(Research Article)

Design Optimization and Characterization of Fluconazole Loaded Emulgel Using Box Behnken Design

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ABSTRACT:

Aim: The aim of the selected study was to develop and statistically optimize fluconazole loaded emulgel to enhance transdermal permeation by incorporating emulsion into gel base by selecting the Box-Behnken model.

Materials & Methods: Fluconazole is an antifungal drug which belongs to BCS class-II with high permeability and choice for topical drug delivery. In the study, the levels of the oil (oil phase), S_{mix} (surfactant mixture) and water (aqueous phase) are selected as independent variables, were varied to study the influence on % entrapment efficiency, *in vitro* drug release and zeta potential as dependent variables. Response surface designs through software Design expert version 13 (Box-Behnken design) is applied for this study and the optimization process was carried out using the desirability plots and point prediction techniques.

Results: Results of the study with the application of a design expert shows that the out of 15 experimental runs with three centre points optimized(F2) drug loaded emulgel with high % entrapment efficiency of 87.83±0.28% as predicted and zeta potential of -32.05±0.22mV indicating good stability of the formulation and *in vitro* drug release of 87.54±2.5% respectively.

Conclusion: Through obtained results, it's concluded that; the independent variable plays a crucial role in optimizing formulation. Study data provided strong evidence that the optimized emulgel formulation through Box-Behnken factorial design can be potential carrier for loading drug of selected category for enhancing transdermal permeation.

KEYWORDS: Fluconazole, emulgel, design of experiment, Box-Behnken design, fungal infections

INTRODUCTION

Emulgel is emerging topical drug delivery systems which gained more popularity in recent days for delivery of both hydrophilic and lipophilic drugs of a selected category of drugs on to the skin to treat various skin related disorders superficially[1-3]. Emulgel are generally a combination of gel and emulsion with type's oil-in-water and water-in-oil emulsion which can be used as vehicle to encapsulate and to deliver various categories of drugs.The topical drug delivery system is mostly preferred when other routes of administration like oral, rectal, parenteral, sublingual fails.The main advantage of preferring emulgel as topical drug delivery system is to overcome the first pass metabolism and inconveniences of intravenous therapy and varied conditions of absorption, presence of enzymes, pH changes, gastric emptying time can be avoided which are common drawbacks with oral route of administration[4,5].

Semisolid dosage forms administered topically for their local action at the site of application can enhance the drug absorption if it has a suitable lipid/water partition coefficient and if it is a non electrolyte. Emulgel (gellified emulsion) generally used where the other systems of drug administration failed to directly treat cutaneous disorders such as fungal infections, acne, psoriasis, etc[6]. Emulgel are generally a combination of gel and emulsion with type's oil-in-water and water-in-oil emulsion which can be used as vehicle to encapsulate and to deliver various categories of drugs on to the skin. Generally emulsions have high ability to penetrate into the skin there by combination gel base with emulsion enhances the rate of permeability [7].Fluconazole is basically a BCS class-II drug with low solubility with high permeability properties. Fungal infections have developed as a threat to the health of human beings over the past few years. In healthy state the human body houses many commensal fungal species, Candida albicans being one of them. In present research work attempt was made to prepare fluconazole loaded emulgel for treating superficial skin related fungal infections by selecting suitable optimization techniques.

MATERIALS AND METHODS

Fluconazole were generously provided as gift sample and castor oil, tween 80, PEG 600, Propylene glycol, carbopol,triethanolamine, methyl and propyl paraben were from Himedia Laboratories Limited, Mumbai., India. All other solvents and reagents utilized in this study were of analytical grades and used without further purification.

PREPARATION METHODS OF EMULGEL [8,9]

A.Co-Emulsification Method: The co-emulsification method is a simple and versatile technique for preparing emulgels. It involves the simultaneous emulsification of two immiscible phases, an oil phase and an aqueous phase, using a surfactant and a co-surfactant. The resulting emulsion is then dispersed into a gel base to form the emulgel.

Procedure for the co-emulsification method:

1. **Prepare the oil phase:** Dissolve the oil in a suitable solvent, such as ethanol or propylene glycol. Add any oil-soluble drugs or excipients to the oil phase.
2. **Prepare the aqueous phase:** Dissolve the water-soluble drugs or excipients in water. Adjust the pH of the aqueous phase to the desired value.

3. **Prepare the surfactant mixture:** Combine the surfactant and co-surfactant in a suitable solvent, such as ethanol or propylene glycol. The ratio of surfactant to co-surfactant will depend on the specific oils and aqueous phases being used.
4. **Emulsification:** Add the surfactant mixture to the oil phase and mix thoroughly. Add the aqueous phase to the oil phase and mix vigorously until a stable emulsion is formed.
5. **Gelation:** Disperse the emulsion into a gel base, such as carbomer or xanthan gum. The concentration of the gel base will depend on the desired viscosity of the emulgel.
6. **Homogenization:** Homogenize the emulgel to reduce droplet size and improve stability.

B. Phase Inversion Temperature Method: This method involves the preparation of a w/o emulsion by heating the emulsifying agent and oil phases at high temperature. To this mixture, the water phase is added slowly while stirring, then mixture is stirred till homogenous w/o emulsion is formed. The gel is obtained by cooling the mixture below the phase inversion temperature.

CALIBRATION OF CURVE OF FLUCONAZOLE [10]

A. Preparation of stock solution:

Accurately weighed 10mg of fluconazole is transferred into a 100ml volumetric flask and dissolved into methanol. It was then sonicated for 10 min and made up to the mark final volume with phosphate buffer pH 7.4 to give a stock solution containing 100µg/ml concentration.

B. Construction of calibration curve of fluconazole :

From the prepared stock solution of fluconazole in methanol concentration, aliquots ranging 2,4,6,8,10µg/ml was prepared.The absorbance values of above solutions were measured in the wavelength at $\boldsymbol{\lambda}_{max}$ 260nm was against methanol as blank and calibration curve was plotted by taking concentration (µg/ml) on x-axis and absorbance on y-axis.

Optimization of formulation using design by selecting dependent and independent variables:

Formulation were evaluated for selected responses like entrapment efficiency (Y1), Cumulative drug release (Y2), Zeta potential (Y3). The values are obtained by evaluating selected responses were fit into different mathematical models using drug design expert software version 13. The best fit model was selected based on comparison of optimization of statistical parameters.

CHARACTERIZATION OF EMULGEL[11,12]

Entrapment efficiency:Precisely weighed quantity of emulgel containing drug was kept in 10ml of methanol buffer solution (pH 6.1) with stirring. Filtered samples were further analyzed at 260 nm next to blank using UV spectrophotometer (Pharmaspec 1700, Shimadzu, Japan). Estimation of entrapment efficiency for all optimized formulations was done using the following formula.

$$\% \text{ Entrapment efficiency} = \frac{\textit{Actual drug content in emulgel}}{\textit{Theoretical drug content}} \times 100$$

In vitro drug release (%)

The *invitro* drug release studies of the emulgel were carried out on Diffusion cell using dialysis membrane. This was clamped carefully to one end of the hollow glass tube of dialysis cell. Emulgel (1gm) was applied onto the surface of dialysis membrane. The receptor chamber was filled with freshly prepared PBS (pH 7.4) solution to solubilize the drug. The receptor chamber was stirred by a magnetic stirrer. The samples (1 ml aliquots) were collected at suitable time intervals for every hour up to 24hrs and replaced with same amount of buffer to maintain sink conditions.Sample were analysed for drug content by UV-visible spectrophotometer after appropriate dilutions. The cumulative amount of drug release across the membrane was determined as a function of time. The cumulative % drug release was calculated using standard formula.

% cumulative drug release = [conc.µg/mL x Dilution factor x Vol. of release medium (mL)] x 100 ÷ Initial dose (µg)

RESULTS AND DISCUSSION

Calibration curve of fluconazole

The results of absorbance for all the prepared concentrations were plotted i.e. Concentration on X-axis and Absorbance on Y-axis. The plotted graph was observed to be linear over the prepared concentration range with the standard equation y=0.0299x-0.0052 and with the Regression value (R^2) of 0.9885. From the calibration data obtained it was found that the regression coefficient was less than 1 which is nearer to its unity. It obeyed beer's law in these concentration ranges. The results were shown in table.1

Table 1 Calibration curve data for fluconazole at 260nm using UV spectroscopy

S.No	Concentration (µg/ml)	Absorbance Mean ± SD
1	0	0
2	2	0.054 ± 0.01
3	4	0.112 ± 0.03
4	6	0.154 ± 0.05
5	8	0.215 ± 0.07
6	10	0.312 ± 0.09
7	12	0.362 ± 0.12

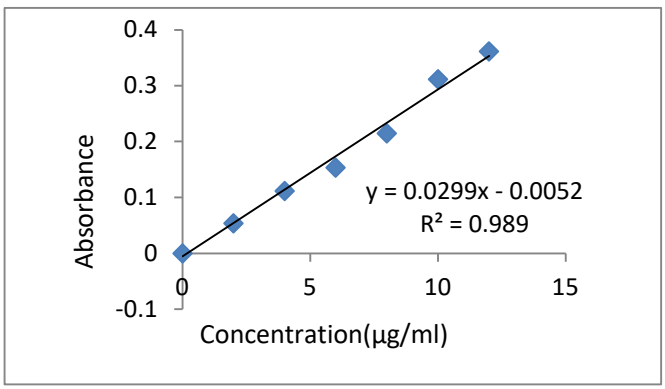

Figure.1 Calibration curve of fluconazole

Table 2. Selection of independent and dependent Variables in Box-Behnken Experimental Design

Independent variables	**Coded symbol**	**Levels**		
		-1	**0**	**+1**
Oil	A (X_1)	10	15	20
Smix	B(X_2)	30	35	40
Water	C(X_3)	40	45	50
Dependent variables	**Coded symbol**	**Levels**		
Entrapment efficiency (%)	Y_1	Maximize		
Cumulative drug release (%)	Y_2	Maximize		
Zeta potential (mV)	Y_3	Maximize		

Table.3 Optimization of fluconazole loaded emulgel using Box-Behnken design:

Standard	Run	Factor 1 A:Oil (X1)	Factor 2 B:Smix (X2)	Factor 3 C:Water (X3)	Response 1 Entrapment efficiency (%) Mean±SD (Y1)	Response 2 Cumulative drug release Mean±SD (Y2)	Response 3 Zeta potential (mV) Mean±SD (Y3)
14	1	15	35	45	61.33±1.52	60.24±1.6	-25.21±0.45
7	2	10	35	50	87.83±0.28	87.54±2.5	-32.05±0.22
5	3	10	35	40	76.66±0.57	73.81±1.5	-24.35±0.10
4	4	20	40	45	62.66±1.15	61.58±2.1	-23.54±0.41
8	5	20	35	50	57.52±3.21	54.36±3.2	-26.01±0.20
6	6	20	35	40	65.66±2.08	60.85±4.2	-25.54±0.49
9	7	15	30	40	73.16±1.53	69.65±0.24	-19.62±0.32
11	8	15	30	50	60.33±0.55	59.43±0.9	-27.85±0.24
10	9	15	40	40	58.33±1.52	57.34±0.9	-24.61±0.18
2	10	20	30	45	64.33±2.51	58.45±2.3	-16.46±0.28
1	11	10	30	45	82.33±3.21	80.85±1.2	-25.45±0.36
3	12	10	40	45	76.45±3.05	75.45±3.6	-23.21±0.47
15	13	15	35	45	63.66±1.62	61.95±3.0	-21.64±0.40
13	14	15	35	45	67.72±1.81	62.05±0.7	-22.44±0.15
12	15	15	40	50	72.54±2.08	68.42±1.9	-25.65±0.23

(*n=3 , SD= Standard deviation)

In the present study fluconazole loaded emulgel were prepared and characterized to find out the effect of oil concentration (X_1), S_{mix} concentration (X_2), and water (X_3) on entrapment efficiency (Y_1), cumulative drug release (Y_2), zeta potential (Y_3) by response surface methodology by selecting Box-Behnken design. The contour plots, 3D response surface plots and cubical representation were generated based on the effect of different factors on responses of fluconazole loaded emulgel results shown in the fig.1 and 2

Entrapment efficiency (Y_1):

Entrapment efficiency was determined for all the prepared fluconazole emulgels. The results of the entrapment efficiency of emulgel were shown in table(3). The entrapment efficiency varied from minimum to maximum of 57.52 ±3.21 % to 87.83±0.28%, indicating this response is affected by selected independent variables. The entrapment efficiency of F5 was found to be minimum and maximum for F2 formulation.

Effect of independent variables on entrapment efficiency (Y_1):

The effect of variables can be explained based on contour, response surface, cubical plots and coefficient of quadratic model shown in the following figures and equation respectively. The mathematical modelling of % entrapment efficiency of optimized emulgel formulation (F2) was represented by following second order quadratic polynomial equation.

$$Y_1 (\%\text{Entrapment efficiency}) = +64.00 - 9.62A + 1.00B + 0.3750C + 1.00AB - 4.25AC + 6.50BC + 6.13A^2 + 0.3750B^2 + 1.12C^2$$

Here; X1= oil concentration (X_1), S_{mix} concentration (X_2), and water (X_3) on entrapment efficiency (Y_1), cumulative drug release (Y_2), zeta potential (Y_3).

In case of Y_1 (Response 1), X_1 represents negative effect indicating increase in oil concentration leads to decrease in % entrapment efficiency, it might be due to higher oil concentration decrease the solubility of drug and increase consistency of emulgel thus leading to decreasing loading and encapsulating efficiency; but X_2, X_3 shows positive effect with increase in the concentration of Smix and water. The % entrapment efficiency increases with increase in surfactants, it might due to reduction in their interfacial surface tension and water decreases the consistency of emulgel, increases entrapment efficiency of drug molecules to permeate and entrap easily.

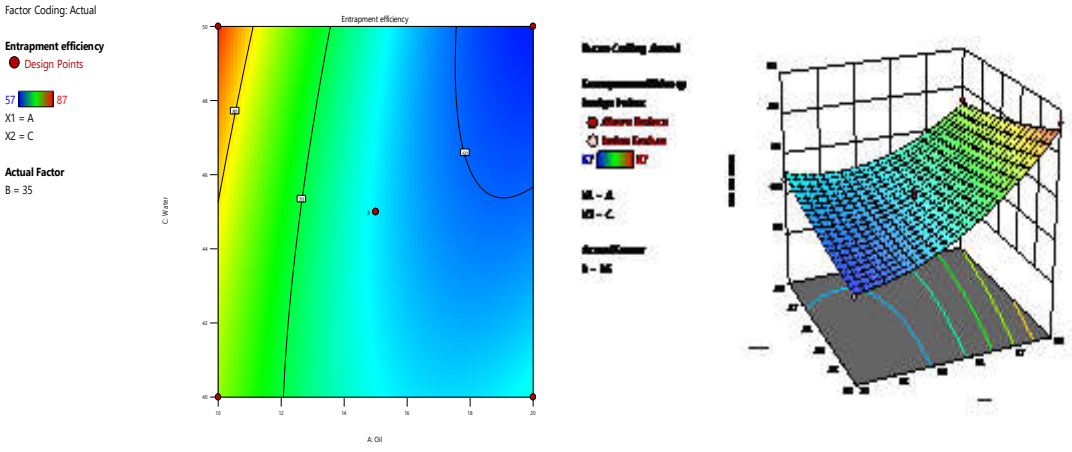

Figure 2. 2DContour plot 3D surface response plots showing the effect of independent variables on entrapment efficiency

Table.4 Statistical ANOVA results of %entrapment efficiency:

Source	Sum of Squares	Df	Mean Square	F-value	p-value	
Model	1135.68	9	126.19	24.99	0.0012	Significant

A-Oil	741.13	1	741.13	146.76	< 0.0001	
B-Smix	8.00	1	8.00	1.58	0.2637	
C-Water	1.12	1	1.12	0.2228	0.6568	
AB	4.00	1	4.00	0.7921	0.4142	
AC	72.25	1	72.25	14.31	0.0129	
BC	169.00	1	169.00	33.47	0.0022	
A^2	138.52	1	138.52	27.43	0.0034	
B^2	0.5192	1	0.5192	0.1028	0.7614	
C^2	4.67	1	4.67	0.9254	0.3803	
Residual	25.25	5	5.05			
Lack of Fit	11.25	3	3.75	0.5357	0.7026	not significant
Pure Error	14.00	2	7.00			
Cor Total	1160.93	14				

***In- Vitro* drug release (Y_2):**

%DR was determined for all the formulations of fluconazole loaded emulgel. The results of the percentage drug release of emulgel were shown in table 3. The *in-vitro* drug release varied from 54.36±3.2 % to 87.54±2.5%, indicating that this response is affected by selected independent variables. The % drug release of F5 was found to be minimum and maximum for F2 formulation.

Effect of variables on *invitro* drug release (Y_2)

The effect of variables can be explained based on contour, response surface, cubical plots and coefficients of quadratic model shown in following figures and equation respectively.The mathematical modelling of % drug release of optimized emulgel formulation (F2) was represented by following second order quadratic polynomial equation.

$$Y_2 \text{ (\% } \textit{In-vitro} \text{ drug release)} = +61.00-10.24A+0.6250B+1.13C-2.00AB-5.00AC+5.25BC+6.38A^2+1.13B^2+1.12C^2$$

Here; X1= oil concentration (X_1), S_{mix} concentration (X_2), and water (X_3) on entrapment efficiency (Y_1), cumulative drug release (Y_2), zeta potential (Y_3)

In case Y_2(Response-2) a positive coefficient of X_2 and X_3 represent increase in % *invitro* drug release with an increase in the concentration of S_{mix} and water negative coefficient of X_1 represents decrease in % *Invitro* drug release with increase in the concentration of oil. The positive effect of S_{mix} and water concentration indicates that, as time increases the layers around drug gets weaken and drug release increases due to selected surfactants which has the effect to reduce interfacial tension between selected composition.The negative effect of oil indicates that, as oil concentration increases the emulgel consistency increase, having high surface area and more time permeate. Increased surface area leads to more contact of drug with dissolution medium leads to decrease in the drug release.

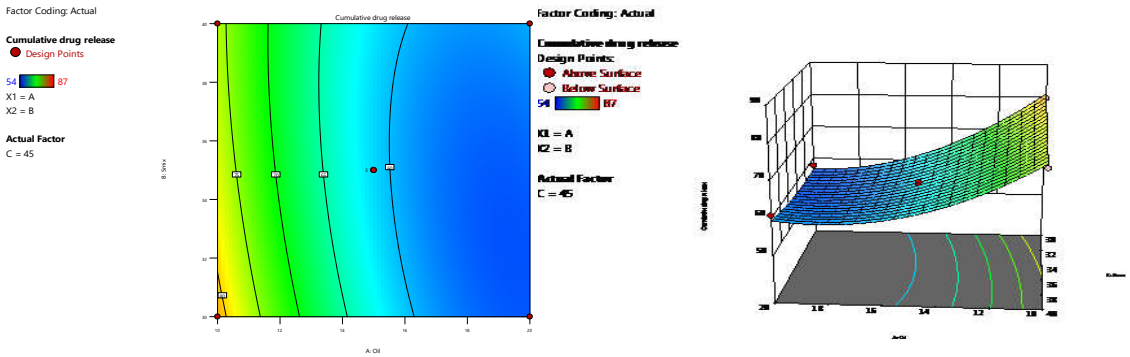

Figure.3 2DContour plot and 3D response surface plots showing the effect of independent variables on cumulative drug release

Table 5 Statistical ANOVA results of % *invitro* drug release

Source	Sum of Squares	Df	Mean Square	F-value	p-value	
Model	1232.85	9	136.98	33.01	0.0006	significant
A-Oil	840.50	1	840.50	202.53	< 0.0001	
B-Smix	3.12	1	3.12	0.7530	0.4252	
C-Water	10.12	1	10.12	2.44	0.1791	
AB	16.00	1	16.00	3.86	0.1068	
AC	100.00	1	100.00	24.10	0.0044	
BC	110.25	1	110.25	26.57	0.0036	
A^2	150.06	1	150.06	36.16	0.0018	
B^2	4.67	1	4.67	1.13	0.3372	
C^2	4.67	1	4.67	1.13	0.3372	
Residual	20.75	5	4.15			
Lack of Fit	18.75	3	6.25	6.25	0.1410	not significant
Pure Error	2.00	2	1.0000			
Cor Total	1253.60	14				

Zeta potential (Y_3):

The zeta potential was determined using Zeta sizer (ZS9, Malvern instrument). Electric field of -120 to 120 mV applies in case of Zeta potential. The optimized formulation for Zeta potential was found to be minimum -16.46±0.28 and maximum -32.05±0.22mV. Based on Oil (x_1) and S_{mix} (x_2), Water (x_3) optimized formulation for Zeta potential was found to be 32.05±0.22mV indicating all the prepared formulations indicating good stability.

The dependent and independent variables were related using mathematical relationships. The mathematical modelling of Zeta potential of optimized emulgel formulation (F2) was represented by following second order quadratic polynomial equation.

$$\mathbf{Y_3\ (\%\ Zeta\ potential) = -22.67 - 1.75A + 1.0000B - 2.25C - 2.25AB + 1.75AC + 1.75BC - 1.04A^2 + 1.96B^2 - 3.04C^2.}$$

Here; X1= oil concentration (X_1), S_{mix} concentration (X_2), and water (X_3) on entrapment efficiency (Y_1), cumulative drug release (Y_2), zeta potential (Y_3)

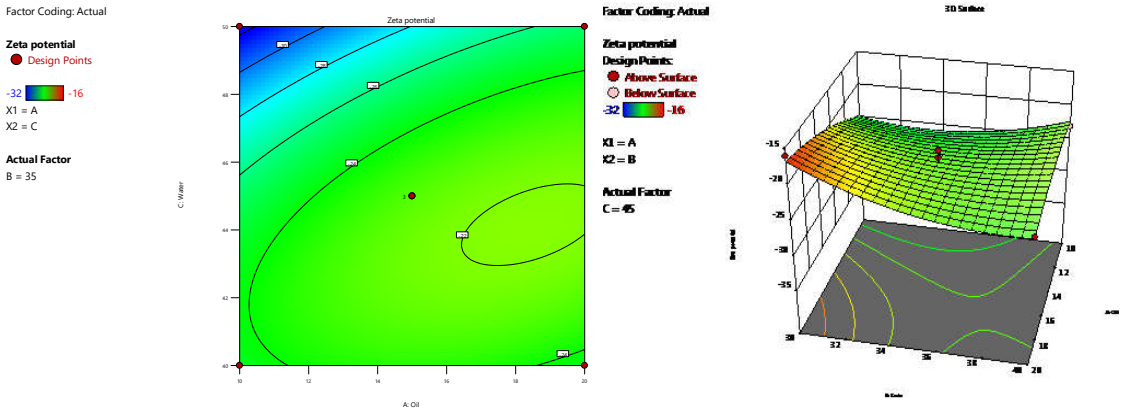

Figure 4. 2DContour plot and 3D surface response plots showing the effect of independent variables on zeta potential

Table 6. Statistical ANOVA for Y_1, Y_2& Y_3 (dependent variables)

Variable	F-value	P- value	R^2 value	Result
Y1(Entrapment efficiency)	24.99	0.0012	0.9783	Significant
Y2 (Cumulative drug release)	33.01	0.0006	0.9834	Significant
Y3 (Zeta potential)	8.62	0.0144	0.9394	Significant

CONCLUSION

A successful attempt was made by incorporating fluconazole into emulgel by selecting Box- Behnken design with 3 centre points it generated total 15 experimental runs, out of which F2 has shown highest entrapment efficiency of 87.83±0.28%, cumulative drug release of 87.54±2.5% and zeta potential of -32.05±0.22%, with optimum pH and good viscosity and spreadability based on this results in which F2 considered as optimized formulation. Through obtained results, it was concluded that; the independent variable plays a crucial role in optimizing formulation. Study data provided strong evidence that the optimized emulgel formulation through Box-Behnken factorial design can be potential optimization technique for loading drug of selected category for topical application.

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