

# Formulation and Evaluation of Liposomes Containing Fluconazole

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*Received: 09th December, 2021; Revised: 25th January, 2022; Accepted: 20th February, 2022; Available Online: 25th March, 2022*

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## ABSTRACT

Liposomal transporters are potential in topical drug delivery. In the current research work, statistical study for the formulation of fluconazole liposomes for topical delivery carried out by factorial design approach. For the preparation of liposomes, phospholipid (DPPC) and cholesterol were taken at 03 different concentrations and preparations were done by using ethanol injection technique. Optimized batch determined by applying experimental design on all nine batches and then optimized batch was characterized for zeta potential, entrapment efficiency, drug release, antimicrobial study and stability studies. From results of analysis it was concluded that vesicle size and entrapment efficiency are dependent on the cholesterol and lipid concentration. Batch F9 shows less particle size and entrapped efficiency was high. Liposome raises the skin penetration compared to marketed formulation. Antifungal activity of liposomal suspension was determined by disk diffusion technique against *Candida albicans*. Diameter of zone of inhibition of liposomal suspension was 24 mm; while zone of fungal growth of marketed gel (Flucos 0.5%) was less than liposomal drug i.e. 20 mm. Batch F9 was analyzed for physical stability studies. Liposomal suspension was stored at 4°C for 1 month. After one month they analyzed for the change in physical appearance, pH, %Entrapment efficiency, particle size of the vesicles, and Zeta potential. There was no change in the physical appearance neither in the formulation consistency of vesicles. Liposomal preparations could be a promising approach in novel drug delivery system.

**Keywords:** Entrapment efficiency, Factorial design, Fluconazole, Liposomes, Particle size analysis.

International Journal of Drug Delivery Technology (2022); DOI: 10.25258/ijddt.12.1.19

**How to cite this article:** Yadav VD, Shinde AD, Jadhav PD. Formulation and Evaluation of Liposomes Containing Fluconazole. International Journal of Drug Delivery Technology. 2022;12(1):98-102.

**Source of support:** Nil.

**Conflict of interest:** None

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## INTRODUCTION

Fungi are dependent microorganisms which create systemic infection to the skin. Apparently dermatologists generally have informed about patients with skin fungal infections. Around 21–26% of human population shows presence of fungal infections to the skin. Usually, fungal infections of skin can be cured with creams, gels and lotions containing antifungal medicaments. Probabilities of drug-drug interactions are irrelevant in the case of topical formulations which are common in orally administered antifungal drugs. Poor skin dispersion of hydrophilic antifungal drugs and high dosing occurrence of conventional antifungal preparations reduces their efficiency against skin fungal pathogens and lipophilic drugs show outstanding skin penetration upon fundamental skin layer, though, their relief rates should be measured to obtain acceptable local concentrations and sustained pharmacological effects.<sup>1</sup> Liposomes are phospholipids based nanocarrier systems in which phospholipid layer use to enclose the aqueous media of drug. This drug delivery described in 1985. Basically, liposomes contain bilayered vesicles having an aqueous media along with one and more phospholipid membranes. Liposomes

have the capacity to deliver hydrophilic as well as lipophilic drug molecules due to their exclusive physical features.<sup>2</sup> Fluconazole is a broad spectrum antifungal agent active against number of fungal infections. Encapsulation of Fluconazole in liposomes provides prolonged drug delivery and minimizes the commonly occurring toxic effects. The objective of present work is to prepare Fluconazole liposomes and study the in vitro drug release and stability studies of prepared liposomes.<sup>3</sup>

## MATERIAL AND METHODS

### Materials

Fluconazole was a gift sample from Ajanta Pharma, Mumbai. Phospholipid (Dipalmitoyl Phosphatidyl choline) was gifted by Torrent Pharmaceuticals Ahmedabad. Cholesterol and ethanol was purchased from Gurudatta chemical suppliers, Satara.

### Methods

*Preparation of Liposomes:*<sup>4</sup> Liposomal formulations were formulated by ethanol injection technique. In ethanol injection technique, the lipidic solution is injected into a spare quantity of aqueous solution. Ethanol mixture was injected slowly to an aqueous solution of the drug that should be encapsulated at

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55–65°C. The removal of ethanol forms liposomes. Liposomes produced are heterogeneous in nature (70–190 nm).

### Evaluation of Fluconazole Liposomal Preparation<sup>5,6</sup>

#### Particle Size Analysis

The size of particle of drug loaded liposomes were defined by (Horiba SZ-100) analyzer, at 28°C. It detects the intensity variations which are caused by particle movement. Each sample was analysed three times.

#### Zeta Potential Measurement

The zeta analysis of Fluconazole liposomal preparation was measured by Nano-particle analyzer (Horiba, SZ-100, Japan) based on the principle by detecting the intensity variations of light which scattered through a particulate dispersed sample. Laser Doppler micro-electrophoresis was done to detect zeta potential.

#### Entrapment Efficiency

Entrapped efficiency of prepared liposomes were analysed by centrifugation method. About 5 mL of liposomal dispersion was added in test tube and centrifuged in cooling centrifuge (REMI-Electronics India) at 15,000 rpm for 45 minutes. After centrifugation the solution was detached and diluted with appropriate solvent. The amount of conc. of drug in solution was determined UV-Visible Spectrophotometrically.

Entrapped efficiency was measured using Equation<sup>1</sup>

$$EE = \frac{W_{\text{initial drug}} - W_{\text{free drug}}}{W_{\text{initial drug}}} \times 100 \quad \dots(1)$$

Where,

$W_{\text{initial drug}}$  = Initial weight of drug in the formulation.

$W_{\text{free drug}}$  = Weight of free drug in the supernatant.

### Application of Experimental Design<sup>5,6</sup>

By using factorial design ( $3^2$ ) the experimental design was obtained as shown in Table 1.

#### Independent Variables

1. Concentration of Lipid ( $X_1$ )
2. Concentration of Cholesterol ( $X_2$ )

#### Response Variables

1. Particle size ( $Y_1$ )
2. Entrapment efficiency ( $Y_2$ )

**Table 1:** Composition of batches of liposomal preparation

Batches	Concentration of lipid ( $X_1$ )	Concentration of cholesterol ( $X_2$ )
F1	50(-)	10(-)
F2	50(-)	20(0)
F3	50(-)	30(+)
F4	60(0)	10(-)
F5	60(0)	20(0)
F6	60(0)	30(+)
F7	70(+)	10(-)
F8	70(+)	20(0)
F9	70(+)	30(+)

### Selection of Optimized Batches

Analysis of design was finding out by using Design Expert V-10 registered software (Stat Ease Inc. Minneapolis). The obtained data were fitted in the software to get polynomial equation, ANOVA, response surface plot (3-D graphs) and contour plots (2-D graphs). Analysis of variance (ANOVA) was used to find irrelevant factors.

### Characterization of Optimized Batch<sup>5,6</sup>

#### In-vitro Drug Diffusion Study

Skin infusion of fluconazole containing liposome was passed out by using Cellulose membrane, employing modified Franz-diffusion cell. The samples were quantified spectrophotometrically at 271 nm.

#### Antimicrobial Activity Susceptibility Test<sup>7-9</sup>

Disk diffusion is adopted technique for the evaluation of antimicrobial activity

#### Stability Studies<sup>10-13</sup>

Liposomal preparations of optimized batch were designated for stability studies for 30 days.

## RESULTS AND DISCUSSION

### Evaluation of Prepared Liposomes (Table 2)

#### Effect of Variables on Size Particle size

Ethanol injection technique produce uni-lamellar and homogeneous population of liposomes.<sup>31,32</sup> The important parameter, which wants to show during liposome preparation its best performance, is the size of vesicle of liposomes. From the number of reports, it was determined that the size of the liposome shows their *in vivo* performance. Particle size decreases as concentration of lipid and cholesterol decreases.

#### Effect of Variables on Entrapment Efficiency

In the present study, the observed entrapment efficiency for each and every batch was in the range of 82.42–86.01%. Entrapment efficiency get increases when concentration of lipid and cholesterol get increases.<sup>14</sup>

### Application of Experimental Design<sup>7</sup>

ANOVA of Particle Size (Figure 1)

**Table 2:** Effect of variables on particle size and entrapment efficiency of Fluconazole Liposomes.

Batches	Conc. of Lipid (%)	Conc. of cholesterol (%)	Particle size (nm)	Entrapment efficiency (%)
F1	1	1	677.9	86.01
F2	1	0	541.9	85.78
F3	1	-1	489.1	85.43
F4	0	1	432	84.6
F5	0	0	380.4	83.89
F6	0	-1	313.6	83.62
F7	-1	1	291.7	83
F8	-1	0	282.8	82.53
F9	-1	-1	163.7	82.42

**Polynomial Equation for Particle size**

$$Y1 = +380.02 + 161.78 * X1 + 73.53 * X2 + 15.20 * X1X2 + 32.5 * X1^2 - 7.02 * X2^2 \quad (2)$$

2D and 3D responses surface plots presented in Figure 2 and 3 shows that concentration of lipid and cholesterol shows positive effect on Particle size.

Entrapment Efficiency by ANOVA (Figure 4)

**Polynomial Equation for Entrapment Efficiency**

$$Y1 = +83.96 + 1.54 * X1 + 0.356 * X2 + 0.00 * X1X2 + 0.1583 * X1^2 + 0.8533 * X2^2 \quad \dots(3)$$

2D and 3D response surface plots presented in Figures 5 and 6

Source	Sum of Squares	df	Mean Square	F-value	p-value	
<b>Model</b>	1.917E+05	5	38349.47	31.51	0.0085	significant
A-Conc. of Lipid	1.570E+05	1	1.570E+05	129.05	0.0015	
B-Conc. of Cholesterol	31566.51	1	31566.51	25.94	0.0146	
AB	924.16	1	924.16	0.7595	0.4476	
A <sup>2</sup>	2114.67	1	2114.67	1.74	0.2790	
B <sup>2</sup>	98.94	1	98.94	0.0813	0.7941	
<b>Residual</b>	3650.62	3	1216.87			
<b>Cor Total</b>	1.954E+05	8				

Figure 1: Particle size by ANOVA

shows that concentration of lipid and cholesterol shows positive effect on Entrapment efficiency.

**Characterization of Optimized Batch<sup>15-17</sup>**

*Particle Size*

Optimized batch i.e., Batch F9 shows least particle size than other batches due to presence of less quantity of lipid and cholesterol, shown in Figure 7.

**Zeta Potential Analysis**

Batch F9 shows negative zeta potential which promises a respectable physical stability of the formulation, avoiding the occurrence of particle aggregation or coalescence. Zeta

Source	Sum of Squares	df	Mean Square	F-value	p-value	
<b>Model</b>	15.16	5	3.03	108.41	0.0014	significant
A-Conc. of Lipid	14.32	1	14.32	512.05	0.0002	
B-Conc. of Cholesterol	0.7633	1	0.7633	27.29	0.0136	
AB	0.0000	1	0.0000	0.0000	1.0000	
A <sup>2</sup>	0.0501	1	0.0501	1.79	0.2730	
B <sup>2</sup>	0.0257	1	0.0257	0.9184	0.4086	
<b>Residual</b>	0.0839	3	0.0280			
<b>Cor Total</b>	15.25	8				

Figure 4: Entrapment efficiency by ANOVA

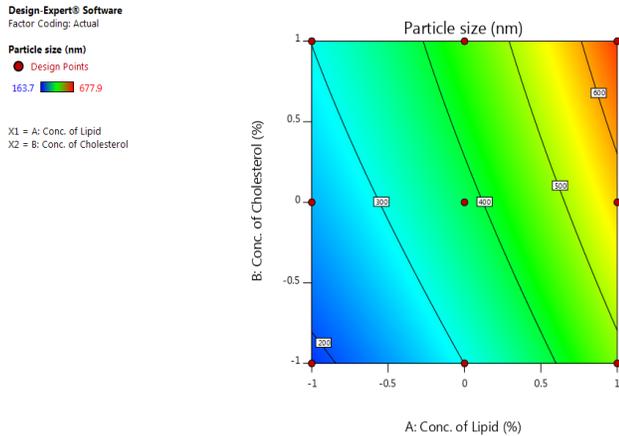


Figure 2: 2D contour graph for particle size for F9 batch

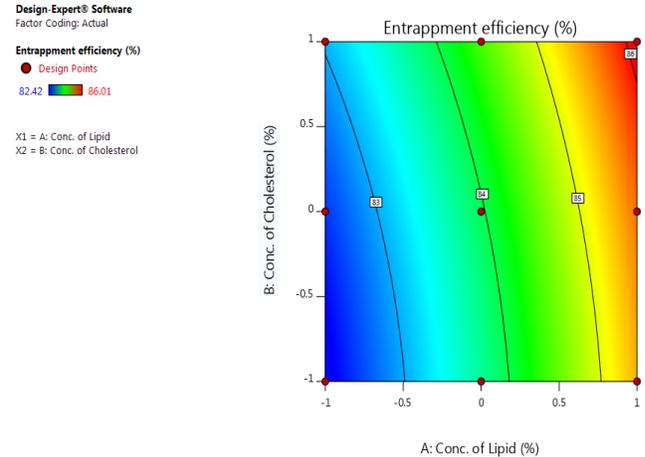


Figure 5: 2D contour graph for entrapment efficiency for F9 batch

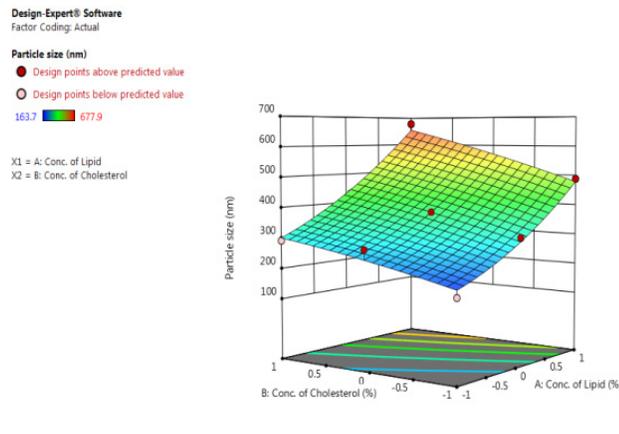


Figure 3: 3D response surface graph for particle size for F9 batch

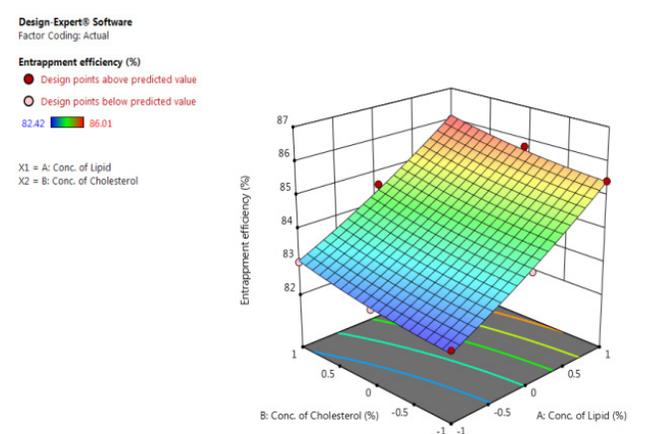


Figure 6: 3D response surface graph for entrapment efficiency of F9 batch

**Cumulant Operations**

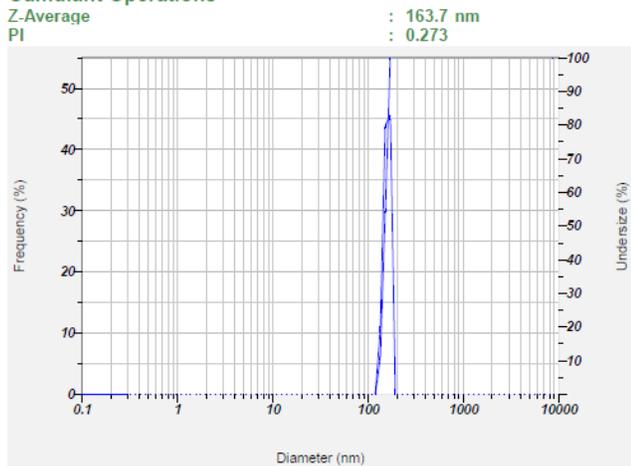


Figure 7: Particle size for optimized batch i.e., F9

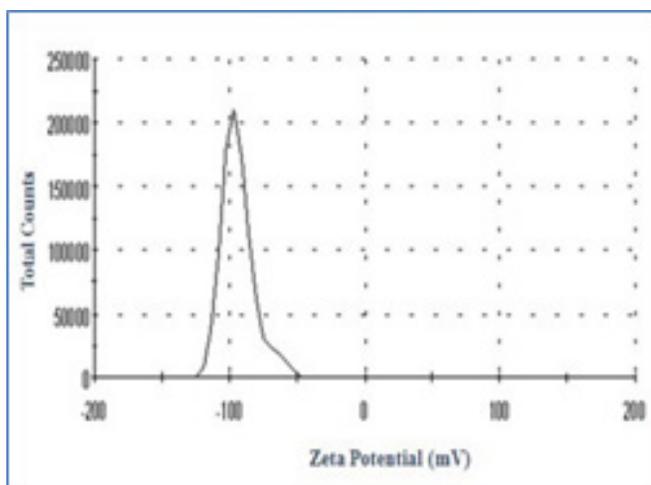


Figure 8: Zeta potential (mV) of optimized batch

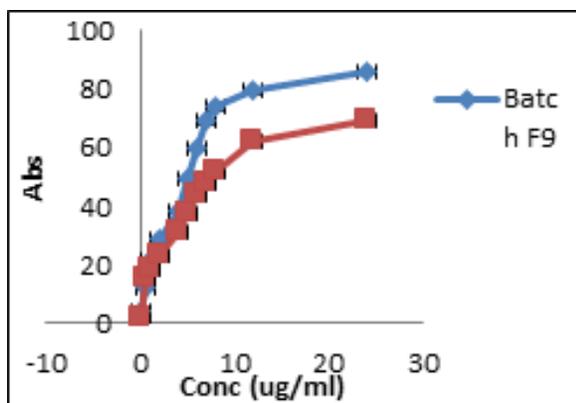


Figure 9: *In-vitro* drug release study in buffer (pH 7.4)

potential for optimized batch i.e. F9 was -98.4 mV as shown in Figure 8.

***In-vitro* Drug Diffusion**

Data of release profiles indicates that developed Liposomal formulation were able to release Fluconazole in controlled approach as the cumulative release percentage of Fluconazole over 24 hours ranged from 2 to 86.1%. Batch 9 shows almost

**Table 3:** Zone of inhibition

Formulations	Diameter of ZI in mm against <i>Candida albicans</i>
Liposomal preparation (Batch F9)	24 mm
Marketed formulation	20 mm
Control	12 mm

**Table 4:** Physical stability study observations initial and after 1 month

Parameters	Initial observation	Final observation (After 1 month)
Physical appearance	Milky white	Milky white
pH	6.9	6.9
Particle size	163.7 nm	164 nm
Entrapment efficiency	82.42%	82.38%
Zeta potential	Negative	Negative
<i>In-vitro</i> drug release	86.10%	86.0%



Figure 10: Zone of inhibition for Batch F9, Marketed formulation and control against *C. albicans*

80% drug release within 24 hours, whereas marketed preparation shows 68% drug release, as shown in Figure 9.

**Antifungal Activity<sup>18,19</sup>**

Table 3 and Figure 10 show antifungal activity of Liposomal preparation, marketed formulation and control on selected fungi. Marketed formulation shows less activity than liposomal preparation.

**Physical Stability Studies**

In the present study, stability was performed on the selected formulation. It was stored at 4–5°C ± 1°C. After 1 month it was showed that there was no any variation. This result showed the high stability and suitability of liposomal preparation for the topical fluconazole delivery (Table 4).

**CONCLUSION**

FLZ-entrapped liposomes were successfully prepared by using ethanol injection method to improve efficacy of drug,

to enhance penetration of drug molecules to the skin and to reduce the possible toxic effects. Study also focus on increasing stability of liposome preparations. The prepared liposomes were found morphological properties. The percentage entrapment efficiency and drug release were optimized after studying the effect of various factors by applying factorial design. Incorporation of phospholipid forms a bilayer and cholesterol increase the rigidity of bilayer. Decreasing amount of phospholipid and cholesterol shows less particle size. Optimized batch further studied for antifungal activity against candida albicans by comparing with marketed formulation. Liposomal drug shows more drug release than marketed gel. It can be concluded from all the above observations that, these studies suggest a better drug delivery through liposome carriers with an added advantage of improved drug release, drug efficacy, and stability and reduce toxicity and having affinity of encapsulating hydrophilic, hydrophobic as well as lipophilic drugs.

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