

Design and Characterization of Fluconazole-Loaded Egg Lecithin Liposomes in Treatment of Fungal Infection

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Abstract: Liposome have emerged as one of the most promising vesicular drug delivery systems due to their biocompatibility, structural versatility, and ability to encapsulate both hydrophilic and lipophilic therapeutic agents. Discovered by Bangham and Horne in 1961, liposomes have since been extensively explored for topical drug delivery, where enhancing dermal penetration while minimizing systemic exposure is a major therapeutic challenge. The liposomal drug delivery system are developed promising and effective drug delivery for antifungal therapy to enhance therapeutic effects of fluconazole and reduce side effects. Fluconazole -loaded liposomes was prepared by thin film hydration method using egg lecithin as the main lipid component. This study focuses on preparation of liposomes from egg lecithin used as phospholipid. The encapsulation of liposomes protects the drug degradation, and sustained release. Liposomal formulations enhanced skin penetration, improved drug retention in the epidermis / dermis layers in skin. The pre-evaluation test such as Calibration curve, FTIR study, Melting point and post-evaluation test pH, Entrapment efficiency and SEM analysis was performed. FL-3 batch was considered as optimized liposomal formulation for topical antifungal therapy of fluconazole.

Keywords: Fungal Infection, Fluconazole, Thin Film Hydration, Liposomes, Egg Lecithin.

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I. INTRODUCTION

The fungal infections are incredibly familiar and can be current as well as systemic. The incidence of superficial fungal infections of skin, hair and nails has been increased in worldwide. It has been predictable that about 40 million people have suffered from fungal infections in developing and under developed nations. The succession of fungal infections can be rapid and serious due to compromising with immune function. Dermatophytes are one of the majority frequent causes of tinea and onychomycosis. Candidal infections are also among the most widespread superficial cutaneous fungal infections. Even, candida can occupy deeper tissues as well as the blood which leads to life-threatening systemic candidiasis, when the immune system is weakened. The fungal infections can be treat by topically applied medicines as well as by oral administrations. However, oral use of medicine is not much significant in treating local fungal infections and also has systemic side effects. Fungal infections of the skin are also known as 'mycoses. They are

universal and generally mild. However, in very sick or otherwise immune suppressed people, fungi can sometimes cause serious disease.(1)

The term liposome" is derived from the Greek words lipos (fat) and soma (body), reflecting their structural nature as lipid-based vesicles.(2) liposomal systems are widely studied in dermatological, cosmetic, vaccine, and pharmaceutical fields.

The ability to fine-tune size, lamellarity, surface charge, lipid composition and surface modifications give formulation scientists significant control over their behaviour. They can carry hydrophilic drugs [in core] and hydrophobic drugs [in bilayer], Liposomes are spherical vesicles composed of one or more phospholipid bilayers that enclose an aqueous core. Their structural similarity to biological membranes, physicochemical properties, and ability to encapsulate both hydrophilic and hydrophobic agents make them exceptionally versatile carriers. Over the past few decades, liposomal

formulations have exceptionally versatile carriers. Structurally, liposomes are formed from amphiphilic lipids that self-assemble in aqueous environments into bilayer vesicles. The number of bilayers and size, liposomes are classified as small unilamellar vesicles (SUVs), large unilamellar vesicles (LUVs), and multilamellar vesicles

(MLVs).(3) Unlike plant-derived soy lecithin, egg yolk phospholipids have a composition that is closely related to human cell membranes, providing better physiological tolerance and skin-penetration effects in topical applications.(4)

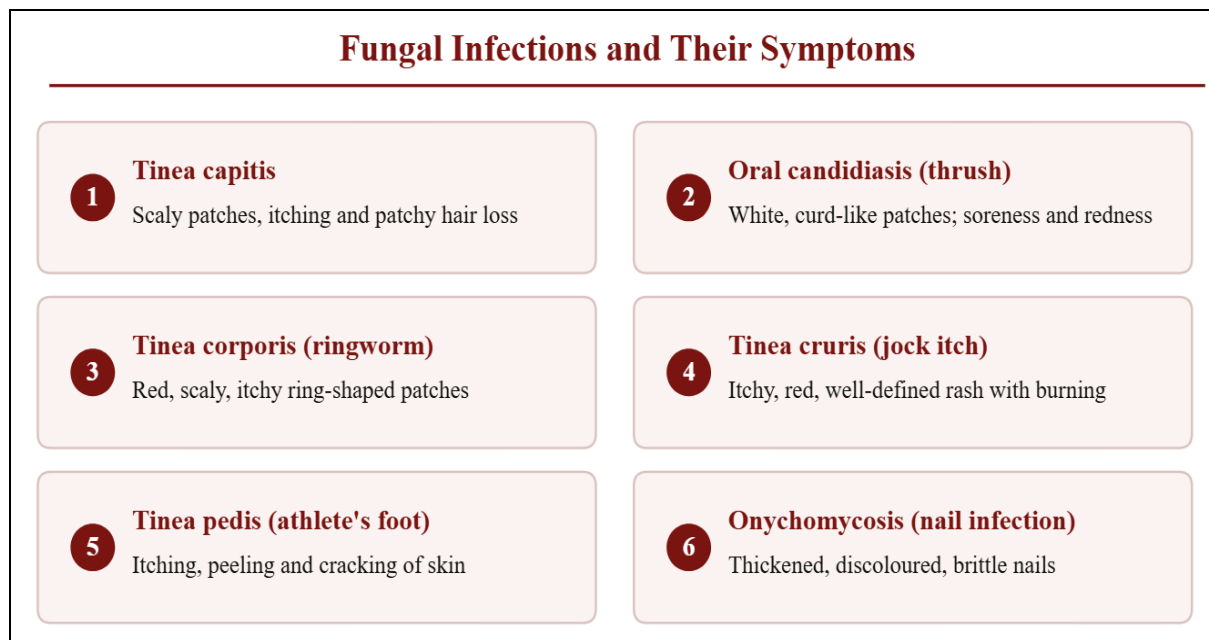


Fig 1 Types of Fungal Infection

➤ Mechanism Action of Fluconazole:

Fluconazole is a very selective inhibitor of fungal cytochrome P450 dependent enzyme lanosterol 14- α -demethylase. The enzyme convert lanosterol to ergosterol, which is necessary for fungal cell wall synthesis. The free nitrogen atom located on the azole ring of fluconazole binds with a single iron atom located in the heme group of lanosterol 14- α -demethylase. This prevents oxygen activation and, as a result, inhibits the demethylation of lanosterol, halting the process of ergosterol biosynthesis. Methylated sterols are then found to accumulate in the fungal cellular membrane, leading to an arrest of fungal growth. These accumulated sterols negatively affect the structure and function of the fungal cell plasma membrane. Maintaining the Integrity of the Specifications. (5)

II. MATERIAL AND METHODS

Fluconazole was procured from Dhamtec Pharma and Consultants, Navi Mumbai, Maharashtra, India. Cholesterol, Tween-80, Methanol were procured from Loba Chemie Pvt. Ltd., Mumbai, Maharashtra, India. egg lecithin was prepared by isolation from Eggs yolk All chemicals and reagents used in the study were of analytical grade.

➤ Preparation Method of Egg Lecithin:

Break the egg carefully and separate the egg yolk from egg white, remove the outer membrane of yolk gently, spread yolk in a glass and dry at 40-45°C in hot air oven for 24-48 hours. After complete drying, grind the yolk into fine powder

and Adding the acetone in to the powder stir for 30-60 minutes, filter the mixture and repeat the process 3 times to remove fats and cholesterol, Dry the residue. Adding the ethanol or chloroform into the dried residue stir for 1 hour and filter it. Evaporate solvent using rotary evaporator or water bath at low temperature. Then semi solid yellowish lecithin, was prepared and store at airtight container at 4°C.(6)

➤ Preparation of Egg Lecithin Liposomes:

The liposomal suspension (50 ml) was prepared by the thin film hydration method, a widely used technique for the formation of vesicular drug delivery systems. Weighed quantities of phospholipid (phosphatidylcholine) and cholesterol in ratio of 1:1,1:2, and 2:1 along with a fluconazole drug. Where they are dissolved in suitable organic solvent methanol. The clear solution was transferred into a clean and dry round bottom flask. This flask was attached to a rotary evaporator, and the organic solvents were evaporated under reduce pressure at a controlled temperature of approximately 40-45°C with a rotational speed of about 100-150 rpm. As the solvent evaporated, a thin uniform lipid film was formed on the inner wall of the flask. (7)

The dried lipid film was hydrated with 50 ml of phosphate buffer of PH 7.4 maintained at a temperature above the phase transition temperature of lipid around 40 °C. The hydration process was carried out by gentle rotation of the flask for 30-60 minutes, allowing the lipid film to swell, and form multilamellar vesicles (MLVs), then the milky

suspension was formed. For size reduction of more vesicular liposomes was sonicate in bath sonication for 15-30 minutes

and probe sonication for 5-10 minutes. Small unilamellar vesicles (SUVs) was formed after sonication.(8)

Table 1 Formulation Table of Fluconazole-Loaded Egg Lecithin Liposomes

Batch	Ratio	Phospholipid	Cholesterol
FL-1	1:1	250 mg	250 mg
FL-2	1:2	250 mg	500 mg
FL-3	2:1	500 mg	250 mg

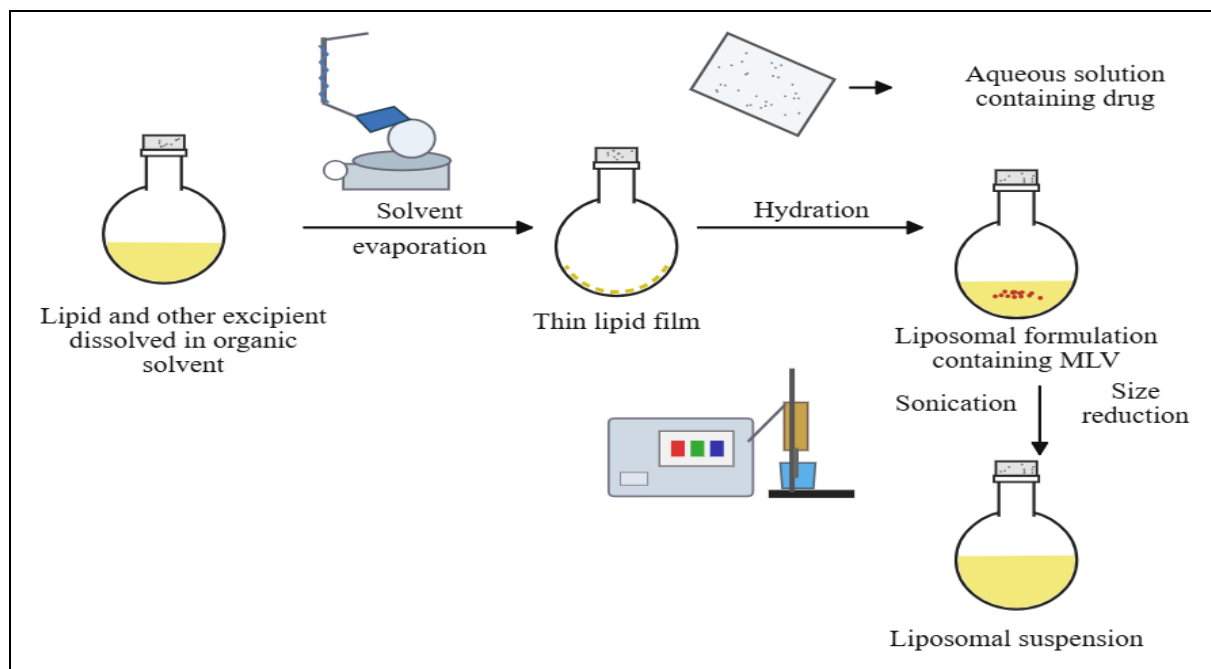


Fig 2 Liposome Preparation Method

III. RESULT AND DISCUSSION

➤ Pre- Characterization Test:

• Organoleptic Properties :

Table 2 Organoleptic Properties

Sr. No	Parameters	Description
1]	Appearance	Powder
2]	Colour	White
3]	Odour	Odourless
4]	Taste	Slightly bitter
5]	Physical state	Solid powder

• Melting Point:

Melting point of fluconazole is carried out by capillary tube method found to be 160 °C

• Calibration Curve:

Table 3 Concentration & Absorbance

Sr. No	Concentration (µg/ml)	Absorbance
1]	0	0000
2]	0.2	0.0044
3]	0.4	0.0082
4]	0.6	0.0121
5]	0.8	0.0163

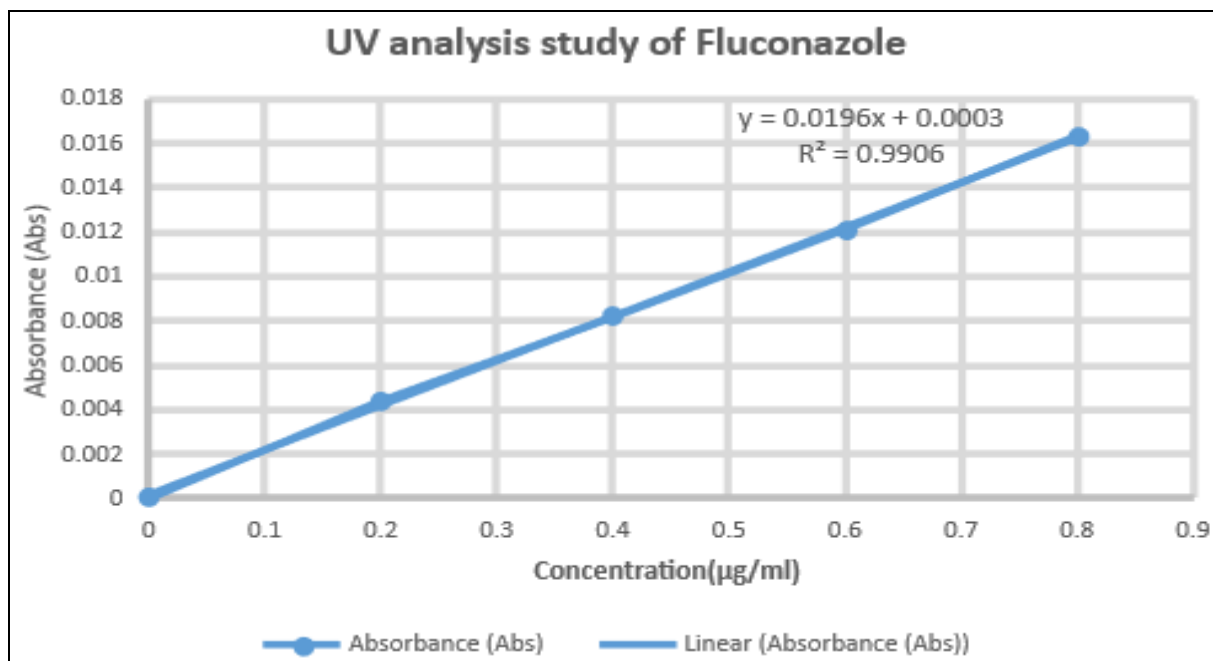


Fig 3 Calibration Curve of Fluconazole

➤ *FTIR Analysis of Fluconazole:*

Fluconazole is a triazole antifungal drug commonly analyzed by FTIR (Fourier Transform Infrared Spectroscopy) to identify its characteristics functional groups and confirm drug purity, compatibility and structure. The chemical structure of fluconazole contain following functional groups.

- Two triazole rings
- Difluorophenyl ring
- Hydroxyl (-OH) group
- Carbon- fluorine (C-F) bond

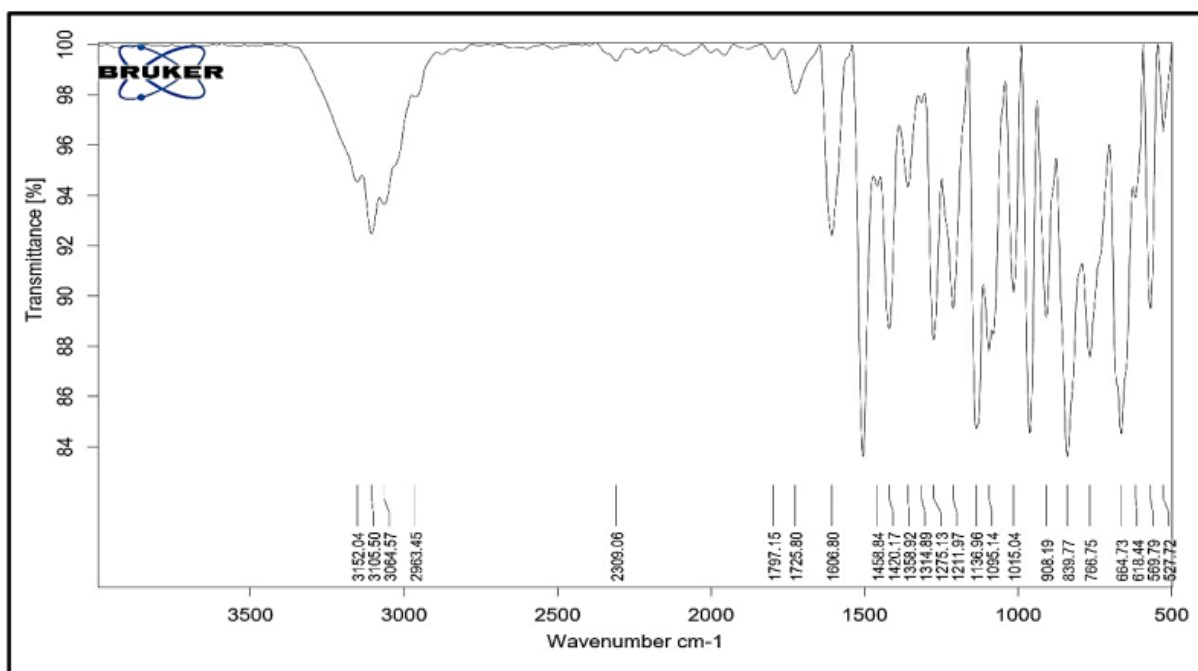


Fig 4 FTIR Spectrum of Fluconazole

Table 4 FTIR Study of Fluconazole

Sr. No	Functional group	Observed Wavelength (cm-1)
1]	O-H group	3152.04, 3105.50, 3064.57
2]	C-H group	2963.45
3]	C=N group	1606.80
4]	C=C group	1458.92, 1420.17

5]	C-N group	1358.92, 1314.89
6]	C-O group	1275.13, 1211.97
7]	C-F group	1136.96, 1095.14
8]	C-Cl group	664.73, 618.44
9]	Skeletal vibrations	569.79, 527.72

➤ *FTIR of Fluconazole + Egg Lecithin:*

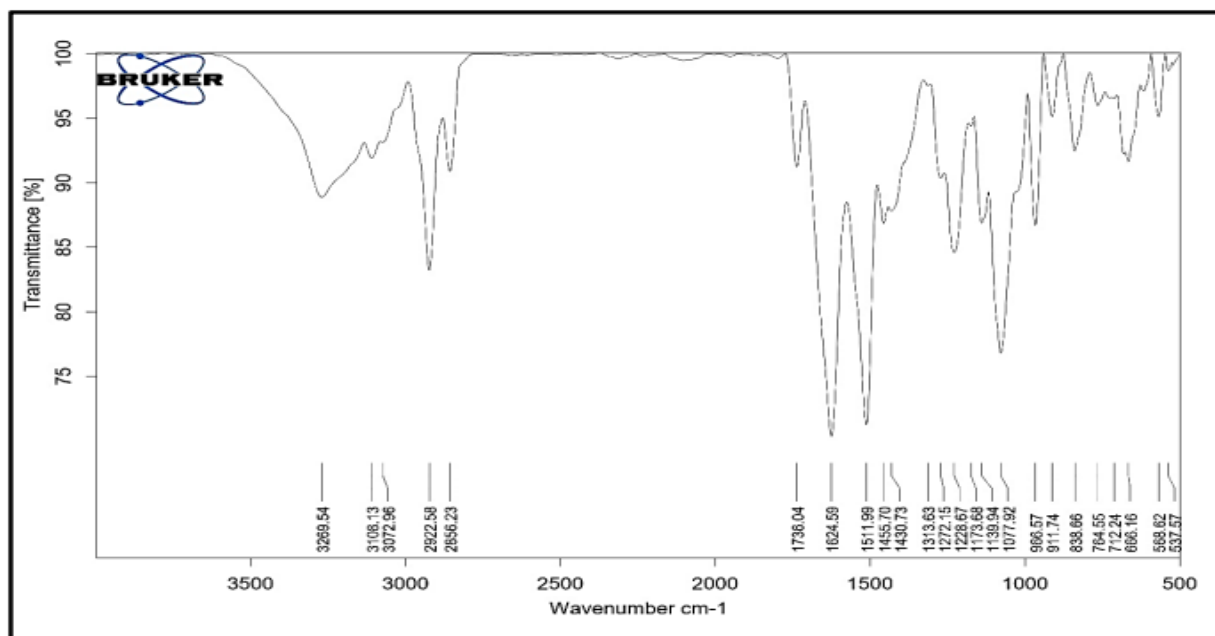


Fig 5 FTIR Spectrum of Fluconazole + Egg Lecithin

Table 5 FTIR Study of Fluconazole + Egg Lecithin

Sr. No	Functional group	Observed Wavelength (cm-1)
1]	O-H group	3269.54
2]	Triazole and aromatic ring	3108.13, 3072.96
3]	CH ₂ and CH ₃ group	2922.58, 2856.23
4]	C=O Stretching of lecithin	1736.04
5]	Phospholipid phosphate group of lecithin	1228.67
6]	C-O-C Ester linkage in lecithin	1173.68, 1139.94
7]	C-F group	966.57, 911.74

The FTIR spectrum of fluconazole with egg lecithin showed characteristics absorption peaks corresponding to both drug and phospholipid components. The major peaks observed at 3269.54 cm⁻¹, 1736.04 cm⁻¹, 1228.67 cm⁻¹ and 1077.92 cm⁻¹ confirmed the presence of hydroxyl, aromatic, ester carbonyl, phosphate and phospholipid functional groups respectively.

➤ *Post- Characterization Test:*

• *Entrapment Efficiency:*

Fluconazole associated with liposome was separated from untrapped drug using centrifugation method .

Liposomes were centrifuged at 20000 rpm for 1 h at o 11 controlled temperature of 4°C Supernatant containing untrapped fluconazole was withdrawn and measured UV spectrophotometrically at 260 nm against phosphate buffer saline (pH 7.4). The amount of fluconazole entrapped in liposome was determined as follow.(9)

$$\text{Entrapment Efficiency (EE \%)} = \frac{(\text{Total drug} - \text{Free untrapped drug}) \times 100}{[\text{Total drug}]}$$

Table 6 Entrapment Efficiency of Fluconazole-Loaded Egg Lecithin Liposomes.

Batch no.	EE%
FL-1	96.50%
FL-2	91.32%
FL-3	98.50%

• *SEM Analysis:*

- ✓ The SEM analysis of the fluconazole-loaded liposomes showed the formation of nearly spherical and uniformly distributed vesicles.

- ✓ The particle size was observed in the nanometer range, approximately 51-132 nm, indicating successful preparation of nanosized liposomes.
- ✓ The SEM results confirm smooth surface morphology and successful formation of fluconazole-loaded liposomal particles suitable for topical drug delivery.(10)

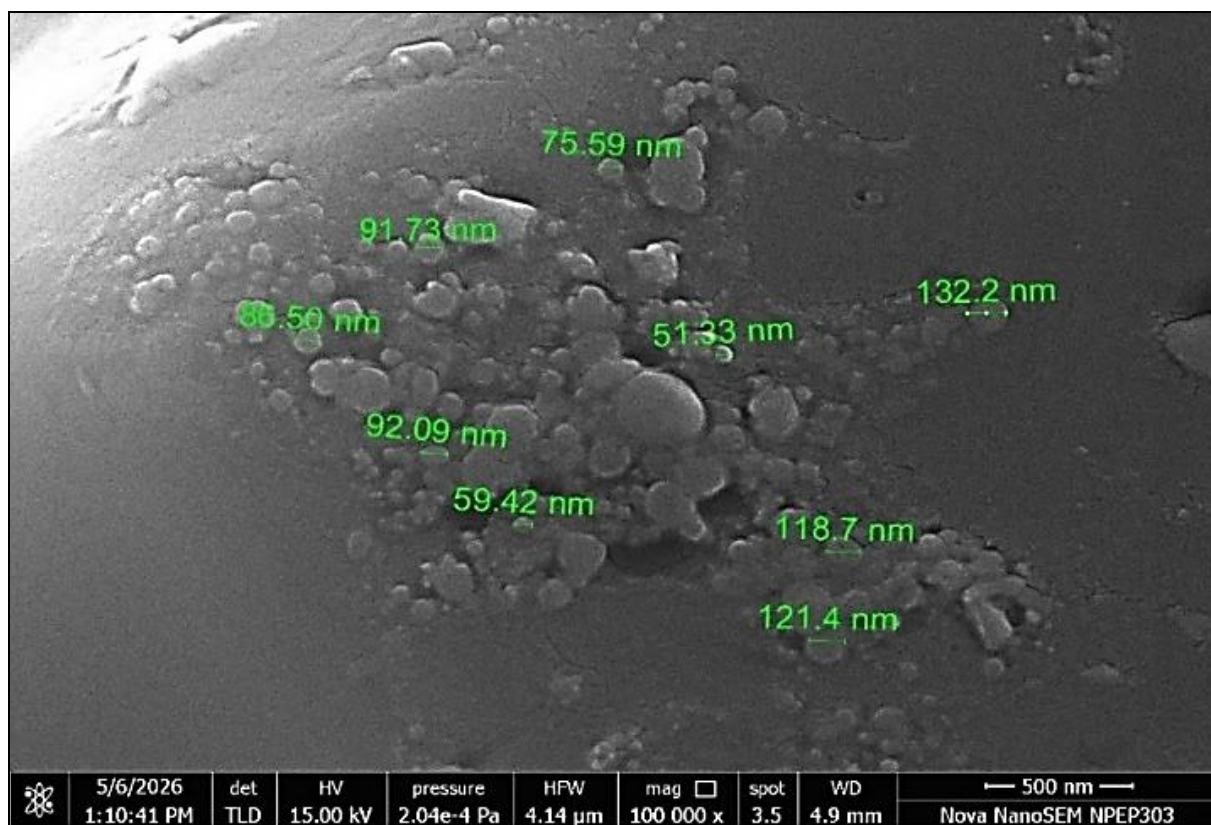


Fig 6 SEM Analysis of Fluconazole-Loaded Liposomes

• *In Vitro Permeation Test of Fluconazole-Loaded Liposomal Gel:*

The permeation of fluconazole from the liposomal gel was studied using a Franz diffusion cell (diffusion area about 1.77 cm², receptor volume about 12 mL). Excised skin, or a synthetic membrane such as Strat-M, was soaked in phosphate buffer (pH 7.4) for 30 min and fixed between the donor and receptor compartments with the stratum corneum facing up. If animal or human skin is used, state the ethics approval in your paper. The receptor compartment was filled with phosphate buffer (pH 7.4). It was kept at 37 ± 0.5 °C and stirred at 100 rpm with a magnetic bead. After 30 min of equilibration, gel equivalent to 5 mg of fluconazole was spread evenly on the skin in the donor compartment, which was then covered to prevent evaporation.

At set times (0,5,10,15,30), 1 mL of receptor fluid was withdrawn and replaced with the same volume of fresh buffer to maintain sink conditions. The samples were filtered and analyzed by UV spectrophotometry at 261 nm. Each formulation was tested in triplicate, and a plain fluconazole gel was run as the control.

The cumulative amount permeated per unit area (μg/cm²) was plotted against time. Steady-state flux (J_{ss}) was taken from the slope of the linear portion. The permeability coefficient was calculated as $K_p = J_{ss} / C$, where C is the drug concentration in the donor. Results are given as mean ± SD.(11,12)

Table 7 In Vitro Test for Liposomal Gel

Sr. No	Time(min)	FL-1	FL-2	FL-3
1]	0	0	0	0
2]	5	11.34	9.68	21.56
3]	10	23.23	16.74	39.02
4]	15	31.77	25.96	53.19
5]	30	47.01	31.27	75.75

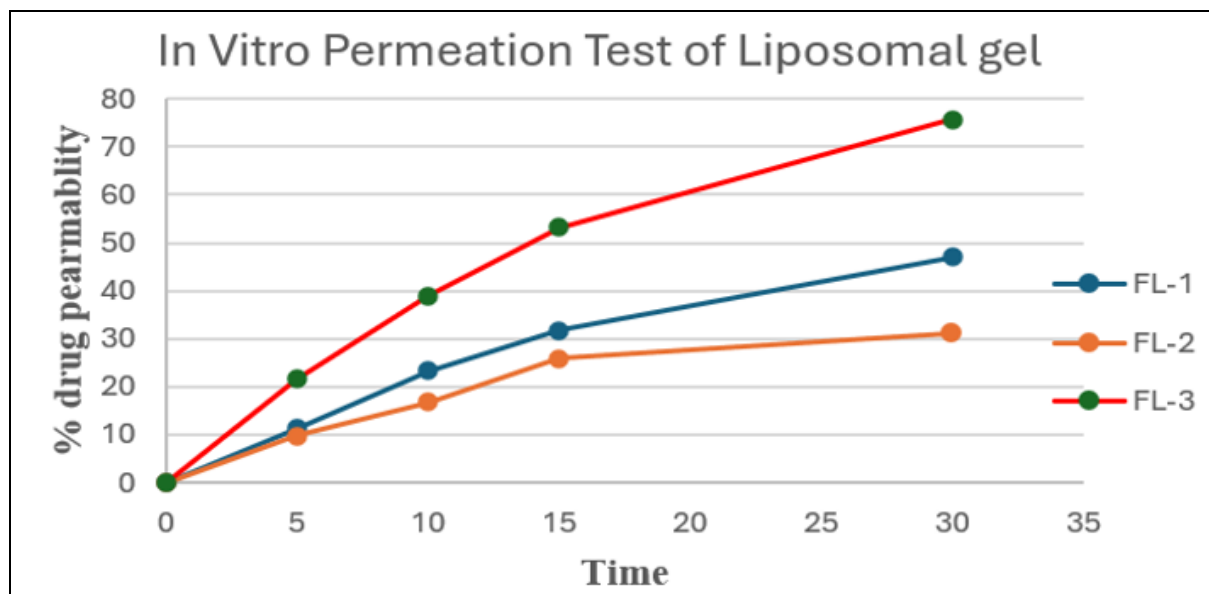


Fig 7 In Vitro Permeation Test for Liposomal Gel

The in vitro permeation study confirmed that the fluconazole-loaded liposomal gel can effectively sustain the release of fluconazole, which may improve therapeutic effectiveness and reduce dosing frequency. FL-3 batch showed controlled or sustained release of fluconazole.

IV. DISCUSSION

The developed liposomal formulation of fluconazole has demonstrated promising properties for the topical delivery of the antifungal drug. The calibration curve was prepared to determine the absorbance of fluconazole at different concentrations based on the evaluated formulation parameters like . FTIR analysis was performed to identify functional groups in the sample and to evaluate the possible interactions between the drug and the excipients. The pH of the liposomal suspension was within acceptable limits for topical application. Entrapment efficiency study confirmed the successful encapsulation of fluconazole in the liposomes. SEM analysis was used to investigate the shape and surface morphology of the synthesized liposomes. Based on the evaluation results, batch FL-3 was chosen as the optimized formulation due to its higher entrapment efficiency and sustained drug-release profile. In general, the results show that the optimized fluconazole-loaded liposomes may be useful as topical antifungal delivery systems for drugs, but further studies are required to evaluate their stability, skin permeation and antifungal activity.

V. CONCLUSION

In this work, fluconazole-loaded liposomes were prepared by the thin film hydration method using egg lecithin and cholesterol in three ratios (FL-1, FL-2 and FL-3), with the aim of developing a topical carrier for fungal skin infections. The melting point (160 °C) and FTIR spectra of the drug alone and with egg lecithin showed that fluconazole was identified correctly and that its characteristic peaks remained visible alongside those of the

lipid, so no major drug-lipid incompatibility was seen. The pH of all three batches was between 5.75 and 6.57, which suits application on the skin. Entrapment efficiency was high in every batch (91.32% to 98.50%). SEM images showed smooth, nearly spherical vesicles of about 51 to 132 nm. Of the three batches, FL-3 (phospholipid to cholesterol 2:1) did best, with the highest pH (6.57), the highest entrapment (98.50%) and the greatest drug release in the permeation study (75.75% at 30 minutes, against 47.01% for FL-1 and 31.27% for FL-2). A higher lecithin proportion therefore seems to high drug loading and release in this formulation. FL-3 is taken as the optimized batch and appears to be a promising liposomal carrier for topical fluconazole.

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