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Design Development and Evaluation of Nanostructured Lipid Carrier Loaded Emulgel of Voriconazole

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ABSTRACT

Voriconazole is a second generation triazole derived from fluconazole. Voriconazole is used for the treatment of various fungal infections. Though it has excellent antifungal activity, there are several side effects associated with it. In order to overcome these limitations, nanostructures may be an excellent carrier for antifungal drugs. This study aimed to develop topical preparations of voriconazole for the treatment of mycotic infections of the skin. A nanostructured lipid carrier based emulgel formulation was developed and its physical characteristics, in vitro skin permeation and retention profiles were examined. A Voriconazole loaded NLC dispersion, consisting of stearic acid, oleic acid, SLS, Carbopol 940 was prepared by high pressure homogenization, and embedded into Carbopol 940 hydrogel. The lipid nanoparticles in the emulgel were approximately 272.8nm in size, with a spherical shape and zeta potential of -22.40 mv. Amongst all the formulations, NLC loaded emulgel prepared with the 60% Stearic acid was found to have better encapsulation efficiencyi.e. 97.5%. All the formulations were found to have good spreadability.The percentage of drug content was found to be within 95-99 %. The novel topical formulation reported here represents an alternative treatment for skin infections such as candidiasis, with less potential for systemic adverse effects than oral therapy.

Keywords: Voriconazole, NLC, emulgel, topical delivery, candidiasis.

I. INTRODUCTION

Voriconazole is a second generation triazole. It is derived from fluconazole. It is a broad-spectrum antifungal agent. It inhibits the vital step in cell membrane ergosterol synthesis i.e., it inhibits Cyt P450 dependent 14-alpha lanosterol synthesis. [(1)]Voriconazole is active against all candida species that have acquired resistance against fluconazole. Currently, it is used for the treatment of Aspergillosis and Candidiasis infections of abdomen, wounds, kidney, bladder wall, and skin.[(2)]

Despite its advantages, systemic exposure to Voriconazole as a result of oral and/ or intravenous administration can result in several side effects. These side effects are not usually fatal, but are considerable. The side effects include abdominal pain, vomiting, diarrhoea, photopsia, visual hallucinations, increased levels of hepatic enzymes. [(3)]Besides its side effects, the drug has low water solubility and it is prone to high first pass metabolism.Thereafter a need exists for a topical delivery system for VOR to overcome limitations in oral and intravenous administration. [(4)]

Topical drug application offers several advantages like higher tissue levels, more rapid drug delivery and lower systemic exposure.[(5)] But because of the poor solubility of VOR, solubilization of the drug is necessary for delivery in a topical formulation.[(6)]In addition, a penetration enhancer required to achieve therapeuticconcentrations in stratum corneum and deeper skin layers.[(7)] Topical drug delivery is an excellent route for local and systemic treatment. A unique aspect of the dermatological delivery is the direct availability of the skin.

NLC have emerged as a promising topical delivery system for pharmaceuticals in recent years, especially for delivery of lipophilic drugs. [(8)] Different categories of drugs can be applied to the skin like anti-inflammatory, antiseptic, antibacterial, antifungal, antiviral, antiacne and anaesthetic, etc. This delivery system improves the therapeutic efficacy by different mechanisms like sustained drug release, targeting potential to macrophage &deeper skin layer, enhanced solubility, enhanced permeation through subcutaneous layer (9,10). NLCs ensures close contact to the stratum corneum because of its unique lipid composition and smaller particle size thereby enhancing drug flux through the skin.[(11, 12)]

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Also because of solidified lipid matrix, a controlled release of the moiety from these carriers is possible.[13] Bioavailability of drug, solubility of the insoluble drug are 2 main criteria which may be enhanced with the formulations like NLCs.[14] NLCs are prepared with a blend of solid and liquid lipid which are completely biodegradable. The size of NLCs ranges from 40-1000 nm.[15]

Advantages:[16-17]

The several advantages of NLCs are as follows:

1.NLC has excellent biocompatibility

2.NLC's providesthe control or targeted drug release.

3. Higher drug loading capacity.

4. Physical and chemical stability avoids expulsion of drug during storage.

5. Physiological and biodegradable lipid are used as a carrier.

6. Exhibits low systemic toxicity and low cytotoxicity.

7. NLC's delivers higher drug content as related to other carriers available.

8. Transports both lipophilic and hydrophilic drugs on same time.

Components of NLC

The various components of NLC's are as follows:

Solid lipids–Beeswax, Carnauba wax, Cetyl palmitate etc.

Liquid lipids – Oleic acid, Soyabean oil, Miglyol, Castor oil, Palm oil, etc.

Emulsifying agents/ Surfactants – Sodium lauryl sulphate, Span 20, 80, 85, Tween 20, 80, Poloxamer, etc.The purpose of current study is to formulate the NLC dispersion based emulgel with VRC for facilitating drug delivery into the deeper layer of skin including subcutaneous and epidermis.[18]A voriconazole loaded lipid carrier system was successfully prepared using a high-pressure homogenization technique and incorporated into a gel base for topical application.The NLC dispersion was characterized with respect to particle size, surface charge, drug entrapment efficiency and amount and morphological features.[19,20]

II. MATERIALS AND METHODS

2.1 Materials

Voriconazole was procured from Dr. Reddy's Laboratories Ltd. (Hyderabad, India). The used lipids Stearic acid, Precirol ATO 5, Compritol 888 ATO, Glyceryl monostearate, Beeswaxwas purchased from the Research- Lab Fine Chem. Industry, Mumbaiand Loba chemie Pvt. Ltd, Mumbai. Castor oil, Almond oil, Sunflower oil and olive oil were obtained from local market, Colaba (South Mumbai India). Sodium Lauryl Sulphate and Carbopol were obtained from Molychem, Mumbai. Methanol, Ethanol and Triethanolamine were purchased from Research- Lab Fine Chem Industry, Mumbai. Distilled water was prepared in laboratory.

2.2 Methods

Screening of Solid and liquid lipid[21]

The screening of lipid was performed to select the best lipid to formulate NLC. The selection was performed on the basis of the highest solubility of a drug into a lipid. The appropriate quantity of each solid lipid (Precirol ATO 5, Compritol 888 ATO, Glyceryl monostearate, stearic acid, beeswax) was taken in glass vials and melted above 5^0 C of the melting point. An excess quantity of Voriconazole was added to the melted lipid. Similarly,the liquid lipids (Olive oil, Castor oil, Sunflower oil, Labrasol, Cremophore EL, Almond oil) were taken in glass vials. An excess of Voriconazole was added to the liquid lipid. Both the solid and liquid lipids were vortexed for complete solubilization and was allowed to stand for 72 hr. The mixture was then centrifuged at 6000 rpm for 30 min and the supernatant separated. The supernatant was appropriately diluted and the concentration of Voriconazole dissolved in lipids was measured by a UV-Spectrophotometer.

Compatibility study

The primary purpose of this work is to determine the drug- excipient compatibility. The drug is in close contact with one or more excipient in its solid dosage form, and this may affect drug stability. FT-IR technology has been used to study chemical interaction of drug, lipid and surfactant. In order to detect any appearance or disappearance of peaks, the IR spectrum of the mix was compared with the pure drug, lipids and surfactants and peak fitness were determined.

Preparation of NLC Dispersion

An NLC aqueous dispersion was prepared using a High –pressure homogenization technique. Briefly, the lipid phase was prepared by mixing the required amounts of Stearic acid and Olive oil, and the resultant mixture was heated upto 70^0C to melt the solid lipid completely and the weighed amount of Voriconazole was added to the melted lipid. The aqueous phase was prepared by adding the desired amounts of Sodium lauryl sulphate into distilled

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water and the same temperature was maintained as lipid. The resultant mixture was heated up to 70^0C with continuous stirringto dissolve the surfactant completely. The melted lipid phase was slowly added into the aqueous phase under high pressure homogenization at the speed of 8,000 rpm for 2 minute to form a primary emulsion The primary emulsion then converted to the NLC dispersion using a high-pressure homogenizer as shown in. The NLC dispersion was cooled down at room temperature.

Preparation of NLC- Gel

NLC embedded emulgel was prepared to increase the viscosity and improve skin applicability. Carbopol 934P (1%) served as a gelling agent and was dispersed in the NLC aqueous dispersion. Next, the dispersion was homogenized at 11000 rpm for 2 min. Finally, the dispersion was neutralized by addition of 0.1 % Triethanolamine to adjust the pH of the gel formulation within the range of 5.5 to 6.5.

Table 1. Composition of Voriconazole loaded NLC's formulations as per 3^2 full factorial design

Formulations (Batch No.)	Drug (% w/w)	Lipid		Surfactant (% w/w) SLS	Water (% v/v)
		Stearic acid (%w/w)	Oleic acid (%v/v)		
VOR-NLC 1	1	351.75		1.3	100
VOR-NLC 2	1	35 1.75		1.3	100
VOR-NLC 3	1	351.75		1.3	100
VOR-NLC 4	1	47.51.75		1.3	100
VOR-NLC 5	1	47.51.75		1.3	100
VOR-NLC 6	1	47.51.75		1.3	100
VOR-NLC 7	1	60	1.75	1.3	100
VOR-NLC 8	1	60	1.75	1.3	100
VOR-NLC 9	1	60	1.75	1.3	100

Table 2. Variables in Optimization study

Variables	Code	Factor
Independent	X1	Stearic acid
	X2	Speed of homogenizer
Dependent	Y1	Antifungal activity
	Y2	Entrapment efficiency

III. CHARACTERIZATION OF NLC AND NLC GEL

Physical characterization

The NLC based formulations were visually inspected for colour, homogeneity, consistency.
The particle size and zeta potential of the optimized formulations were determined using the Zetasizer.

pH

The pH of the dispersion was measured using pH meter (33). 100 mg of the gel formulations was weighed in a 50 mL volumetric flask and then volume was made up with distilled water up to 50 mL. The electrode was then dipped in to NLC based gel formulation and constant reading was noted.

Viscosity

The viscosity of the different NLC formulations was determined by Brookfield viscometer at 10 rpm at the temperature of 37 +/- 0.5^0C.

Entrapment efficiency

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Entrapment efficiency of the Voriconazole in VOR-NLC's were determined to check the amount of VOR loaded and entrapped in the prepared NLC's. Drug encapsulation efficiency of the VOR-NLC system was determined by an ultrafiltration centrifugation method. VOR-NLC's were filled in a centrifugation tube and centrifuged at 15000 rpm for 30 min. The supernatant was separated and diluted further to evaluate the unentrapped amount of drug in NLCs by measuring the absorbance with the help of a UV spectrophotometer. The concentrations of Voriconazole were measured using HPLC, and the entrapment efficiency (%) was calculated using following formula:

EE% =W (Total) – W(Free)/ W (Total) X 100

Where, EE % = Entrapment efficiency

W (Total) = total amount of drug added

W (Free) = amount of free drug detected in the aqueous phase

Spreadability

0.5 gmof gel was placed within a circle of 1 cm diameter premarkedon a glass plate. Upon this plate, a second glass plate was placed. A weight of 500 gm was allowed to rest on the upper glass plate for 5 min. The increase in the diameter due to gel spreading was noted. The spread ability was calculated by using following formula: S = M x L / T

Where, S = Spreadability

M = Weight in the pan (tied to the upper slide)

L = Length moved by the glass slide

T = Time taken to separate the slide completely.

In Vitro Drug Release Study

The release of VOR from NLC and NLC-gel was evaluated using a Franz diffusion cell mounted with a dialysis membrane. The receptor compartment was filled with phosphate buffered saline (PBS, Ph 7.4) and water jacketed qat 32 ^{0}C to mimic human skin. The dispersion was applied to the donor compartment. Aliquots were withdrawn from the receptor compartment at predetermined time intervals and immediately replaced with an equal volume of freshPBS solution. Using the UV Spectrophotometer, the withdrawn samples were examined for drug content by measuring the absorbance at 255 nm.

Antifungal activity

The antifungal activity was analyzed by cup plate diffusion method using Sabouraud's dextrose agar medium. The fungal broth culture (Candida albicans and Aspergillus fumigatus) was standardized in the growth medium. The required quantity of medium was preparedand transferred in a clean sterilized Petri plate at 121 ^{0}C. The plates were kept aside for 15 min to solidify the media. The wells (6 mm) were prepared using a sterilized stainless-steel borer in the sterilized condition. The samples of VOR-NLC-gel were placed into the wells. The plates were kept for 2 hr at room temperature to diffuse the samples into the medium and then transferred into the incubator. The zone of inhibition (mm) was measured at 12 hr and 24 hr for each test sample and compared with the standard.

Optimization study

Optimization of the formulations was studied by 3^2 full factorial designs. The amounts of Stearic acid (X_1) and Speed of homogenizer (X2) were selected as independent variables and the dependent variables were Antifungal activity (Y1) and Entrapment efficiency (Y2) The data obtained was treated using Design expert software (version 7.0) and analyzed statistically using analysis of variance (ANOVA). The data was also subjected to 3-D response surface methodology to study the effect of Stearic acid and speed of homogenizer on the dependent variables. i.e., Entrapment efficiency and Antifungal activity.

Stability study

Test conditions for stability studies as per ICH guidelines are shown in the table 2.

Table 3: Test conditions for Stability study

Test conditions	
Duration of study	3 months
Temperature conditions	40 ^{0}C ± 2 ^{0}C
Relative humidity conditions	75 % RH ± 5 % RH
Frequency of testing	3 months

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IV. RESULTS AND DISCUSSION

Compatibility study

Fourier Transform Infrared Spectroscopy

The FTIR spectrum of physical mixture retained all characteristic peaks visible in thespectrum of the drug alone. The spectrum of physical mixture is shown in figure.1

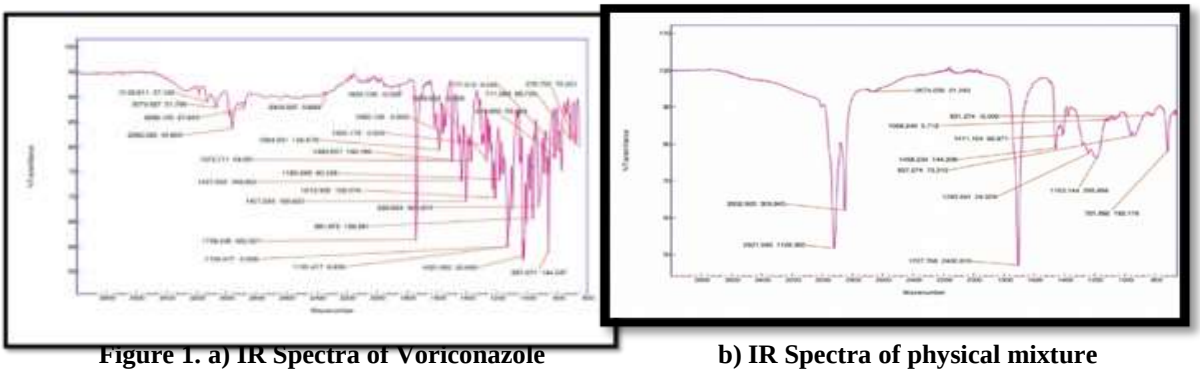

Figure 1. a) IR Spectra of Voriconazole **b) IR Spectra of physical mixture**

The physical mixture of drug with polymer doesn't show disappearance of the peak. All functional groups werefound to be present in the physical mixture which confirms the compatibility of the drug with the polymers.

Formulation and Development of the Nanostructured lipid carrier

The various formulations prepared by 3^2 Full Factorial Design have been shown in the figure 2.

Fig. 2 Formulations F1 to F9

Evaluation of Nanostructured Lipid Carriers

Particle size and Zeta potential

The particle size and zeta potential of the optimized batch F9 is given in the table 4:

Table 4: Particle size and zeta potential of NLC

Formulation code	Particle size	Zeta potential
F9	272.8	-22.40

Entrapment Efficiency

The maximum Entrapment efficiency was found to be 97.5% and the minimum Entrapment efficiency was found to be 70%.It hasbeen observedthat drugentrapment efficiency was highest for optimized batch (F9).

Table 5: Entrapment efficiency of Formulation F1 to F9

Sr. no.	Formulation code	% Entrapment efficiency (± SD n=3)
1.	F1	70.0 ± 0.03
2.	F2	78.5 ± 0.05
3.	F3	80.5 ± 0.02
4.	F4	84.5 ± 0.05
5.	F5	87.5 ± 0.02
6.	F6	90.0 ± 0.03
7.	F7	92.5 ± 0.02

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8.	F8	95.0 ± 0.05
9.	F9	97.5 ± 0.05

Table 6: pH, Viscosity, Spreadability and Drug content of Formulations F1 to F9

Sr.no	Formulationcode	pH	Viscosity	Spreadability	Drug content
1.	F1	5.8 ± 0.05	10481 ± 0.05	14.10 ± 0.39	14.10 ± 0.39
2.	F2	6.1 ± 0.05	9940 ± 0.08	15.60 ± 0.43	15.60 ± 0.43
3.	F3	6.0 ± 0.01	9850 ± 0.07	15.88 ± 0.26	15.88 ± 0.26
4.	F4	6.2 ± 0.05	9985 ± 0.05	15.50 ± 0.40	15.50 ± 0.40
5.	F5	6.7 ± 0.05	9925 ± 0.02	15.66 ± 0.43	15.66 ± 0.43
6.	F6	6.4 ± 0.05	9896 ± 0.017	15.76 ± 0.26	15.76 ± 0.26
7.	F7	5.9 ± 0.05	1032 ± 0.011	1 5.00 ± 0.44	1 5.00 ± 0.44
8.	F8	6.0 ± 0.05	1021 ± 0.05	15.20 ± 0.26	15.20 ± 0.26
9.	F9	6.6 ± 0.05	988 ± 0.012	15.80 ± 0.19	15.80 ± 0.19

The pH of various emulgel formulations is shown in the table 24, which was found to be in rangeof 5.8- 6.4. This range matches with the normal pH range of the skin and thus do not produceany skin irritation. The viscosity is resistance to flow which is an important physical propertyfor topical preparations. Spreadability of an emulgel is very important in the topicalemulgel formulations. Formulations F5, F7 and F9 have good Spreadability.

The drug content was carried out to ascertain that the concentration of drug in each formulation was uniform. The percentage drug content of all prepared emulgel formulations were found to be in the range of 95-98.60 %.

Invitro drug release study

The in vitro drug release of the drug from NLC gel formulation was studied amongst all 9 formulations. The drug release of optimize formulation shows 98.60 %.

Table 7. Invitro drug release of various gel formulations

Time (hours)	% Cumulative drug release of F9 formulation± SD
1	22.3 ± 0.225
2	50.10 ± 0.225
3	59.34 ± 0.175
4	71.56 ± 0.225
5	79.42 ± 0.222
6	81.56 ± 0.22
7	87.52 ± 0.225
8	89.82 ± 0.225
9	91.65 ± 0.225
10	93.48 ± 0.222
11	96.34 ± 0.225
12	98.60 ± 0.225

-± SD (n=3**)**

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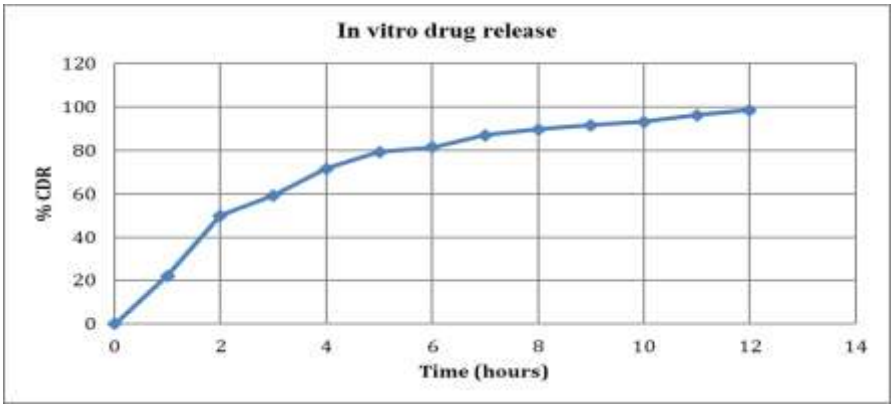

Figure 3. Drug release profile of optimized formulation

Antifungal activity

Table 8: Antifungal activity of formulation F1 To F9

Sr. no.	Formulation code	Zone of inhibition (mm)
1	F1	18.2
2	F2	20.6
3	F3	16.4
4	F4	17.2
5	F5	22
6	F6	15.5
7	F7	19.5
8	F8	20.4
9	F9	21.4

The antifungal activity of VOR-NLC gel was analyzed on Candida albicans by cup plate method. The optimized formulation F9 shows the zone of inhibition of 21.4mm.

Statistical analysis

Optimization

The experimental design in correlation with optimization study has been described in the table 9. To study the effect of independent variables on responses, Design expert 7.0 software was used. Experimental design layout developed for 9 possible batches of Voriconazole emulgel is shown in table 30. X1 and X3 are the amounts of Stearic acid and Speed of homogenization, and Y1 and Y2 are Entrapment efficiency and Antifungal activity respectively. Out of the various models such as Linear, 2FI, Quadratic and cubic, which fit well was suggested by software and was tested for analysis of variance (ANOVA). Regression polynomials were calculated for the individual independent variables and then one factor, 3D and perturbation graphs were obtained for each individual dependent variable or response (R) and expressed as equation 1-2. X1 and X2 are the main effects which represent the average result of changing one factor at a time from its low to high value. X1, X2 are interaction terms that shows how the response changes when two factors are simultaneously changed. The result of Antifungal activity (Y1) and entrapment efficiency (Y2) was added to the software to interpret the results.The actual antifungal activity and entrapment efficiencywere compared to the predicted value generated by the software and the result was found to be closer value. ANOVA value was found to be significant.

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Table 9:Experimental design and Optimization study

Runs	Factor X1 A: Stearic acid (%)	Factor X1 B: Speed of Homogenizer (RPM)	Response Y1 Antifungal Activity (mm)	Response Y2 Entrapment efficiency (%)
1	47.5	15000	18.2	79.44
2	47.5	25000	20.6	95.89
3	35	20000	16.4	82.66
4	35	25000	17.2	92.44
5	60	25000	22	98.12
6	50	15000	15.5	78.17
7	35	20000	19.5	85.78
8	35	15000	20.4	81.15
9	35	20000	21.4	86.99

In order to compare the results, ANOVA (Design expert version 7.0) was used.

Results for the Antifungal activity of DOE:

1. **Fit Summary:**After entering the data in Design-Expert software, fit summary applied to the data after which the "Linear vs Mean" was suggested by the software. Data was expressed in table 10.

Table 10: Fit summary table for Antifungal activity of DOE

Source	Sum of Squares	df	Mean Square	F value	p- value Prob > F	
Mean vs Total	3256.60	1	3256.60			
Linear vs Mean	20.72	2	20.72	126.11	< 0.0001	Suggested
2FI vs Linear	0.00	1	0.00	0.01	0.9146	
Quadratic vs 2FI	0.79	2	0.39	6.05	0.0885	
Cubic vs Quadratic	0.20	2	0.10	351.00	0.0377	Aliased
Residual	0.00	1	0.00			
Total	3299.02	9	366.56			

1. ANOVA for Antifungal activity of DOE:

The analysis of variance (ANOVA) was performed to identify significant and insignificant factors. The results of ANOVA for the antifungal activity of DOE are as following table 11.

Table 11: ANOVA table for an Antifungal activity of DOE

Source	Sum of Squares	df	Mean Square	F value	p-value Prob> F	
Model	41.43	2	20.72	126.11	< 0.0001	Significant
A- Stearic acid	36.02	1	36.02	219.26	< 0.0001	

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B-Speed of homogenizer	5.42	1	5.42	32.97	0.0012	
Residual	0.99	6	0.16			
Cor Total	42.42	8				

The Model F-value of 126.11 implies the model is significant. There is only a 0.01% chance that a "Model F-Value" this large could occur due to noise.

Values of "Prob > F" less than 0.0500 indicate model terms are significant. In this case A and B are significant model terms.

3. Fit Statisticsfor Antifungal activity of DOE

Table 12: Fit Statistics for Antifungal activity of DOE

Std. Dev.	0.41	R-Squared	0.9768
Mean	19.02	Adj R-Squared	0.9690
C.V. %	2.13	Pred R-Squared	0.9492
PRESS	2.15	Adeq Precision	29.061

The "Pred R-Squared" of 0.9492 is in reasonable agreement with the "Adj R-Squared" of 0.9690. "Adeq Precision" measures the signal to noise ratio. A ratio greater than 4 is desirable. Your ratio of 29.061 indicates an adequate signal. This model can be used to navigate the design space.

4. Final equation in Terms of coded Factors for Antifungal activity of DOE:

Table 13: Final equation in terms of coded factor for Antifungal activity of DOE

Antifungal activity	=
19.02	
2.45	*A
0.95	* B

The equation in terms of coded factors can be used to make predictions about the response for given levels of each factor.

Graphical Presentation:Diagnostics of Antifungal activity for DOE

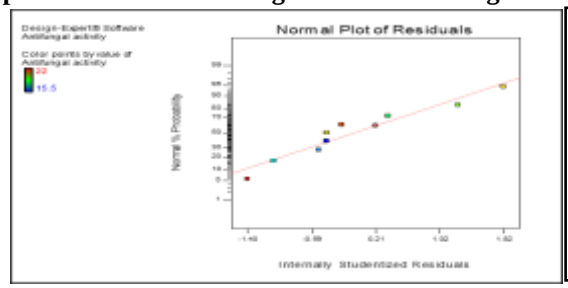

Figure 4: Normal % Probability for DOE of Antifungal Activity

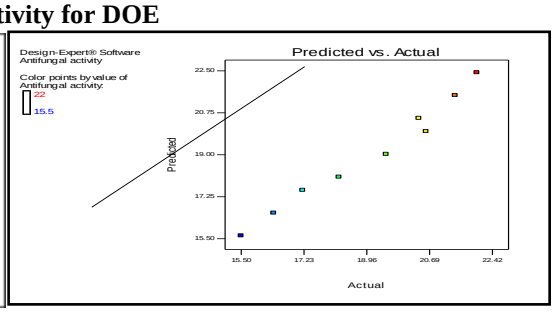

Figure 5: Predicted Vs Actual for DOE of Antifungal activity for DOE

Model Graphs of Antifungal activity: One-factor Graphs of Antifungal activity for DOE:

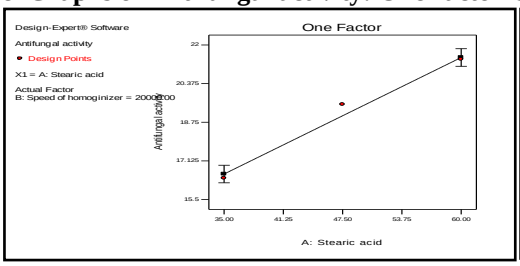

Figure 6: Effect of % Stearic acid on Antifungal activity

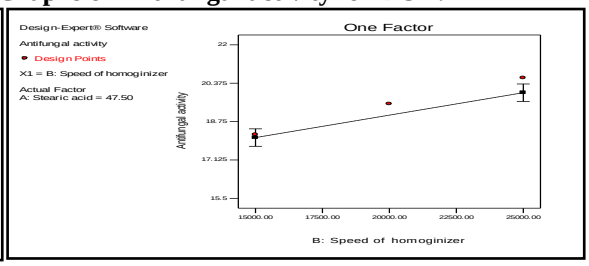

Figure 7: Effect of speed of homogenizer of Antifungal activity

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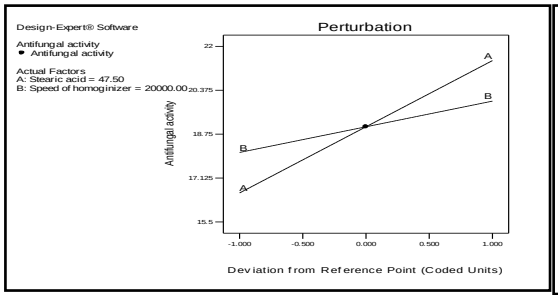

Figure 8: Effect of All 2 independent factors on Antifungal activity

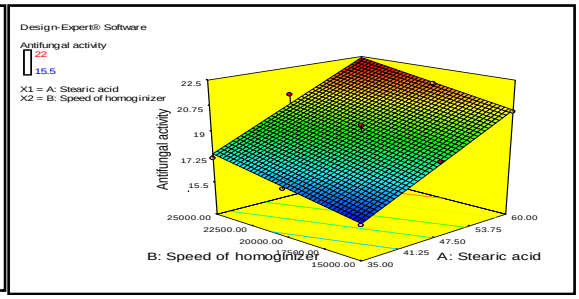

Figure 9: 3D plot of 2 independent factorsfor Antifungal activity

Percentage of Stearic acid and Speed of homogenization in formulation have impact on Antifungal activity of drug. As % stearic acid and speed of homogenization in formulation increases, Antifungal activity increases.

Stearic acid was having high impact on Antifungal activity as compare to speed of homogenization as its P value was very low as compare to speed of homogenization**.**

Results for the Entrapment efficiency of DOE:

1. Fit Summary: After entering the data in Design expert software, Fit summary applied to the data after which the "Quadratic vs 2FI" was suggested by the software. Data was expressed in table 15.

Table 14: Fit summary table for Entrapment efficiency of DOE

Source	Sum of Squares	df	Square Mean	F Value	p-value Prob > F	
Mean vs. Total	67710.98	1	67710.98			
Linear vs. Mean	407.18	2	203.59	86.32	< 0.0001	
2FI vs. Linear	1.82	1	1.82	0.74	0.4292	
Quadratic vs.2FI	11.84	2	5.92	36.52	0.0078	Suggested
Cubic vs. Quadratic	0.23	2	0.11	0.45	0.7265	Aliased
Residual	0.26	1	0.26			
Total	68132.31	9	7570.26			

2. ANOVA for Entrapment efficiency of DOE:

Table 15: ANOVA table for Entrapment efficiency of DOE

Source	Sum of Squares	df	Mean Square	F Value	p-value Prob > F	
Model	420.44	4	105.11	473.28	< 0.0001	significant
A-Stearic acid	28.12	1	28.12	126.63	0.0004	
B-Speed of homogenizer	379.06	1	379.06	1706.79	< 0.0001	

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AB	1.82	1	1.82	8.21	0.0457	
B^2	11.44	1	11.44	51.51	0.0020	
Residual	0.89	4	0.22			
Cor Total	421.33	8				

The Model F-value of 473.28 implies the model is significant.

Table 16: Fit statistics for Entrapment efficiency for DOE

Std. Dev.	0.47	R-Squared	0.9979
Mean	86.74	Adj R-Squared	0.9958
C.V. %	0.54	Pred R-Squared	0.9914
PRESS	3.64	Adeq Precision	57.584

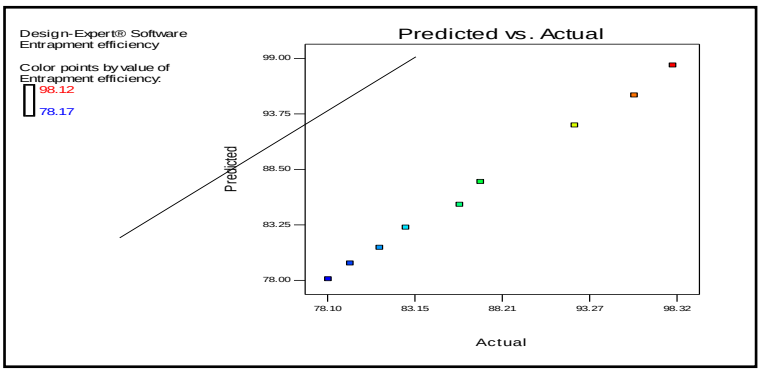

Figure 25: Predicted Vs Actual of Entrapment efficiency for DOE

Model Graphs of Entrapment efficiency: One-factor Graphs of Entrapment efficiency for DOE

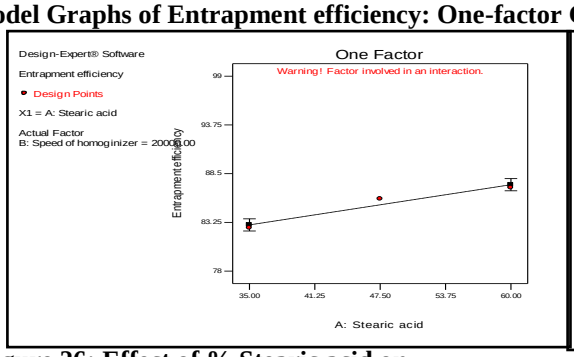

Figure 26: Effect of % Stearic acid on Entrapment efficiency

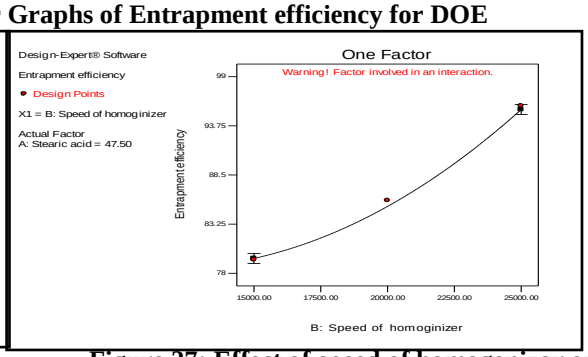

Figure 27: Effect of speed of homogenizor on Entrapment efficiency

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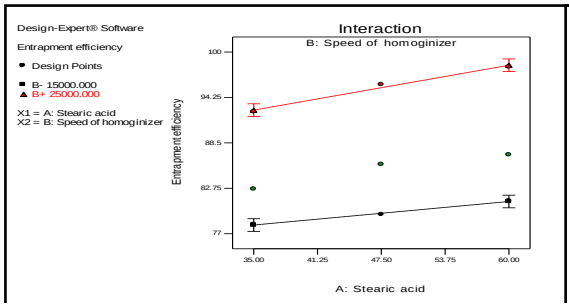

Figure: Effect of All 2 independent parameters on Entrapment efficiency

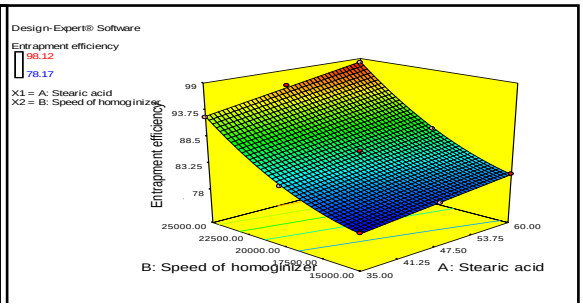

Figure 30: 3D plot of 2 independent factors for entrapment efficiency

Percentage of Stearic acid and Speed of homogenization in formulation have impact on Entrapment efficiency of drug.As % Stearic acid and Speed of homogenization in formulation increases, Entrapment efficiency also increases.

Table 17: Summary of effect of independent variable on dependent variables

Sr. No.	Independent variables	Antifungal activity	Speed of homogenization
1	% Stearic acid in formulation	Directly proportional (As Stearic acid increases, Antifungal activity increases)	Directly proportional (As Stearic acid increases, Entrapment efficiency also increases)
2	Speed of Homogenizer (RPM)	Directly proportional (As speed of homogenizer increases, Antifungal activity increases)	Directly proportional (As speed of homogenizer increases, entrapment efficiency increases)

On the basis of data obtained from evaluation of batches and factorial design model study F9 batch was selected as optimized batch.

Stability studies:

Table 18: Stability studies data for optimized formulation

Sr. no.	Evaluation parameters	Results after 1 month
1.	Physical evaluation	Smooth
1.	pH	6.1 ± 0.1
3.	Viscosity	9785 ± 0.09
4.	Spreadability	15.50
5.	Entrapment efficiency	97.1 %
6.	Drug content	98.05 %
7.	Drug diffusion (%CDR)	97.8 %

±SD (n=3)

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V. CONCLUSION

1.% stearic acid and speed of homogenization have impact on antifungal activity of drug.

As concentration of stearic acid increases, antifungal activity increases.

2. As speed of homogenizer increases, Entrapment efficiency increases.

The present study conclusively demonstrates that the use of a 3^2 full factorial design isvalid for predicting the Entrapment efficiency and Antifungal activity in optimization of emulgel formulations. The developed emulgels were efficacious for the delivery of lipophilic and poorly soluble drugs such as Voriconazole. The results demonstrated that the formulations were stable and showed improved permeation of the drug from the emulgel.

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