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Microemulgel of Voriconazole: an Unfathomable Protection to Counter Fungal Contagiousness

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Background: Fluconazole and ketoconazole both have poor minimum inhibitory concentration than voriconazole. Voriconazole had serious side effects in oral and intravenous doses. It has poor water solubility. The objective of the study was to prepare and optimize microemulgel of voriconazole for topical delivery.

Aim: Formulation, development, and evaluation of voriconazole microemulgel for topical delivery.

Methods: Oil and emulsifiers selected were on the basis of equilibrium solubility study and emulsification property respectively. The pseudo-ternary plot and constrained simplex lattice design were applied for preparation of microemulsions. Microemulsions were subjected to micelle size, zeta potential, polydispersity index, and *in vitro* study. They were optimized by Design–Expert® 9.0.3.1 software. Formulation, development, evaluation and optimization of microemulgel were carried out. Microbial assay of an optimized batch of microemulgel was performed.

Results: Solubility of voriconazole in Parker Neem® oil was 7.51±0.14 mg/g. Acrysol™K-150: PEG-400 in 4:1 ratio had the highest area for microemulsion. 59.2% Acrysol™K–150, 14.8% PEG-400, 11% Parker Neem® oil, 15% rose water, and 1% voriconazole as an optimized batch of microemulsion was selected for preparation of microemulgel. Carbomer 934P found a good gelling agent in 0–2% w/w concentration. An optimized batch of microemulgel had 0.974 desirability value. An optimized batch of microemulgel and Nizral® cream had 37.32±0.63% and 26.45±0.63% zones of inhibition.

Conclusion: Topical antifungal treatment was successfully achieved with voriconazole microemulgel.

INTRODUCTION

Fungal skin infection could be due to yeast fungus that causes candidiasis, ringworm, tinea, etc. that live on the skin, hair or nails. The microemulsion is an isotropic mixture of oil, aqueous phase, and emulsifier sometimes in combination with co-emulsifier.[1] Microemulsion integrates drug in solubilized form and small sized micelles provide a large interfacial area for drug penetration. Moreover, oil portion helps to improve permeability.[2] Emulgel is a combination of both emulsion and gel.[3] Microemulgel is a combination of both gel and microemulsion. Hydrophilic and hydrophobic both types of drugs are incorporated into microemulgel and have privileged of large surface area for drug absorption. The oil portion acts as penetration enhancer.[4] Topically used microemulgel has a wide range of advantages and stability of microemulsion is increased when it is incorporated in gel.[5-7] Voriconazole is BCS (biological classification system) class II drug. It has log p (lipophilicity) 1.65, a molecular weight of 349.310 g/mol. It has better MIC (minimum inhibitory concentration) range (0.078–0.5 μG/mL) than fluconazole (1–64 μG/mL) and ketoconazole (0.002–16 μG/mL) for *candida albicans*. It has MIC_{50} of 0.0313 μG/mL, MIC_{90} of 0.0625 μG/mL, and MIC_{99} is less than 1 μG/mL for *Candida albicans*. MIC value is 8 μG/ mL for the fluconazole-resistant strain of *candida*. However, voriconazole had various serious side effects in oral and intravenous doses.

AIM

Formulation, development, and evaluation of voriconazole microemulgel for topical delivery.

MATERIALS AND METHODS

Voriconazole was gift sample received from Astron lab. Ahmadabad, India. Neem oil was purchased from Parker Biotech Pvt. Ltd., Chennai, India. Acrysol™ K-150 (PG-Polyoxyl 40-hydrogenated castor oil) was gift sample from Corel Pharma, Ahmadabad, India. PEG-400 was purchased from Finar Chemicals Ltd, Ahmadabad, India. Carbomer-934P was purchased from SD Fine Chem. (I) Ltd, India. Propylene glycol (PG) was purchased from Molychem, Mumbai, India. Triethanolamine (TEA) was purchased from Merk Specialties Pvt. Ltd., Mumbai, India. Rose water was purchased from Dabur (I) Ltd. Mumbai, India. Nizral® Cream (the brand of 2% w/w ketoconazole) was purchased from Johnson and Johnson (I) Pvt. Ltd. Blossom™ Active instant Yeast was purchased from Raunak-Eden Enterprises, Thane. Voveran® Emulgel purchased from Novartis (I) Pvt. Ltd. SHY-NM™Gel (Carbomer gel) purchased from Group Pharmaceuticals Ltd, Malur, India.

Preliminary study

Voriconazole was subjected to scan under UV spectrophotometer (UV-1800/1601, Shimadzu Corp. Japan). The standard curve of it was also prepared in methanol. Antifungal oils were subjected to scan under UV spectrophotometer in methanol. Oils that have no absorbance in between 200–400 nm were selected for equilibrium solubility study. The equilibrium solubility of voriconazole was determined by adding it in excess amount in 1 mL of oil and then the mixture was stirred continuously for 1 h at 50 rpm (revolution per minute) and at 25°C in a shaker bath (Innova 4230, Brunswick Scientific, Edison NJ, USA), following attainment of equilibrium. The obtained supernatant was filtered through filter paper (pore size 11 µ). Spectrophotometric measurement of the filtrate was carried out at λ_{max} (wavelength maximum of voriconazole). Solubility study data was used for selection of oil.[8]

Pre-formulation Study

The 300 mg of oil was vortexes (Vortex Mixture, Micro Scientific works, Delhi, India) with 300 mg of each emulsifier and subsequently heated at 40°C to form a homogenous mixture. Microemulsification was checked visually by adding 500 mg of the mixture in 50 mL of distilled water. The selected oil-emulsifier pair from above two steps emulsified with screened co-emulsifier which was selected on the basis of emulsification property.[9] Pseudo-ternary phase diagram was prepared by Statistica® 8 using emulsifier: co-emulsifier ratio and oil at a different range of the ratio of 9:1, 8:2, 7:3, 6:4, 5:5, 4:6, 3:7, 2:8, and 1:9 against the aqueous phase by titration method and formation of spontaneous clear microemulsion was observed visually.

Formulation and development of Voriconazole microemulsion

Seven batches of voriconazole loaded microemulsion in constrained simplex lattice design were prepared. Emulsifier: co-emulsifier (X_1), oil (X_2), and aqueous phase (X_3) were considered as independent factors. Micelle Size (MS), polydispersity index (PDI), the time required for more than 90% voriconazole diffused (t_{90}) were taken as dependent variables. The responses for these micro emulsions were subjected to fit equations of polynomial for constrained simplex lattice design which was predicted results of all possible micro emulsions (Eq. 1).[10]

$$Y=B_1X_1 + B_2X_2 + B_3X_3 + B_{12}X_{12} + B_{13}X_{13} + B_{23}X_{23} + B_{123}X_1X_2X_3 + \varepsilon \quad (1)$$

Where, ε = practical error, Y was the dependent variable B_1,B_2,B_3,B_{12},B_{13},B_{23}, and B_{123} were regression coefficients.

Evaluation of Voriconazole microemulsion

MS, MSD (Micelle Size distribution), ζ (Zeta potential) and PDI

The ζ and PDI were measured to check the stability of microemulsions. Microemulsion was diluted to 50 times and MS as well as MSD and ζ of microemulsion was determined using *Zetatrac* particle size Analyzer, Microtrac Inc., USA

In vitro diffusion of microemulsion

Franz diffusion cell (Durasil®, an effective diffusion area of 3.14 cm^2 and 25 ml cell volume capacity) was used. 1 mL microemulsion was added into donor compartment. Semi-permeable gelatin membrane (impermeable gelatin membrane was heated in 0.1N NaOH for 1 h) was kept in between donor and receptor compartments. Freshly prepared methanol: phosphate buffer (pH 6.8) solution (10%: 90% v/v) was filled in the receptor compartment.[11] The receptor compartment was put on a magnetic stirrer with a hot plate (2 MLH, Remi equipment, India), stirred by magnetic bead at 100 rpm, 37°C,

and analyzed for percentage voriconazole content at suitable time interval by UV spectrophotometer.

Optimization of microemulsion had been carried out under criteria of MS, PDI, and t_{90} with help of Design–Expert® 9.0.3.1 software with the desirability of minimum, 0–1, and minimum respectively. The optimized batches of microemulsion were prepared and all the same parameters of microemulsion were evaluated.

Screening of gelling agent, penetration enhancer, and their concentrations

Different gels were prepared with help of HPMC, Na CMC, sodium alginate, and Carbomer 934P with different concentrations by continuous stirring for a period of 2h.[12] Amongst these gelling agent and its concentration was screened out on basis that gel formed in the least concentration with desired viscosity, which was used for the prospective study. Viscosities were measured using viscometer of Brookfield engineering labs. Inc. at 37±1°C, spindle 6, 50 rpm rotation speed for 30 sec of the time interval. Among the chemical penetration enhancer clove oil, olive oil, PG, isopropyl myristate, eucalyptus oil, laurocapram, urea, dimethyl sulfoxide, citric acid, ascorbic acid, acetone, tween 20, oleic acid, Transcutol P® options were available for use.[13]

Voriconazole-Excipients Compatibility Study

A physical mixture of voriconazole and excipients of microemulgel was prepared geometrically. Several drops of this mixture were dropped onto the liquid cell of FTIR-8400 spectrophotometer (Fourier Transform Infra-Red, Shimadzu Corp. Japan), such that no bubbles were trapped and scanned in the range of 400–4000 cm^{-1} with resolution of 1 cm^{-1}.[14]

Formulation and Development of Microemulgel

Carbomer 934P was left overnight for gelling; finally, TEA was added to adjust pH 7. The optimized microemulsion was added in carbomer 934P gel phase. Six different batches of microemulgel were prepared on the basis of trial and error.

Evaluation of Microemulgel

Physical Appearance

Prepared microemulgels were observed for color, homogeneity, consistency, texture, and pH.

Spreadability Measurement

To determine spreadability of microemulgel, 0.5 g of microemulgel was placed within the circle of 1 cm diameter premarked on a glass plate over which the second plate was placed and 5 g weight was allowed to rest on an upper glass plate for 5 min. Increase in diameter due to weight was spreadability.[15]

Syneresis measurement test

Upon standing sometimes microemulgel shrinks a bit and little liquid would leak out. It is called syneresis. In this test, microemulgel was kept in a cylindrical plastic tube with a perforated bottom which was covered with filter paper (Whatman No. 41). These tubes were then placed in a clinical centrifuge (Remi equipment, Mumbai, India) and centrifuged for 15 min at 1000 rpm. Cylindrical plastic tube and liquid which were separated from microemulgel were weighed. Percentage of syneresis was calculated as per Eq. 2 [16]

$$\% \text{ of Syneresis} = \frac{\text{Weight of liquid separated from microemulgel}}{\text{Total weight of microemulgel before centrifugation}} \times 100 \quad (2)$$

Viscosity

It was determined at 37±1°C by means of Brookfield viscometer. Spindle 6 was taken for measurement; rotation speed was 50 rpm for 30 sec of the time interval.

Extrudability Test

The force required for extruding microemulgel from the tube is called extrudability. Extrudability was measured from the aluminum collapsible tube on the application of weight in g required to extrude at least 0.5 cm ribbon of microemulgel in 10 sec. When more quantity is extruded, extrudability is considered to be better.[17]

In vitro diffusion

In vitro diffusion was carried out in the same manner as that of the microemulsion. However, 500 mg sample was taken into donor compartment. Time at which voriconazole diffusion was started from microemulgel i.e. lag time (t_{leg}) was determined by extrapolating linear portion of the cumulative amount penetrate versus time curve to the x-axis.[18] Since there was a possibility of unpredictable alteration in diffusion studies due to penetration property of Parker Neem® oil, PG and PEG-400 therefore, in order for better approximation of diffusivity of voriconazole diffusion parameter (D/h^2) was derived from lag time by $D/h^2 = 1/(6 \times t_{leg})$ equation.[19] Voriconazole steady state flux permeation rate (J_{ss}) was calculated by dividing slope of graph linear portion with diffusion cell area in $mg/cm^2 min$, a permeability coefficient

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(K_p) was calculated by dividing J_{ss} by the initial concentration of voriconazole in donor chamber in cm^2 min^{-1}. Enhancement ratio (E_r) was calculated as the ratio of J_{ss} to that of Nizral® cream. The t_{mic} i.e. time required to achieve MIC value was also calculated from an *in vitro* study.

Voriconazole release kinetics study

In vitro release results profile obtained for all batches of microemulgel were plotted in models of data treatment as zero-order kinetic model, first-order kinetic model, Higuchi's model, Korsmeyer/ Peppa's model, Hixon-Crowell model.[20]

Optimization of Microemulgel

The desirability of each batch of microemulgel was found individually by way of the manual as Eqs. 3, 4 and 5:

$$d = \frac{Y_i - Y_{min}}{Y_{target} - Y_{min}} \; ; \; Y_i < Y_{target} \qquad (3)$$

$$d = \frac{Y_{max} - Y_i}{Y_{max} - Y_{target}} \; ; \; Y_i > Y_{target} \qquad (4)$$

overall desirability would be:

$$D = \sqrt[n]{d_1} \times d_2 \times .....\times d_n \qquad (5)$$

The desirability of voriconazole microemulgel, colors, physical appearance, homogeneity, syneresis, voriconazole release kinetic, spreadability, extrudability, and texture parameters was in desired range, so its desirability was considered as 1. The desirability of voriconazole content, diffusion parameter, pH, viscosity, Q_{30}, t_{90}, t_{mic}, and t_{leg} were derived. Finally, the batch that had the highest desirability was considered as optimized batch.[21]

Microbial assay of optimized batch of microemulgel

Ditch plate technique was used for evaluation of fungistatic activity of a microemulgel.[22] Previously prepared Sabouraud's agar dried plates were used. One g of the microemulgel was placed in a ditch cut in the plate. Freshly prepared culture loops of *Saccharomyces cerevisiae* (ATCC No.9763) were streaked across agar at a right angle from the ditch to the edge of the plate. Nizral® cream was used for comparison. Negative Control of microemulgel (without voriconazole) was also prepared. After incubation for 72 h at 25°C, fungal growth was observed and % inhibition was measured as per Eq. 6:

$$\% \text{ inhibition} = \frac{L_2}{L_1} \times 100 \qquad (6)$$

where L_1 = total length of the streaked culture, and L_2 = length of inhibition.

Accelerated Stability study of optimized batch of microemulgel

Microemulgel was sealed in the ampoule and then placed in a glass bowl containing desiccators prepared from magnesium hydroxide and calcium chloride then a whole closed bowl of glass was placed in an oven at 30±2°C temperature and 70±5% RH (relative humidity) conditions for 1 month. A duplicate sample was withdrawn at 1 month to evaluate their physicochemical parameters. Physical and chemical stability were evaluated.[23,24] To check the similarity between two *in vitro* diffusion profile similarity factor (f_2) and dissimilarity factor (f_1) were applied. The equation for them are as below Eqs. 7 and 8:

$$f_1 = \frac{\sum_1^n |R_t - T_t|}{\sum_1^n R_t} \times 100 \qquad (7)$$

$$f_2 = 50 \log\left\{\left[1 + \frac{1}{n}\sum\sqrt{(R_t - T_t)}\right]^{-0.5} \times 100\right\} \qquad (8)$$

Where,
R_t = amount of voriconazole released before stability study, W_t = optional weight factor,
T_t = amount of voriconazole released after stability study, n = No. of experimental data.

Statistical Analysis

The study was presented as the mean of three independent variables ± standard deviation (SD) of them for results. Regression analysis, Microsoft excel (Microsoft Excel Worksheet®, version Professional Plus 2010, Microsoft Corporation, USA) was employed to determine control factors that significantly affect responses for microemulsions. Unpaired Student's t-test with equal variance was performed for the solubility of voriconazole between an optimized batch of microemulsion and water and

also for the *in vitro* diffusion profile between an optimized batch of microemulgel and Nizral®cream. The one-way ANOVA was carried out between zones of inhibition of an optimized batch of microemulgel and Nizral®cream. The differences between data were considered statistically significant at 95% of confidence level.

RESULTS

Scanning of voriconazole in methanol and methanolate phosphate buffer at pH 6.8, in the range of 200–400 nm and λ_{max} was found to be 256 nm. Among available antifungal oils, neem oil, black till oil, karanja oil, coconut oil and Parker Neem® oil were found that had no absorbance in between 200–400 nm. Parker Neem® oil and rose water had the solubility of voriconazole 7.51±0.14 mg/g and 0.33±0.05 mg/g. They were selected as oil and aqueous phase for the preparation of micro emulsion.

Acrysol™ K-150: PEG-400 in the ratio of 4:1 ratio was selected for pseudo-ternary phase diagram. The concentration range of Acrysol™ K–150/PEG–400 mixture: 65–80%; Parker Neem® Oil: 5–20% and rose water 15–30% were selected for constrained simplex lattice design for microemulsion preparation (**Table 1**).

MS was found to be in the range of 10–305 nm with PDI value of 0.49–17.21, and ζ of –3.02–5.03. The t_{90} of microemulsion were reported between 5–105 min. The regression analysis lead Eq. 9, 10, and 11:

$$MS=10.22X_1(p=0.000044)+ 118.1X_2(p = 0.000004)+12.37X_3(p=0.00003)+60.8.96X_1X_2 (p=0.000004)+10.02X_1X_3(p=0.00022)+959.06X_2X_3(p=0.000002)-5086.03X_1X_2X_3(p=0.000003)+ \varepsilon(R^2=0.99) \quad (9)$$

The extra design check-point had a response of 31.70 nm; predicted value based on equation was 29.45 nm.

$$t_{90}=10X_1(p=0.009)+30X_2(p=0.003)+60X_3(p=0.001)+220X_1X_2(p=0.002)-120X_1X_3(p=0.003)+ 240X_2X_3(p=0.001)-1110X_1X_2X_3(p = 0.002)+ \varepsilon(R^2=0.999) \quad (10)$$

The extra design check-point had a response of 20 min. The predicted value based on equation was 20 min 20 sec.

$$\zeta=4.5X_2(p=0.002)+6.64X_1X_2(p=0.04)+82.92X_1X_3(p=0.00002)+11.12X_2X_3(p=0.01)-428.11X_1X_2X_3 (p=0.00006) + \varepsilon(R^2=0.998) \quad (11)$$

The extra design check-point had a response of -2.86 mv. The predicted value based on equation was -2.44 mv.

Among all optimized batches of microemulsion (**Table 2**), E12 had the least % bias. The solubility of voriconazole in E12 was 6.96 ± 0.08 mg/g. The micelle size of the E12 batch was 59.8 nm (**Fig. 1**). Carbomer 934P was a good gelling agent as compared to the other gelling agents for preparations of microemulgel formulations even in the low concentration range of 0.5–2% w/w.

Voriconazole-excipients physical mixture exhibit main FTIR peaks at 1616.40 cm^{-1}, 1006.88 cm^{-1}, and 2903.57 cm^{-1}. These were characteristic absorption peaks of voriconazole for C–N stretch of triazole (1616.40 cm^{-1}), C–F Stretching Bond (1006.88 cm^{-1}), and Triazole ring (2914.54 cm^{-1}) respectively (**Fig. 2**).

The difference between batches of microemulgel was the only change in concentration of PG (**Table 3**). The prepared voriconazole microemulgel formulations were yellow, viscous creamy preparation with a smooth and homogeneous appearance. Percentage synergies were zero for all formulations. Viscosities of batches were found in descending order as M3 > M2 > M1. All gel follow pseudoplastic or shear thinning flow behavior. *In vitro* diffusion release of voriconazole from all microemulgel formulation was ranked in descending order as M3 > M2 > M1. The t_{90} of M3, M2, and M1 were 120 min, 210 min, and 300 min respectively. The pH of all formulations was near to 7. The t_{leg} for all formulations were less than 30 sec. M3 batch had overall desirability of 0.974. The other parameters of microemulgels and some marketed brand were reported in **Table 4**.

In the microbial assay, percentage zone of inhibition of M3, voriconazole in DMSO (Dimethyl sulfoxide); M3 without voriconazole, DMSO, Nizral® cream were found to be 37.32±0.63, 27.17±1.09, 10.14±0.63, 0, 26.45±0.63, respectively (**Fig. 3**).

After one-month stability study M3 remained as yellow in color, transparent homogenous, no syneresis developed, texture remained smooth, with slightly acidic. For the *in vitro*, voriconazole release profile, f_2, and f_1 were found to be 99.46 and 0.37. J_{ss}, K_p, E_r, extrudability, spreadability all were slightly decreased but almost identical. Viscosity was improved followed the pseudoplastic flow.

Table 1. Formulation of microemulsion

Batch Code	Code Value			Decoded Value		
	Acrysol™ K–150: PEG–400 4:1 Ratio	Parker Neem® Oil	Rose Water	Acrysol™ K–150: PEG–400 4:1 Ratio	Parker Neem® Oil	Rose Water
E1	1	0	0	80%	5%	15%
E2	0	1	0	65%	20%	15%
E3	0	0	1	65%	5%	30%
E4	0.5	0.5	0	72.5%	12.5%	15%
E5	0.5	0	0.5	72.5%	5%	22.5%
E6	0	0.5	0.5	65%	12.5%	22.5%
E7	0.33	0.33	0.33	70%	10%	20%
E8*	0.5	0.25	0.25	72.5%	8.75%	18.75%

*Check Point batch

Table 2. Optimized batches of microemulsion

Batch Code	Code Value			Decoded Value			Desirability
	Acrysol™ K–150: PEG–400 4:1 Ratio	Parker Neem® Oil	Rose Water	Acrysol™ K–150: PEG–400 4:1 Ratio	Parker Neem® Oil	Rose Water	
E9	0.8	0.2	0	77%	8%	15%	0.775
E10	0.7349	0.2651	0	76%	9%	15%	0.7245
E11	0.7	0.3	0	75.5%	9.5%	15%	0.7009
E12	0.6	0.4	0	74%	11%	15%	0.6468

Desirability was reported from Design-Expert® 9.0.3.1.

Micelle size was 60 nm and desirability was 0.95.

DISCUSSION

Calibration curves for voriconazole showed linear relationships in between concentration range of 5–35 μG/mL in methanol and in methanolate phosphate buffer pH 6.8. pH values of microemulsions had stated that these formulations were acidic due to voriconazole and neem oil, which were not comfortable with skin, so need to formulate in one type of formulation system i.e. gel or emulgel which could be comfortable with skin. Centrifugation of microemulsions stated that these formulations were kinetically stable.[15] Dilution Test of All microemulsions stated that these formulations were thermodynamically stable.[16] Limpidity (percentage transmittance) was showed that these formulations were stable.[17,18] Small micelles size was anticipated to provide large surface area for enhanced *in vivo* penetration of voriconazole. It was found from the results that the MS increases significantly as Neem oil concentration increases. Generally, an increase of electrostatic repulsive forces between microemulsion micelles prevents the coalescence. On the contrary, a decrease of electrostatic repulsive forces would cause phase separation. A positive value of ζ could be attributed to high unsaturated or saturated fatty acid content of neem oil. Predicted values based on equations were very close to the observed value for MS, PDI, and ζ. The study was confirmation of adequacy of the equation as a predictor of MS, PDI, and ζ of the microemulsion.[13] E12 batch had more than 50% of Acrysol™ K–150, so it was incorporated into a gel in at least in 4:1 ratio of microemulsion:

Table 3. Formulation of voriconazole loaded microemulgel

Ingredients	% of Ingredients in different Batches					
	M1	M2	M3	M4	M5	M6
Voriconazole	1	1	1	1	1	1
Acrysol™ K–150	47.36	47.36	47.36	47.36	47.36	47.36
PEG–400	11.84	11.84	11.84	11.84	11.84	11.84
Parker Neem® Oil	8.8	8.8	8.8	8.8	8.8	8.8
PG	0	5	10	0	5	10
Carbomer 934P	1	1	1	1.5	1.5	1.5
Rose Water	30	25	20	29.5	24.5	19.5
Triethanolamine	q.s.*	q.s.*	q.s.*	q.s.*	q.s.*	q.s.*

q.s.*: Quite sufficient to obtain pH 6.8-7

gel for the preparation of microemulgel, because of Acrysol™ K–150 had sensitivity up to 50% range only, beyond this limit cause irritation to the skin.[25] The statistical analysis concluded that optimized batch of microemulsion increased the solubility of voriconazole as compared to water.

Carbomer 934P gel showed higher viscosity as compared to Na CMC, HPMC and sodium alginate gels and pseudo plastic flow, so carbomer 934P was selected as the gelling agent for a prospective study. For optimization of the concentration of carbomer 934P, different concentrations of carbomer 934P (0–2%) were tried for gel preparation and gave acceptable physical properties, from a study it was found that carbomer 934P was a good gelling agent as compared to the other gelling agents for preparations of microemulgel formulations even in the low concentration range. It was concluded that in carbomer 934P gel, with an increase in carbomer 934P concentration, the viscosity was increased. Minimum 20,000 cp viscosity was required for efficient gel preparation and as viscosity was inversely proportional to the release of drug from the gel. The lesser carbomer 934P content the higher would be the voriconazole release from microemulgel, so at least 1% w/w carbomer 934P was used for the preparation of microemulgel.

Citric acid, ascorbic acid, and acetone could not increase penetration as good as PG, while penetration of voriconazole in formulation is directly proportional to concentration of PG, moreover penetration ability of neem oil was dependent over solvent in which it was solubilized[26] and PG solubilized both hydrophilic and hydrophobic penetration enhancers too and it inhibits partition of penetration enhancer in SC, so PG was used as penetration enhancer in concentration of 1–10% w/w or solvent for penetration enhancer for prospective study.

The pH values of all prepared formulation were in range, which was considered acceptable to avoid the risk of irritation upon application to skin because the skin where fungal infection happened had pH in the range of 6 to 6.8. The values of spreadability indicated that the microemulgel was easily spreadable by the minimum amount of shear stress as like Voveran® Emulgel. Percentage synergizes showed that selection of carbomer 934P as the gelling agent and at least 1% w/w concentration was appropriated for the preparation of microemulgel of voriconazole.

As the concentration of carbomer 934P was constant in all preparations, the viscosity of all microemulgel was almost the same but the addition of PG increased the viscosity of microemulgel. The values of extrudability indicated that microemulgel was easy to extrude from the collapsible tube like SHY-NM™Gel. Proper *in vitro* diffusion release of voriconazole from all microemulgel formulation was observed.

J_{ss}, K_p, E_r, were higher, while T_{90}, t_{mic}, t_{leg}, % *dislodgeable* dose after 4 h were lowest with respect to Nizral® cream in the case of M3 than M2 and M1. These were because of the presence of PG

Table 4. Evaluation of voriconazole microemulgel and some marketed brands

Evaluation parameters	M1	M2	M3	Nizral® Cream	*Sapat plus malam®*	*Voveran®* Emulgel	*SHY-NM™*Gel
Q_{30}*	5.03±0.051	5.49±0.101	29.85±0.149	30±1	6h±1h	n/d	n/d
D/h^2 (h^{-1})	0.138±0.001	0.125±0.005	0.16±0.01	0.135±0.003	0.0056±0.0001	n/d	n/d
J_{ss}($mgcm^{-2}$ min^{-1})	0.0733±0.004	0.1295±0.01	0.2141±0.009	0.1231±0.0025	0.019825±0.005	n/d	n/d
E_r**	0.5955±0.005	1.052±0.06	1.7392±0.07	1	0.1611±0.002	n/a	n/a
K_p(cm/min)	0.0075±0.0009	0.0131±0.0008	0.0217±0.009	0.0137±0.0003	0.000247813±0.0000625	n/d	n/d
% dislodgeable dose after 4 h	65±5	5±1	0	0.25±0.001	80±2	n/d	n/d
Extrudability (g/(cm-sec))	14.5±0.5	15.33±0.577	15.67±0.577	25±2	35±3†	13±2	10±2
t_{mic}	110 sec	120 sec	40 sec	16 min	30 h	n/a	n/a

Q_{30}*= CPR at 30 min; n = 3; Mean±SD; h=30 μG; n/a = not applicable; n/d= not derived;
E_r** respect to Nizral®Cream; B-Tex® Super Ointment†.

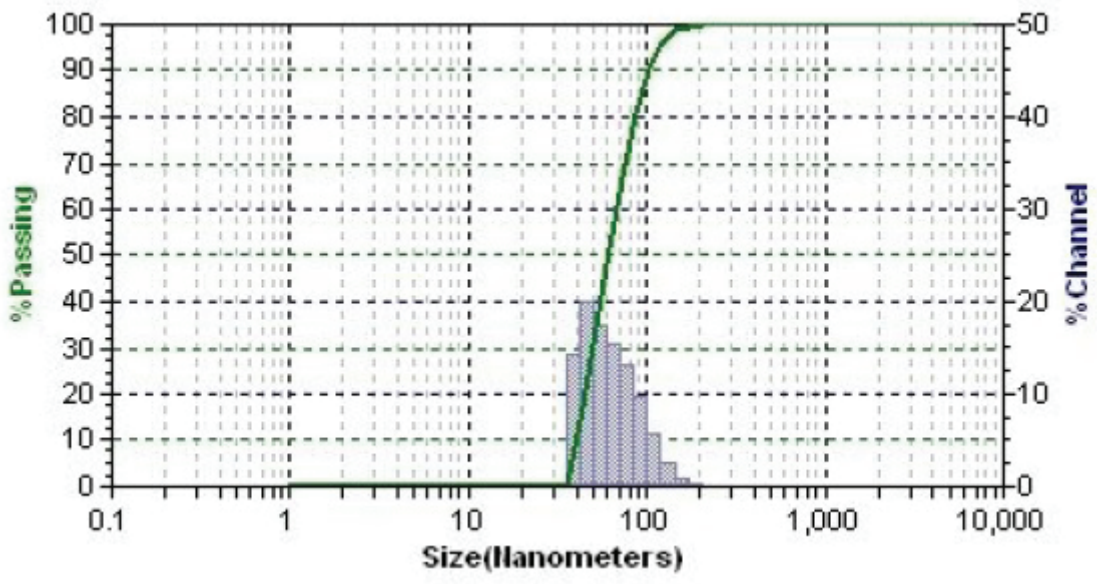

Figure 1. Micelle size and micelle size distribution graph of E12 batch.

10%, 5%, and 0%, respectively, which increased penetration of voriconazole from the membrane by a synergistic penetrating effect with neem oil. Microemulgel provided both intracellular diffusion and intercellular penetration of voriconazole, while Nizral® cream had a penetration of ketoconazole due to intracellular diffusion only.[27]

The sum of the square of residual value found to be least in Korsmeyer–Peppas Model so it was predicted that microemulgel followed Korsmeyer–Peppas model of kinetics. Release kinetic study followed anomalous transport and rate as function

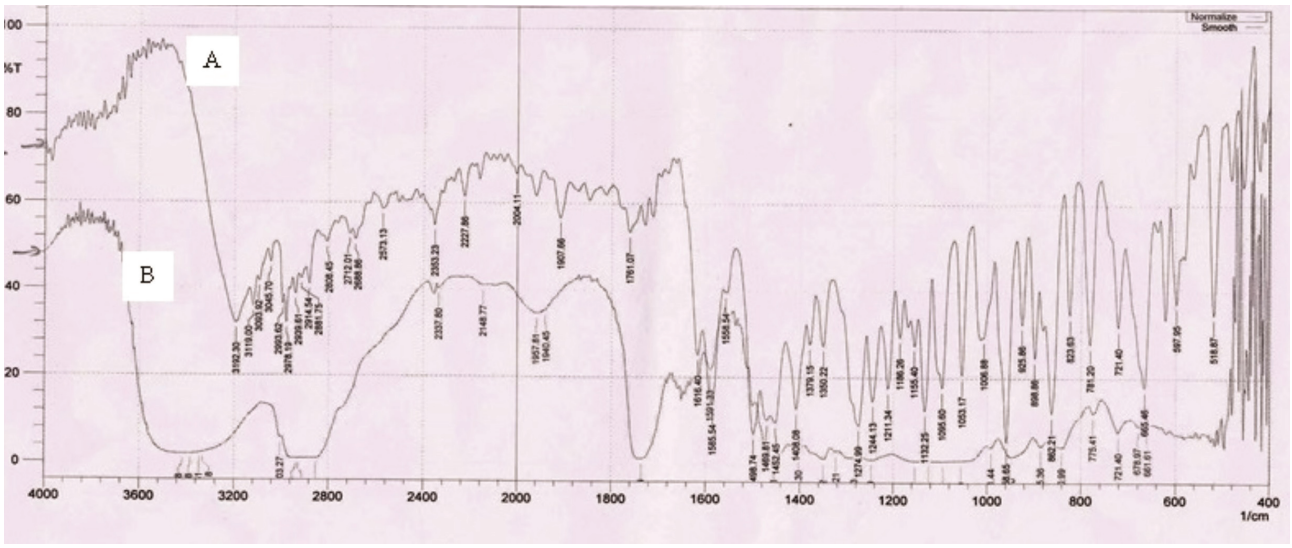

Figure 2. A: FTIR Spectra of voriconazole; **B:** FTIR Spectra of physical mixture of voriconazole and excipients of microemulgel.

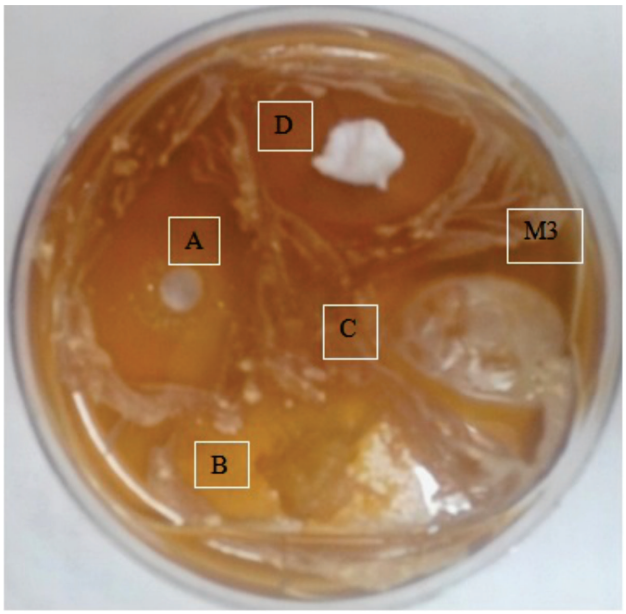

Figure 3. An *ex vivo* study by ditch technique method. A: Voriconazole in DMSO (Dimethyl sulfoxide); B: optimized batch of microemulgel without voriconazole C: DMSO; D=Nizral® Cream; M3: optimized batch of microemulgel, n=3; Mean ± SD.

of time were t^{n-1} because PG as penetration enhancer in microemulgel.[28]

The statistical analysis concluded that *in vitro* voriconazole diffusion was significantly higher than ketoconazole of Nizral® cream.[29]

The study demonstrated that there was significant difference between the zones of inhibition of an optimized batch of microemulgel (M3) and Nizral®cream ($p < 0.05$). The probable reason for that was the synergistic action of voriconazole and Neem oil in the M3.

M3 would be stable when packed in a proper container and stored in cool and dry place. The shelf-life was predicted to be 2 years with storage condition at the cool and dry place. *In vitro* diffusion profile before and after results indicated that voriconazole diffusion of the formulation before stability and after one-month stability studies was identical and superimposable.[28]

In limitations of the study, for example, the microemulgel was not validated for *in vivo* study. The micro analyst and microbiologist selected for MS, MSD, and microbial assay were not blind for the study.

CONCLUSION

The investigation on voriconazole microemulgel concluded that microemulgel successfully increased the solubility of voriconazole and permeability across stratum corneum. They were characterized, found safe and effective than Nizral® cream. Moreover, it had shelf-life of 2 years with patient compliance features. This investigation was also validated for physical parameters against SHY-NM™ Gel and Voveran® emulgel.

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Вориконазол микроэмульгель: всесторонняя защита от грибковых инфекций

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Ключевые слова: Ограниченный симплекс-центроидный план, микроэмульгель, на основе микроэмульсии, масло нима, вориконазол

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Введение: И флуконазол, и кетоназол отличаются худшей минимальной ингибирующей концентрацией по сравнению с вориконазолом. Вориконазол проявляет серьёзные побочные эффекты при пероральном и интравенозном применении. Обладает и плохой растворимостью в воде. Целью данного исследования является изготовление и оптимизация микроэмульгеля из вориконазола для локального применения.

Цель: Разработка формулы, изготовление и оценка микроэмульгеля для локального применения.

Методы: Масла и эмульгаторы были выбраны на основе исследования равновесной растворимости и эмульгирующих свойств. Были использованы псевдо-трёхкомпонентная фазовая диаграмма и ограниченный симплекс-центроидный план для изготовления микроэмульсии. Микроэмульсии были исследованы на предмет установления величины мицелиев, электрокинетического потенциала, индекса полидисперсности и были проведены исследования in vitro. Оптимизация эмульсии была доработана с использованием программного обеспечения Design® expert 9.0.3.1. Была разработана формула эмульгеля, он был изготовлен, оценен и оптимизирован. Был осуществлён микробный анализ оптимизированной партии микроэмульгеля.

Результаты: Растворимость вориконазола в масле нима Parker® составила 7.51 ± 0.14 мг / г. Acrysol ™ K-150: PEG-400 в соотношении 4 : 1 имела самую высокую площадь для микроэмульсии. 59.2% Acquisol ™ K-150, 14.8% ПЭГ-400, 11% масла нима Parker® 15% розовой воды и 1% вориконазола в качестве оптимизированной партии микроэмульгеля была выбрана для производства эмульгеля. Carbomer 934P оказался подходящим желирующим агентом при концентрации 0–2 % w/w. Оптимизированная партия микроэмульгеля и крема Nizral® обладала 37.32 ± 0.63 % и 26.45 ± 0.63 % зонами ингибирования.

Заключение: Вориконазол микроэмульгель является успешным средством локальной антигрибковой терапии.