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DEVELOPMENT AND *IN VITRO* CHARACTERIZATION OF FLUCONAZOLE INCAPSULATED NIOSOMES

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Abstract

The current study focuses on the development and in vitro characterization of fluconazole encapsulated niosomes to improve drug delivery and therapeutic efficacy. Niosomes, which are non-ionic surfactant vesicles, offer significant advantages in enhancing the bioavailability of drugs with poor water solubility. Fluconazole, an antifungal agent with limited water solubility, was encapsulated in niosomes using the thin-film hydration method. The niosomes were characterized through particle size analysis, zeta potential measurement, encapsulation efficiency, in vitro drug release, and stability studies. The results revealed that the niosomes had a uniform particle size distribution, high encapsulation efficiency, and sustained drug release profile. Stability studies indicated that the niosomes remained stable under various storage conditions. This study demonstrates that fluconazole encapsulated niosomes could potentially enhance the bioavailability and therapeutic performance of fluconazole, paving the way for further in vivo studies and clinical applications.

Keywords: Fluconazole, Niosomes, Ether injection method, Site-specific delivery, Drug delivery system

Introduction

Fluconazole, a triazole antifungal agent, is widely recognized for its efficacy in treating a variety of fungal infections, including those caused by Candida species and Cryptococcus neoformans[1,2]. It works by inhibiting the fungal cytochrome P450 enzyme 14α-demethylase, crucial for converting lanosterol to ergosterol, an essential component of the fungal cell membrane[3]. This inhibition disrupts the integrity of the fungal cell membrane, leading to cell death. Fluconazole's clinical applications are extensive, encompassing the treatment of oropharyngeal and esophageal candidiasis, systemic Candida infections, cryptococcal meningitis, and prophylaxis against fungal infections in immunocompromised patients[4,5]. Despite its widespread use, fluconazole presents several challenges that limit its therapeutic potential. One of the primary issues is its poor water solubility, which translates to low bioavailability. When administered orally, fluconazole's absorption is incomplete and variable, often requiring high doses to achieve therapeutic plasma concentrations[6,7]. This can lead to increased risks of adverse effects, such as gastrointestinal disturbances and hepatotoxicity. Additionally, the emergence of drug-resistant fungal strains necessitates the development of more effective delivery systems to enhance fluconazole's antifungal activity and minimize resistance[8,9]. Niosomes, non-ionic surfactant-based vesicles, have garnered significant attention as a promising drug delivery system. Structurally similar to liposomes, niosomes are composed of non-ionic surfactants and cholesterol, forming bilayer vesicles capable of encapsulating both hydrophilic and lipophilic drugs[10]. The versatility and biocompatibility of niosomes make them an attractive option for enhancing the delivery and bioavailability of therapeutic agents. Niosomes offer several advantages over conventional drug delivery systems[11]. Firstly, their ability to encapsulate drugs in both aqueous and lipid environments allows for the protection of the drug from degradation and enhances its stability. Secondly, niosomes can improve the solubility and bioavailability of poorly water-soluble drugs, such as fluconazole, by encapsulating them within their bilayer structure[12]. This can lead to a reduction in the required dose and associated side effects. Furthermore, the surfactants used in niosome formulation can modulate the drug release profile, allowing for sustained and controlled drug release[13]. This property is particularly beneficial for chronic conditions requiring long-term drug administration. Niosomes have been explored for a wide range of applications in drug delivery, including the delivery of anticancer agents, antibiotics, vaccines, and genetic material[14,15]. Their ability to enhance the bioavailability and therapeutic efficacy of drugs has been demonstrated in various preclinical and clinical studies[16]. For instance, niosomal formulations of anticancer drugs have shown improved targeting to tumor cells, reducing the side effects associated with conventional chemotherapy. Similarly, niosomes have been investigated for the delivery of antibiotics, demonstrating enhanced antibacterial activity and reduced resistance[17,18]. This study focuses on the development and in vitro characterization of fluconazole encapsulated niosomes to enhance its delivery and therapeutic performance.

Materials and Methods

Materials

Niosomal suspensions were novel drug carriers used for the entrapment of hydrophilic, lipophilic, or both types of drugs. The vesicles could be unilamellar or multilamellar and could deliver the drug transdermally, orally, or intravenously. These vesicular carriers offered better stability over conventional dosage forms and could overcome drug-related side effects. Materials used included diethyl ether, cholesterol, fluconazole, phosphate buffer pH 7.4, and Span 60.

Methods

Preformulation Studies

Preformulation investigations involved analyzing the physical and chemical characteristics of the pharmacological ingredient, both by itself and in combination with excipients. Preformulation applied biopharmaceutical principles to the physical properties of a drug substance to aid in the creation of dosage forms. Prior research guided this process to develop stable and bioavailable formulations.

Physical Identification

The obtained sample of fluconazole was identified for its physical appearance through visual inspection. Characteristics such as color, odor, and physical appearance were carefully noted.

Solubility Analysis

Solubility was defined as the maximum concentration of solute dissolved in a given solvent under standard conditions of temperature, pressure, and pH. For the solubility analysis, 10 mg of fluconazole was dissolved in 10 ml of solvent followed by shaking. Solvents were then checked using ultraviolet spectroscopy to identify which solvent had the maximum solubility for fluconazole.

UV Spectrophotometry of Fluconazole

UV-visible spectrophotometry was commonly used in the pharmaceutical industry for analysis. It measured the amount of UV or visible radiation absorbed by a substance in solution. UV-VIS spectrophotometers measured the intensity ratio between two beams of light in the UV-VIS range. An ultraviolet-visible spectrophotometer was used to measure absorbance at a specific wavelength to determine the concentration of fluconazole in a solution.

Preparation of the Stock Solution

A standard stock solution was prepared by dissolving 100 mg of fluconazole in 100 ml of solvent. Then, 1 ml of this stock solution was taken and diluted in a 100 ml volumetric flask to create a working stock solution. Further dilutions were prepared in concentrations of 2-10 µg/ml.

Angle of Repose

The angle of repose referred to the maximum angle formed between a pile of powder and a horizontal surface. For most pharmaceutical drug powders, the angle of repose ranged between 25-45°. The formula for calculating the angle of repose was:

$$\tan(\theta) = h/r$$

where h was the height of the pile and r was the radius of the pile. To measure the angle of repose, a fixed funnel was placed such that its tip lay approximately 2 cm above the surface. Powder was poured through the funnel until it reached the tip. The height and radius of the resulting pile were then measured.

Melting Point

The melting point was the temperature at which a substance transitioned from a solid to a liquid. The melting point of pure crystalline solids was distinct and precise. Melting point determination was required for assessing product purity. To determine the melting point, a capillary tube was

filled with the sample, and the powder was pushed to the bottom by gently tapping the tube against a hard surface. A sample height of around 2-3 mm was recommended. The filled capillary tube was placed into a melting point apparatus, and the temperature at which the drug started to melt was recorded[19].

FT-IR Spectroscopy

Fourier Transform Infrared (FT-IR) Spectroscopy was used for the structural analysis of the drug. FT-IR used infrared radiation to scan the sample. An FT-IR spectrophotometer contained a single beam assembly with an interferometer located between the source and the sample. The KBr disc method allowed for the quantitative measurement of fluconazole by IR spectroscopy. Dehydrocholic acid was used as an internal standard. Absorption bands at 960 and 675 cm-1 for fluconazole and at 1705 cm-1 for dehydrocholic acid were selected. Beer's law requirements were met by the concentration range in the KBr disc, which was between 0.4 and 1.6% w/w[20].

Preparation of Niosomal Suspension

The niosomal suspension was prepared using the ether injection method. Surfactant, cholesterol, fluconazole, and chloroform were accurately weighed and mixed. This mixture was slowly dropped into the aqueous phase (phosphate buffer pH 7.4) maintained at 60-70 °C using a needle and syringe. Initially, span 60 and cholesterol were accurately weighed and dissolved in an appropriate amount of fluconazole. Fluconazole was then dissolved in this lipidic solution. The solution was then taken into a 16 or 14-gauge needle and injected into 20 ml of aqueous phase in a beaker maintained at constant temperature. The vaporization of chloroform at constant temperature led to the formation of niosomes[21].

Table 1: formulation table

Run	Factor A (Ratio)	Factor B (Surfactant)	Factor C (Hydration Time)
1	1:1	Span 60	30
2	1:1	Span 60	60
3	1:1	Tween 60	30
4	1:1	Tween 60	60
5	2:1	Span 60	30
6	2:1	Span 60	60
7	2:1	Tween 60	30
8	2:1	Tween 60	60

Evaluation of Niosomal Suspension

Following are the parameters used for the evaluation of niosomes:

Viscosity Studies

The viscosity of the prepared niosomal suspension was analyzed using a Brookfield viscometer.

pH Determination

The pH of the prepared formulation was analyzed using a digital pH meter. One gram of the prepared formulation was dissolved in 100 ml of distilled water. The pH was then determined by first calibrating the pH meter with pH 4 and pH 7 buffers[21].

FT-IR Spectroscopy

FT-IR spectroscopy was conducted for compatibility studies between the drug and the excipient. The sample was submitted to the Sophisticated Analytical Instrumentation Facility at Panjab University, Chandigarh, to carry out FT-IR spectroscopy.

Drug Content

A niosomal suspension equivalent to 5 mg of fluconazole was taken into a 10 ml volumetric flask, and the volume was made up with methanol to disrupt the vesicles. The sample was sonicated for 40 minutes. One ml of the sample was withdrawn, and the volume was made up to 50 ml in a volumetric flask. The concentration of the drug was then analyzed using a UV spectrophotometer[22].

Drug Entrapment Efficiency

The drug entrapment efficiency was measured by separating the unentrapped drug from the prepared dispersion by centrifugation. The dispersion was centrifuged at 17,000 rpm for approximately 30 minutes. The amounts of unentrapped and entrapped drug were estimated using a UV spectrophotometer[23].

Percentage Drug Entrapment=(Amount of drug taken−Amount of unentrapped drug/Total amount of drug taken)×100

Determination of Particle Size

Vesicle size was determined using a Beckman Coulter zeta sizer.

In Vitro Drug Release

The in vitro release studies were conducted using a Franz diffusion cell. The prepared solution was applied to the surface of a cellophane membrane, which was then placed between donor and receptor compartments. A phosphate buffer of pH 7.4 was used as the dissolution medium. The temperature was maintained at 37°C using a circulating water jacket. The assembly was placed on a magnetic stirrer, and the solution was agitated. At constant time intervals, a 5 ml sample was withdrawn, and the same amount of phosphate buffer of pH 7.4 was added. The sample was analyzed spectrophotometrically at 260 nm[24].

Transmission Electron Microscopy (TEM)

The sample was submitted to the Sophisticated Analytical Instrumentation Facility/Central Instrumentation Laboratory at Panjab University, Chandigarh, for TEM analysis.

Stability Studies

The stability study aimed to identify the effect of various environmental factors on the formulation. The niosomal formulation was stored at room temperature and refrigerator temperature for a period of 3 months. Samples were observed monthly for any changes in the physical parameters of the formulation[25].

Results And Discussions

Preformulation studies

Physical appearance: The physical appearance of the drug was done by checking its physical identification i.e. colour, odor and appearance of the fluconazole drug. The results so obtained are discussed in below table:

Table 2: physical appearance of fluconazole

Sr. no.	Test	Specification	Observation
1	**Colour**	White	White
2	**Odour**	Odourless	Odourless
3	**Appearance**	Crystalline	Crystalline

Detrmination of solubility: The solubility of fluconazole was observed and obtained result gives highest absorbance for fluconazole in methanol. The solubility of fluconazole in methanol was found to be maximum with absorbance of 1.192. The solubility of fluconazole into different solvents was discussed in the table given below:

Table 3: solubility analysis of fluconazole

Sr.no	Solvent	Solubility
1	Chloroform	Soluble
2	Methanol	Freely soluble
3	Ethanol	Sparingly soluble
4	Distilled water	Insoluble

U.V. dtermination

Preparation of standard curve

U.V. scanning of fluconazole in methanol has maximum absorbance at 266 nm. The standard λmax of fluconazole is 266 nm.

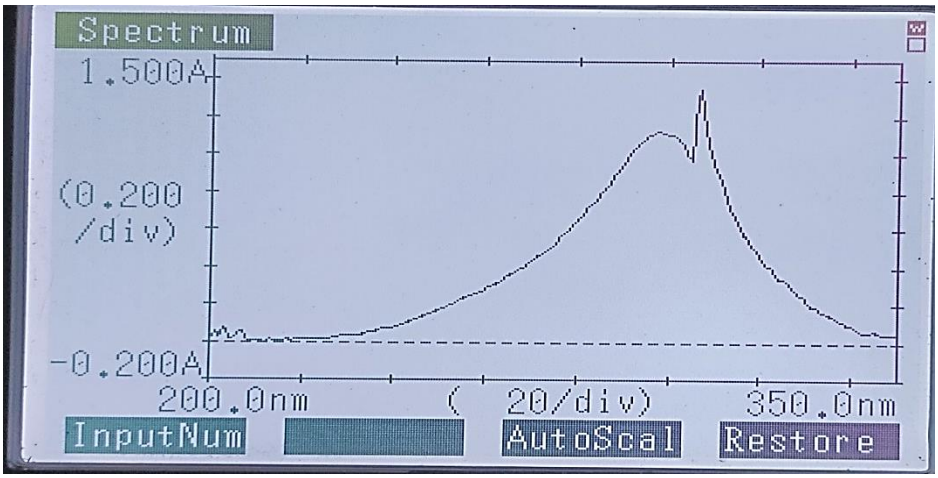


Fig. 1 Standard graph of fluconazole

Preparation of calibration curve

Different concentrations of 10, 20, 30, 40, 50 µg/ml in methanol was analyzed in the region of 200nm-350nm. All the concentration were found to follow beers lambert law. As per to this method the correlation coefficient for fluconazole was found to be 0.9891. The calibration data was tabulated in the table no. 6 and calibration graph so obtained is given in fig. 2.

Table 4: Calibration data of fluconazole

Sr. no.	Concentration(µg/ml)	Absorbance
1	10	0.114
2	20	0.152
3	30	0.169
4	40	0.210
5	50	0.239

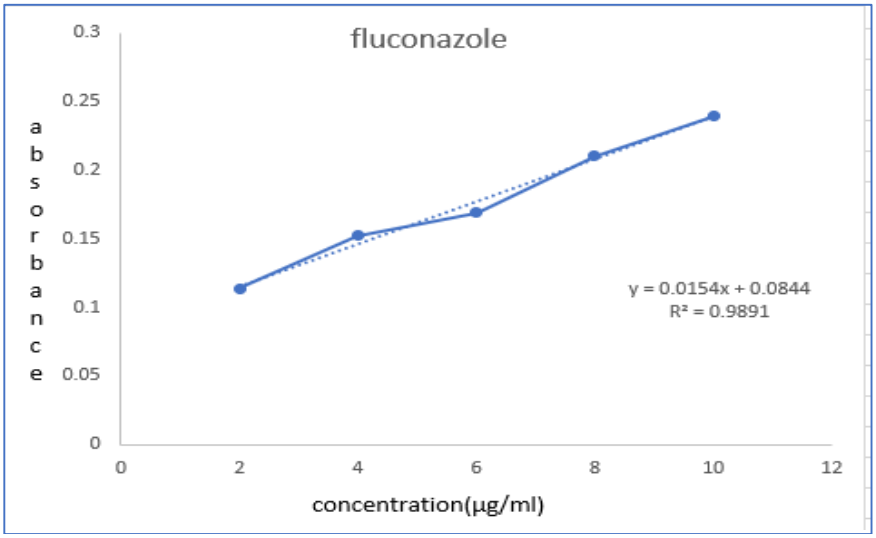


Fig. 2 Standard calibration curve of fluconazole

Angle of repose

The angle of repose of the fluconazole was found to be 18.77 which is less than 25% which concludes to be the excellent kind of flow for fluconazole powder.

Table 5: Standard for angle of repose

Sr. no	Angle of repose(degrees)	Flow property	Result
1	Less than 25	Excellent	18.77
2	25-30	Good	
3	30-45	Fair to Passable	
5	45-55	Poor	
6	56-65	Very poor	
7	Greater than 66	Very very poor	

Melting point

Melting point was obtained by mean of the three values i.e. 138, 139, 138 °C. Therefore, the melting point of fluconazole was found to be 138.33°C. as shown in the table 6:

Table 6: Standard and observed melting point of fluconazole

Standard	observed	Mean
138- 140 °C	**138 °C** **139 °C** **138 °C**	**138.33 °C**

FT-IR SPECTRAL ANALYSIS OF FLUOCNAZOLE AND SPAN 60

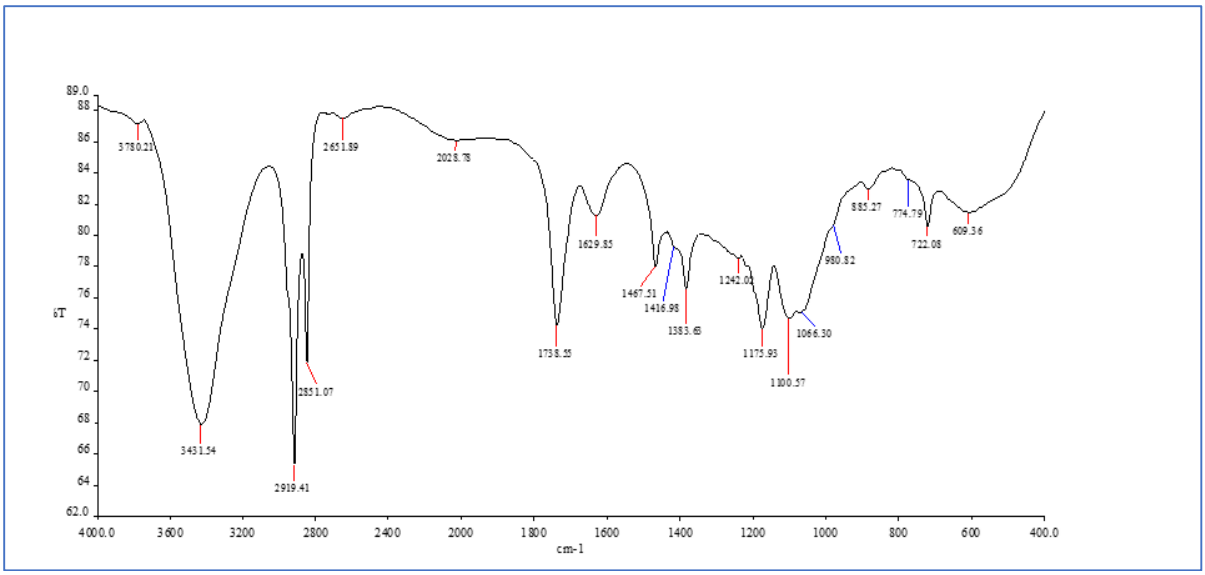


Fig. 3 FTIR spectrum of span 60

Table 7: Interpretation of FTIR spectrum of span 60

Sr. no.	Functional group	Reference peak	Observed peak
1	C=O	1750-1650	1738.55
2	OH	3500-3200	3431.54
3	CH	1421-1415	1416.98

The FTIR spectrum of span-60 showed the characteristic peak at 1738.55 which confirmed the presence of CO group, characteristic peak at 3431.54 confirmed the presence of OH group and a characteristic peak at 1416.98 which confirmed the presence of CH group. The observed spectrum of span 60 conformed the purity of the span 60.

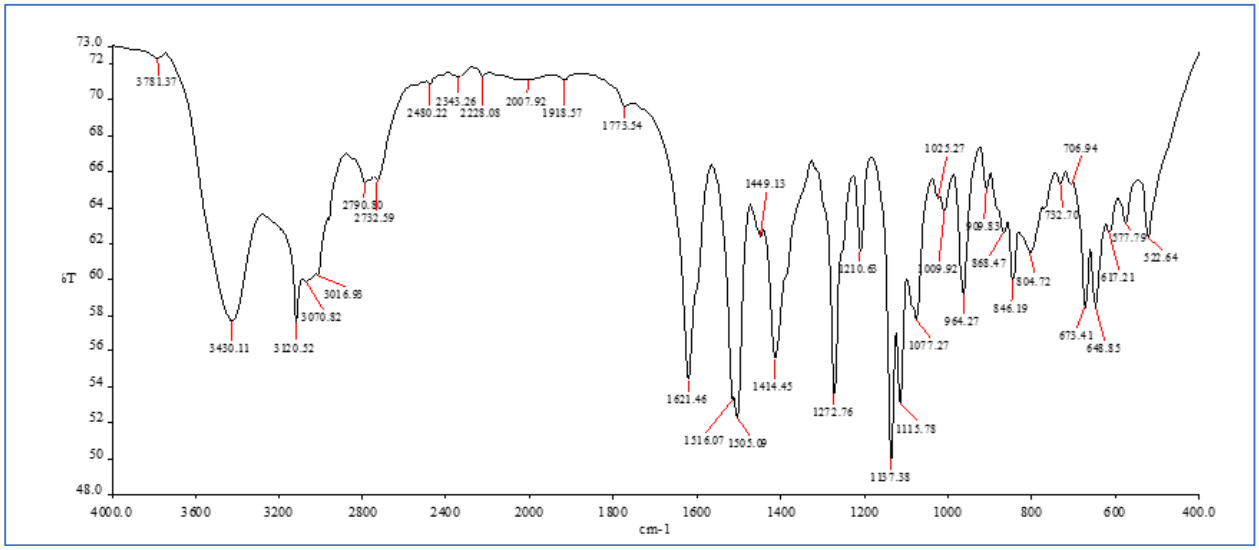


Fig. 4 FTIR spectrum of fluconazole

Table 8: Interpretation of FTIR spectrum of fluconazole

Sr.no	Functional group	Reference peak(cm^{-1})	Observed peak(cm^{-1})
1	O-H	3500-3200	3430.11
2	N-H	1650-1580	1621.46
3	C-F	1400-1000	1272.76, 1137.38
4	C-O	1124-1050	1115.78
5	C-N	1342-1266	1272.76

The FT-IR spectrum of fluconazole shows characteristic peaks at 3430.11 confirms the presence of OH group, characteristic peak at 1621.46 confirms presence of NH group, 1272.76 and 1137.38 confirmed the presence of C-F group, characteristic peak at 1115.78 confirmed presence of CO group, characteristic peak of 1272.76 confirmed the presence of C-N group respectively. The observed FT-IR spectra confirmed and identify the presence of functional group and purity of the drug.

Preparation of niosomal suspension

All the ingredients were weighed and taken for preparation of niosomal suspension of fluconazole. The preparation of the niosomal suspension is shown in the below figure:

EVALUATION OF FORMULATED NIOSOMAL SUSPENSION

Determination of viscosity

All the formulations were subjected to viscosity analysis. The viscosity of the F4 was found to be maximum with 1.57 cp.

The viscosity of all the formulation were tabulated below:

Table 9: Viscosity analysis of niosomal suspension

Sr. no.	Formulation	Viscosity
1	F1	1.34 cp
2	F2	1.56 cp
3	F3	1.55 cp
4	F4	1.57 cp

pH DETERMINATION

All the formulation were subjected to pH determination. The standard pH of the niosomal suspension should be in the range from as that or oral suspension i.e. 5-8. pH of all the formulations lies within this range.

The pH of all the formulation is tabulated below:

Table 10: pH determination of niosomal suspension

SR. No	Formulation code	pH
1	F1	6.7
2	F2	6.6
3	F3	6.6
4	F4	6.4

FT-IR OF NIOSOMAL SUSPENSION OF FLUCONAZOLE

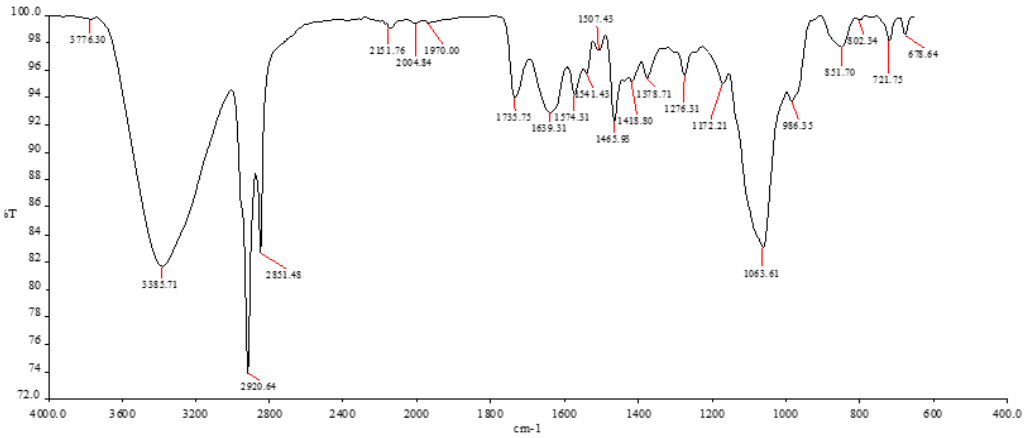


Fig. 5 FTIR Of Niosomal Suspension of Fluconazole

Table 11: Interpretation of FTIR spectrum of niosomalsuspensiom fluconazole

Sr.no	Functional group	Reference peak	Observed peak
1	O-H	3500-3200	3385.71
2	N-H	1650-1580	1639.31
3	C-F	1400-1000	1276.31,1172.21
4	C-O	1124-1050	1063.61
5	C-N	1342-1266	1276.31

The FT-IR spectrum of fluconazole shows characteristic peaks at 3385.71 confirms the presence of OH group, characteristic peak at 1639.31 confirms presence of NH group, 1272.76 and 1137.38 confirmed the presence of C-F group, characteristic peak at 1063.61 confirmed presence of CO group, characteristic peak of 1276.31 confirmed the presence of C-N group respectively. The observed FT-IR spectra confirmed that there is no significant interaction between the drug and excipient used in the niosomal suspension.

DRUG CONTENT

The drug content of all the formulation were found to be greater than 85%, the maximum drug content was found to be of F4 which was 97.14. the drug content of all the formulation were tabulated in the table given below:

Table 12: Drug Content

Sr. no	formulation code	drug content
1	F1	83.70
2	F2	94.48
3	F3	96.10
4	F4	97.14

DRUG ENTRAPMENT EFFICIENCY

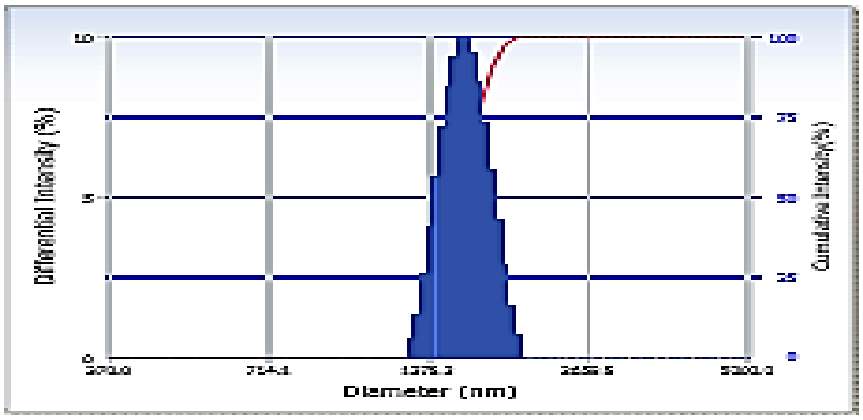
The drug entrapment efficiency of all the formulation were found to be greater than 85%. The maximum entrapment efficiency was of F4 and was found to be 88.35%. The drug entrapment efficiency for the various formulation were tabulated in table 13:

Table 13: Drug Entrapment Efficiency Data

SR.NO	FORMULATION CODE	% DRUG ENTRAPMENT
1	**F1**	88%
2	**F2**	87.7%
3	**F3**	87.35%
4	**F4**	88.35%

DETERMINATION OF PARTICLE SIZE NIOSOMAL SUSPENSION

The particle size determination of the niosomal suspension was done and the average diameter of the particles were found to 1603.7 nm. which is characteristic particle size for suspension as suspension have particle size larger than 1000nm. Also, for niosomes the particle size greater than 100 nm suggests to be of large unilamellar vesicles type.



Distribution Results (Contin)

Peak	Diameter (nm)	Std. Dev.
1	1,603.7	152.7
2	0.0	0.0
3	0.0	0.0
4	0.0	0.0
5	0.0	0.0
Average	1,603.7	152.7

Fig. 6 Particle Size Determination of niosomal suspension

IN-VITRO DRUG RELEASE

The in-vitro drug release of prepared niosomal formulation was determined. The collected sample show characteristic release of the drug from the niosomal formulation. The release rate of F4 was found to be maximum that consist of the maximum concentration of the span 60 polymers. The release of F4 is found to be 79.34 %.

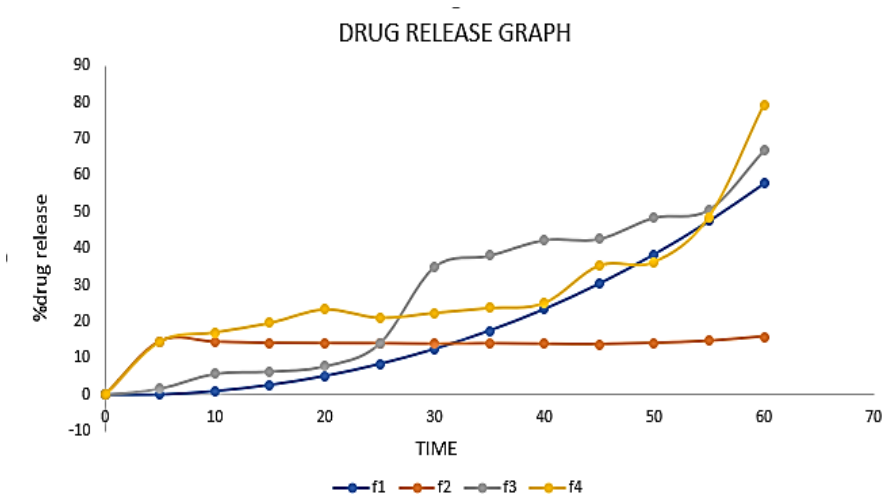


Fig. 7 Drug Release Graph of different formulation

Table 14: Invitro Percentage Drug Release Data of Formulations

Time	% drug release f1	% drug release f2	% drug release f3	%drug release f4
0	0	0	0	0
5	0	14.59	1.69	14.59
10	0.94	14.45	5.79	17.1
15	2.63	14.12	6.39	19.63
20	5.08	14.06	7.82	23.36
25	8.37	14.03	14	21.08
30	12.5	13.89	35	22.41
35	17.52	14.02	38.1	23.75
40	23.44	13.89	42.38	25.1
45	30.41	13.81	42.7	35.34
50	38.44	14.14	48.51	36.31
55	47.56	14.69	50.68	48.67
60	57.83	15.91	66.88	79.34

TRANSMISSION ELECTRON MICROSCOPY

The morphology of the particles was observed using TEM. Most Particle in the niosomal suspension were found to be spherical with few irregular shaped particles.

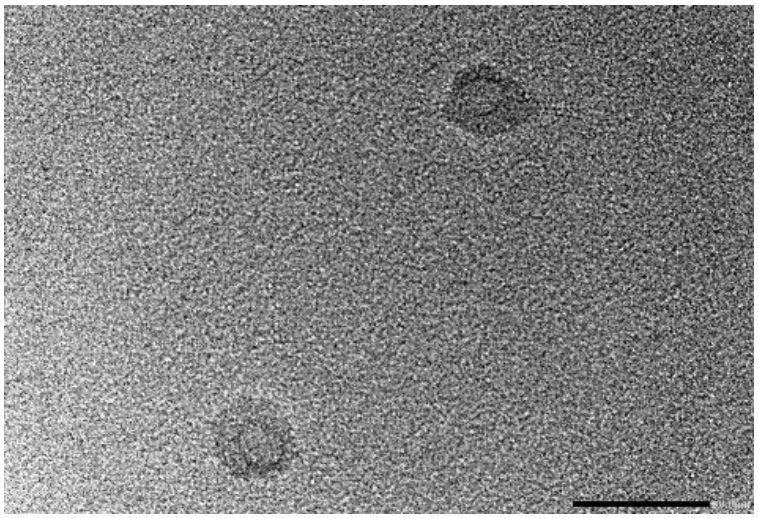


Fig. 8 TEM image of niosomal suspension

STABILITY STUDIES

The stability studies are the important parameter which identifies whether the formulation was able to bear any change in temperature. The stability studies were done for 3 months. The formulations were stable for 3 months without any significant change in their physical appearance. But increase in pH was observe in all the formulations.

The results for stability studies are discussed in the table below:

Table 15: Stability Studies for Niosomal Suspension (physical appearance)

Temperature	**Physical appearance(visual)**		
	After One Month	**After 2 Month**	**After 3 Month**
At room temperature	No Change	No Change	No Change
At refrigerated temperature	No Change	No Change	No Change

Table 16: Stability Studies for Niosomal Suspension(pH)

Temperature	**Change in pH**		
	After 1 month	**After 2 months**	**After 3 months**
	F1	**F2**	**F3** **F4**

At Room Temperature	6.8	6.8	7.2	7.4
At Refrigerated Temperature	7.0	7.2	7.5	7.5

Conclusion

In this study, a niosomal suspension of fluconazole was developed using the ether injection method. The formulation was evaluated for vesicle size, entrapment efficiency, in vitro release, and drug content. The results indicated that niosomal delivery of fluconazole could be a viable alternative to conventional dosage forms, providing site-specific delivery and potentially reducing drug-associated side effects. Niosomes proved to be promising drug carriers for fluconazole in treating fungal infections. Preformulation studies confirmed the identity and purity of fluconazole, showing it as a white, crystalline, odorless substance with good solubility in methanol and a melting point of 138.33°C. The UV spectrum showed a λmax at 266 nm, and the calibration curve demonstrated a positive correlation between concentration and time. The niosomal suspension, prepared with diethyl ether, phosphate buffer (pH 7.4), cholesterol, and Span 60, exhibited suitable viscosity and a pH range of 6.4-6.7. FTIR analysis indicated no interaction between the drug and the polymer. Formulation F4 had the highest drug content (97.14%) and entrapment efficiency (88.35%), with particle sizes in the nanometric range (1603.7 nm). In vitro release studies showed a consistent release over time, with F4 performing best. Transmission electron microscopy revealed spherical niosomes in the nanometric range. Stability studies showed no significant changes in physical appearance, though a slight increase in pH was noted. Overall, niosomal suspensions offer potential improvements in fluconazole delivery and therapeutic efficacy for fungal infections. Overall, the study confirmed that niosomal suspensions are a promising vehicle for fluconazole, offering potential improvements in drug delivery and therapeutic efficacy for fungal infections.

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