254 nm

6.1.5 Drug Polymer Compatibility Study

The below table show the Drug and polymer are compatible to each other no changes can show it in table no. 4

Table 4: Drug Polymer Compatibility Data

Ingredient	Ratio	Tem. condition	Parameter	Initial	1 Month	2 Month	3Month
API	1	25°C±2°C/75%±5%	Appearance	W.P.	W.P.	W.P.	W.P.
			Color change	No	No	No	No
		40°C±2°C/75%±5%	Appearance	W.P.	W.P.	W.P.	W.P.
			Color change	No	No	No	No
API+ Carbopol 934	1:1	25°C±2°C/75%±5%	Appearance	W.P.	W.P.	W.P.	W.P.
			Color change	No	No	No	No
		40°C±2°C/75%±5%	Appearance	W.P.	W.P.	W.P.	W.P.
			Color change	No	No	No	No

Tem.: Temperature
W.P.: White Powder

FT-IR spectroscopy was used in the compatibility between drug and polymer show it in table no. 5 and fig 4

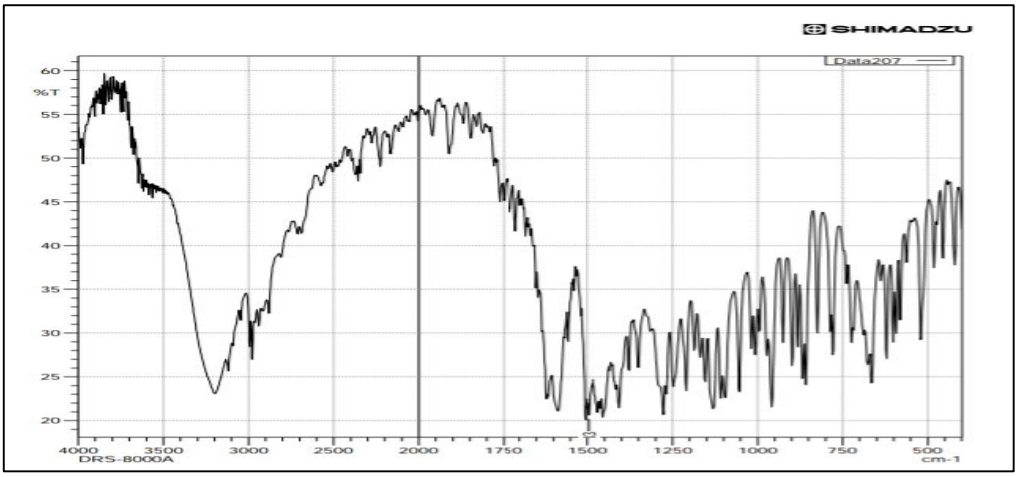


Figure 4: IR Spectra of Voriconazole +Carbopol 934

Table 5: Interpretation of IR Spectra of Voriconazole + Carbopol 934

Wave number (cm-1)	Observed group
3600	OH Stretching
3208	N-H bend Stretching
3102	C–H Stretching
1723	C═O Stretching
1598	C= C Stretching
1207	C–O–C Stretching (acrylate)
1142	C–O–C Stretching (ethereal)
957	=C-H Stretching
822	C-H Stretching
523	C- F Stretching

From above IR spectra of voriconazole + Carbopol 934 show the characteristics peak. In the graph of combination of voriconazole and Carbopol 934 exhibited all functional group of voriconazole. This suggested that voriconazole compatible with Carbopol 934.

6.1.6 Screening of Excipients

Voriconazole dissolve in various oil surfactant co-surfactant and check the solubility mentioned in table no. 6

Table 6: Solubility Study of Voriconazole in Various Oils, Surfactant and Co- Surfactant

Name of Excipient	Solubility (mg/ml)
OIL	
Isopropyl Myristate	4.02
Coconut oil	2.75
Almond oil	1.30
Olive oil	5.64
Castor oil	3.28
SURFACTANT	
Span 20	3.69
Span 60	2.30
Tween 20	2.88
Tween 60	1.41
Tween 80	4.27
Co- SURFACTANT	
Transcutol	2.85
Polyethylene Glycol 400 (PEG 400)	4.73
Polyethylene Glycol 600 (PEG 600)	3.34

6.1.7 Differential Scanning Calorimetry (DSC)

DSC was used for Thermal Analysis of drug. Sample was weighed directly in the pierced DSC aluminum pan and its scanned at temperature range 30- 350°C under atmosphere of dry nitrogen. Heating rate of 10°C/min was used and obtained thermos-grams were observed for any interaction. Sample upto 2mg used for analysis. Showed in fig 5

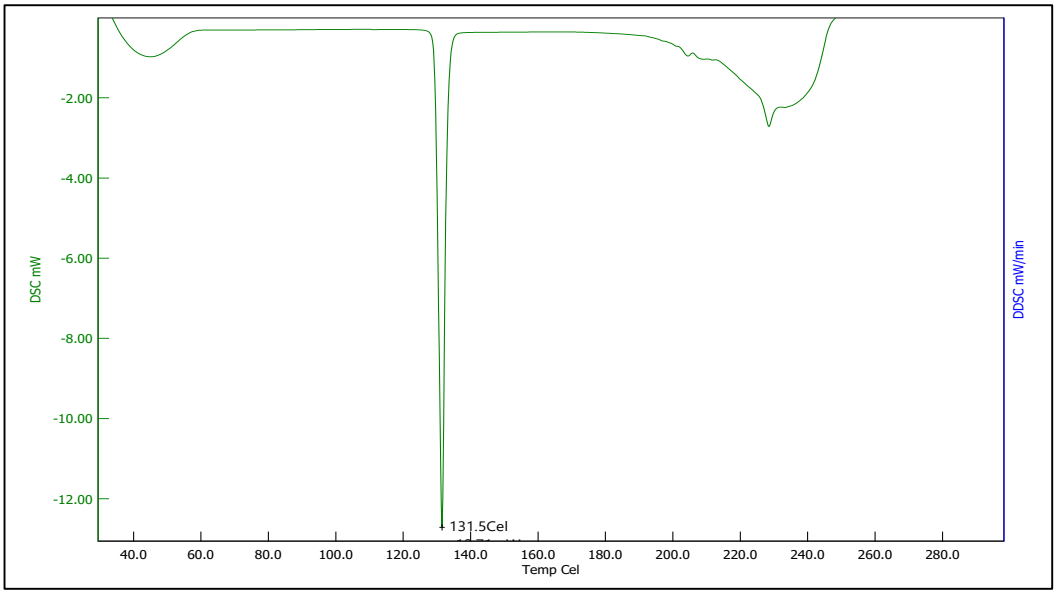


Figure 5: DSC Thermogram of Voriconazole

6.2 Evaluation of Nanoemulsion

6.2.1 The central composite design (CCD) is applied for determining the number of trials that must be evaluated for variable and response optimization. Central composite design was used to optimize Vesicle size, % Drug Contain and help in selection of optimized batch. Mentioned in table 7 and fig 6.

Table 7: Batches of Nanoemulsion in DOE Software

Batch	colour	Homogeneity	Phase separations
F1	Pale yellow	Excellent	No separation
F2	Transparent	Excellent	No separation
F3	Pale yellow	Excellent	No separation
F4	Transparent	Excellent	No separation
F5	White	Excellent	No separation
F6	Transparent	Excellent	No separation
F7	Transparent	Excellent	No separation
F8	Pale yellow	Excellent	No separation
F9	Pale yellow	Excellent	No separation
F10	Pale yellow	Excellent	No separation

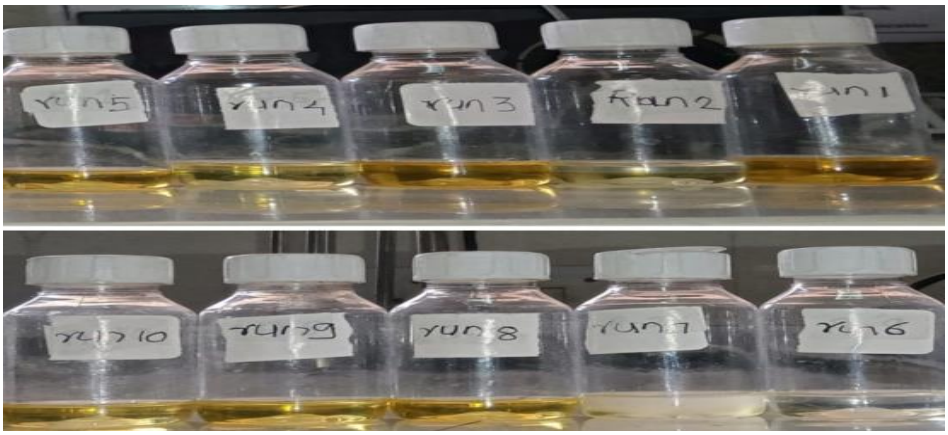


Figure 6: Batches of Emulsion

F6 selected as an optimized batch.

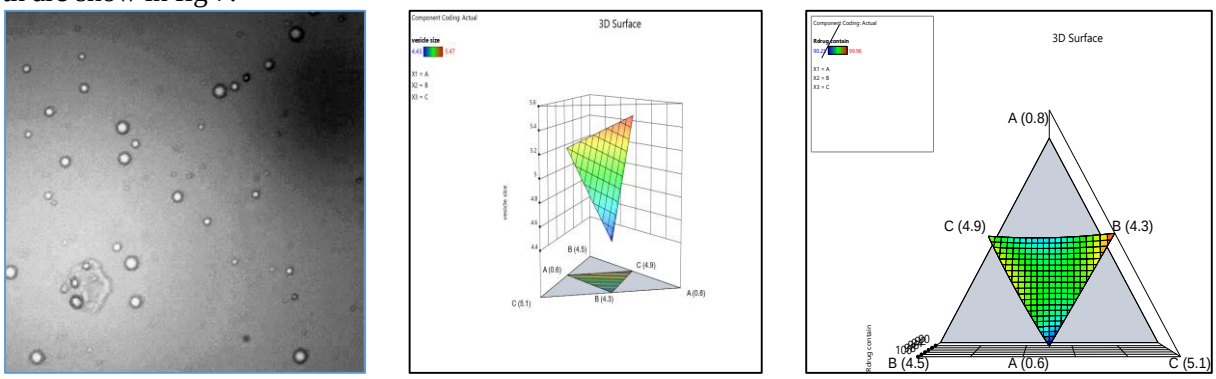
a) Vesicle size

According to Design of Expert software different formulation can prepared. All this formulation determined the vesicle size with help of Pixel Pro Software. Take the average of 50 sample.

b) % Drug Contain

An voriconazole 100 mg were added to 2ml of the vesicle size contain preparation

Both are show in fig 7.



(A) **(B)** **(C)**

Figure 7: (A) Vesicle Size Under Pixel Pro Software (B) 3d Surface Graph Of Vesicle Size (C) 3d Surface Graph Of Drug Contain

Evaluation of Optimized Nanoemulsion

6.1.2 Clarity, pH

The clarity of formulation was determined by under visual inspection. The optimized preparation was found to be clear. pH of the formulation was found to be 5.2

6.1.3 Stress Stability Study

Sample was put in different temperature condition and passed all condition doesn't show any physical instability.

6.1.4 Percentage Transmittance

On 100- times dilution, the percentage transmittance of Voriconazole loaded nanoemulsion was found to be 98%. This approved the good transparent nature of Voriconazole loaded nanoemulsion.

6.1.5 Droplet Size and Poly Dispersity, Zeta Potential Determination

The droplet size of an F6 formulation batch was measured by using the light scattering technique i.e. Zetasizer. The particle size has been found to be 187.6 nm, which falls in the nanometer range. The PDI value of 0.390, that's less than 1, shows that the nanoemulsion is a more stable one. The zeta potential describes what kind of charge present on the surface of the nanoemulsion. This assures that the resulting formulation is stable. The optimized nanoemulsions zeta potential was found to be -17.8mv. A high negative zeta potential values suggested that the nanoemulsion was electro kinetically stable.

6.1.6 Stability Studies of Nanoemulsion

The goal of stability research was to predict a product's shelf life under optimal temperature and relative humidity conditions. Results are shown in the following Table 8.

Table 8: Stability Studies of Nanoemulsion

Storage Condition	No. Of Days	Drug Content (%)	Phase separation	pH
25 ± 2°C/60 ± 5% RH	0	90	No Separation	5.2
	15	90	No Separation	5.2
	30	89.45	No Separation	5.2
	45	89	No Separation	5.2
	60	88.72	No Separation	5.2
40 ± 2°C/65 ± 5% RH	0	92.20	No Separation	5.2
	15	92	No Separation	5.2
	30	92	No Separation	5.2
	45	92.84	No Separation	5.2
	60	91	No Separation	5.2
60 ± 2°C/75 ± 5% RH	0	91.00	No Separation	5.2
	15	90.64	No Separation	5.2
	30	90	No Separation	5.2
	45	89.62	No Separation	5.2
	60	89	No Separation	5.2

6.3. Evaluation of Nanoemulgel

6.3.1 Appearance of Nanoemulgel

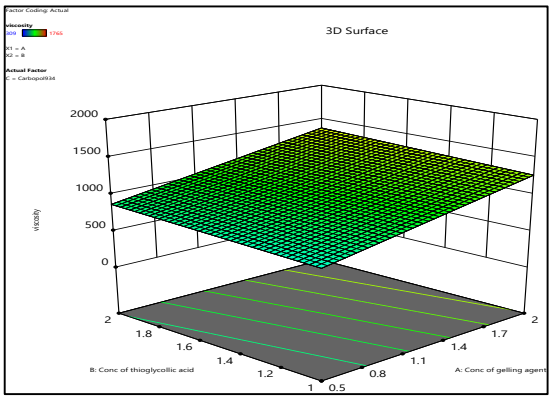
No formulation shows phase separation, and all exhibit great homogeneity and outstanding consistency, which points to strong and stable formulations

6.3.2 pH, Viscosity, Spreadability

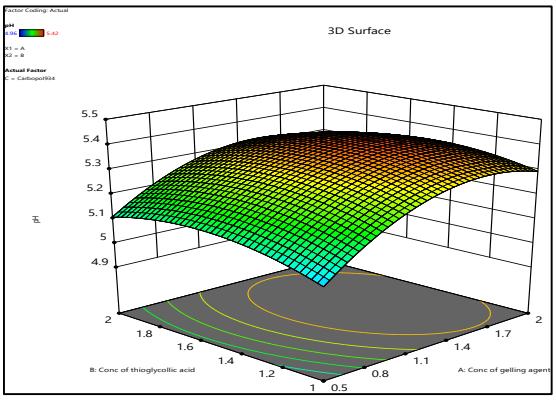
pH, Viscosity, Spreadability, of nanoemulgel was done as per standard procedure.

6.3.3 In-Vitro Drug Release Study of Nanoemulgel, Drug content

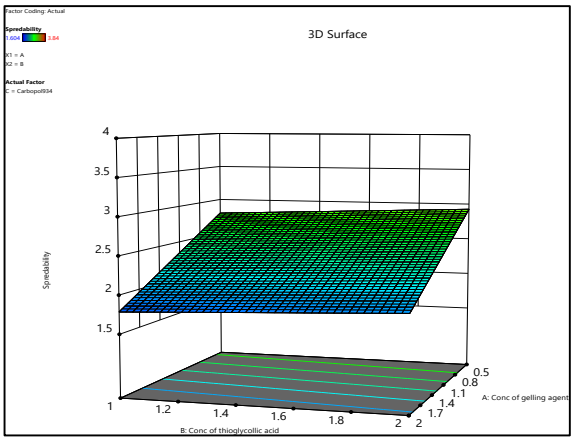
The dissolving profile of Voriconazole nanoemulgel demonstrates entire drug release within 8 hours, with formulation F14 having a much higher drug release profile than other formulations. The kind and concentration of the gel-forming agent, polymers, oil, and surfactant employed in nanoemulgel define its medicine release profile. Shoe in fig 9. The drug content of the nanoemulgel was calculated according to protocol. Above all parameter show in fig 8



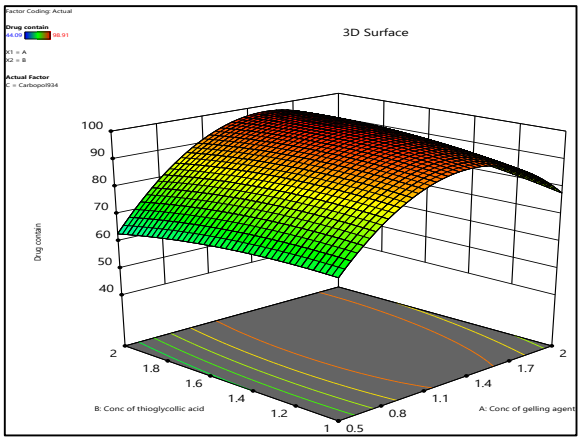
(A)



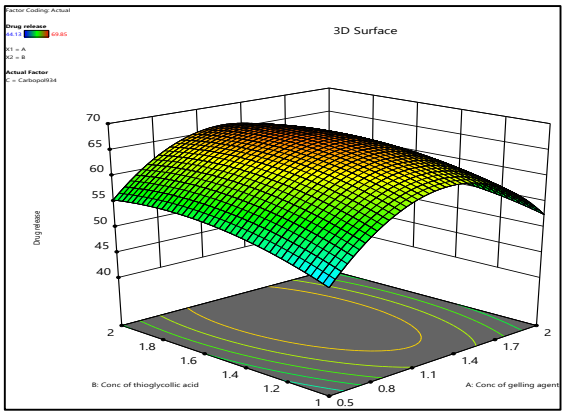
(B)



(C)



(D)



(E)

Figure 8: (A) 3d Surface Graph of Viscosity (B) 3d Surface Graph of pH (C) 3d Surface Graph of Spreadability (D) 3d Surface Graph of Drug Content 3d (E) Surface Graph of In-Vitro Study

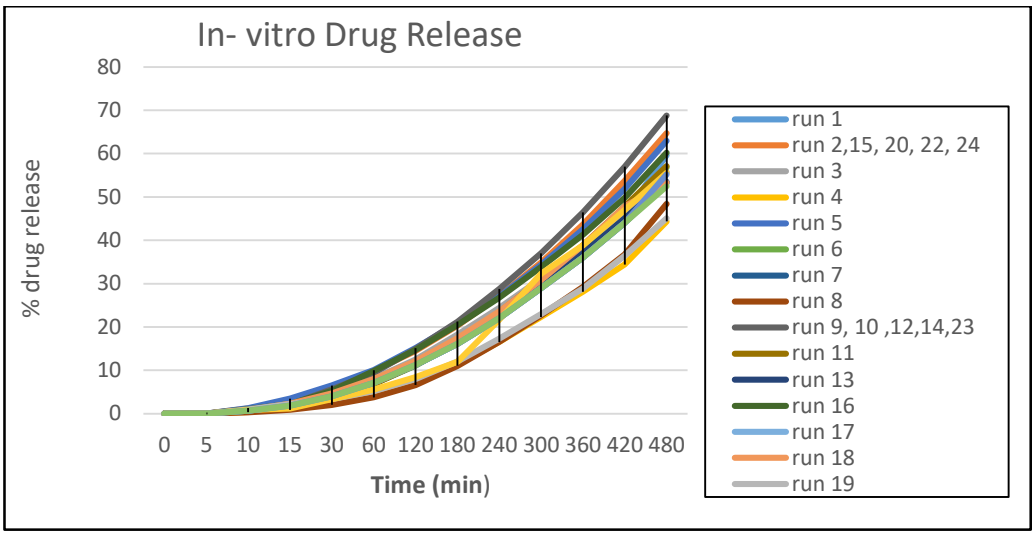


Figure 9: In- Vitro Drug Release

Evaluation of Optimized Batch

6.3.4 Transungual Permeation Study

The ex vivo diffusion tests were carried out on the goat's hoof membrane (it's a part of goat foot). Permeation of drug from optimized batched was found to be 68.1200, in 8 h. Showed in fig 10.

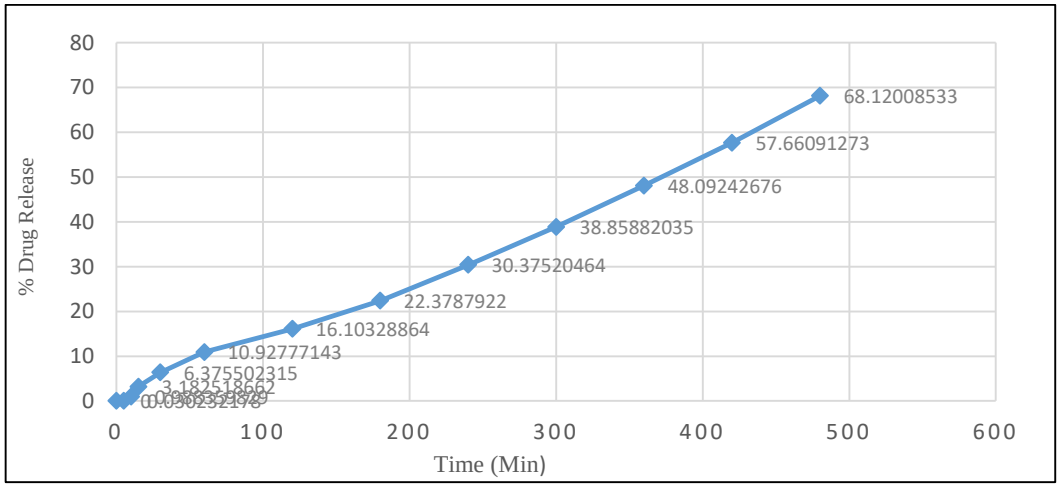


Figure 10: Transungual Permeation Study

6.3.5 Antifungal Activity

Voriconazole in DMSO and nanoemulgel in water were used for testing antifungal activity on Candida albicans after a period of 48 hours of incubation. To compare the real antifungal activity of voriconazole and its formulation, the zone of inhibition of DMSO (antifungal in nature) was also evaluated as standard. However, area of zone of inhibition explained in table 9 and fig. 11

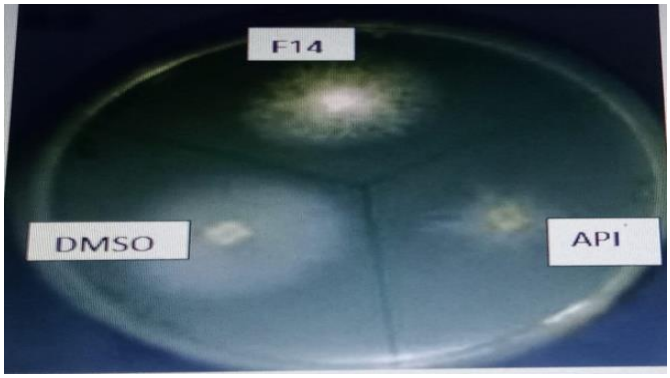


Figure 11: Antifungal Study of Nanoemulgel

Table 9: Antifungal Study Parameter

Parameters	Result		
Name of method	Disk diffusion method		
Stain	Candida albicans		
Agar Media	Sabouraud dextrose agar		
Zone of inhibition (after 48 hours)	DMSO	Drug	Nanoemulgel
	7.4 mm	10mm	9.8 mm

7. Conclusion

Voriconazole-loaded nanoemulsion was created in the current study by utilizing olive oil, Tween 80, and PEG 400. The optimized batch of nanoemulgel (F16) was discovered using viscosity, Spreadability, pH, drug content, and drug release after the optimized nanoemulsion (F6) was mixed into the nanoemulgel. The optimized formulation shows the particle size in nanometer range. The F16's in vitro drug release and transungual permeation investigation produced positive results. As a result of the drug's antifungal effectiveness against C. albicans, the F16 may be able to alleviate onychomycosis symptoms more quickly than with traditional treatment. Thus, nanoemulgel is a formulation for the topical treatment of onychomycosis that is safe and effective.

List of Abbreviations

API= Active Pharmaceutical ingredient
Tem. = Temperature
W.P.= White Powder
C. albicans = Candida albicans

Declarations

Competing Interests

The authors declare that they have no competing interests

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Not provided.

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Ethics approval and consent to participate

Not applicable.

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