Original Article

Formulation And In Vitro Evaluation Of Fluconazole Niosomal Gel For Topical Drug Delivery

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Doi: 10.47750/Pnr.2022.13.S08.498

Abstract

Fluconazole, a macrolide antibiotic, is employed to treat certain susceptible bacteria. Niosomes play a significant role in drug delivery since they can alter pharmacokinetics and bioavailability characteristics, as well as minimise toxicity. Niosomes are becoming more and more crucial in the administration of drugs. While decreasing the drug's systemic absorption, topically administered niosomes can lengthen the duration that medications remain in the stratum corneum and epidermis. Fluconazole-loaded topical gel niosomes are intended to be developed and evaluated in this study. Span 20, 40, 60 (as a non-ionic surfactant) and cholesterol were used to create niosomes by the thin film hydration method (as stable vesicle forming agent). Different dosages of the drugs, surfactants, and cholesterol were used to make niosomes (0.30:1:0.6, 0.6:1:0.6, 0.9:1:0.6). The vesicle size, surface shape, % entrapment effectiveness, drug content, and in vitro drug release of the niosomal dispersion were examined. Using a UV spectrophotometer, the drug concentration and entrapment efficiency were determined at 262 nm. A range of 77.650.25 to 94.120.48 was discovered for the entrapment efficiency. A maximum entrapment efficiency of 94.120.48 was shown for Formulation FS5, which contains Span 60, and 93.900.70 was shown for Formulation FT4, which contains Tween 60. Carbopol 940, glycerol, triethanolamine, and distilled water were used to make fluconazole niosomal gel. Niosomal gel's evaluation was based on its outward appearance, pH, viscosity, drug content, entrapment effectiveness, and in-vitro permeation investigations. The amount of medication released from the niosomal gel was discovered to be 80.76%. The aforementioned data show that encapsulating a fluconazole-loaded niosomal topical gel lengthens drug release, increases drug retention in skin, and enhances cellular permeability.

Keywords: Niosomes; fluconazole; carbopol 934, fungal infection

INTRODUCTION

Clinicians and patients have access to a variety of carriers, including solids, semisolids, and liquid formulations, for skin care and the topical treatment of dermatological diseases. Antimicrobial topical medications are a helpful treatment option for infections of the skin and soft tissues [1]. To enable therapeutic targeting of sick cells, various nanocarrier-based delivery methods for antifungals are developed. With the goal of reducing side effects and enhancing drug therapy effectiveness, nanosized carriers have been given specific consideration. The efficacy of several nanosized delivery methods in antifungal therapy has already been established [2].Some medications have a range of optimal concentrations where they provide the greatest benefit, while dosages above or below this range might be harmful or have no therapeutic benefit. Another approach for treating severe diseases that involves a multidisciplinary approach to getting medications to the target site is deliberate progression in treatment efficacy. Various drug delivery and drug targeting methods are now being developed to reduce medication loss and degradation, to prevent negative side effects, to maximise drug bioavailability and drug accumulation in the needed zone. Carriers must possess the drug in order to obtain the required medication delivery. Carriers include micelles, liposomes, lipoproteins, microcapsules, cell ghosts, soluble, biodegradable, or insoluble polymer microparticles, etc. These carriers may slow degrade, react to a stimulus (temperature, pH), or be targeted (like conjugating with particular Ab) [3].Liposomal and niosomal drug delivery technologies have unique advantages over traditional dose forms. These devices can operate as drug reservoirs and deliver controlled release of the active ingredient. Targeting may also be possible by altering their surface or composition. Prior research have demonstrated that focusing specifically on lipid-based vehicles, which take into account the unique characteristics of fungal parasites, leads to better antifungal action [4].Vesicle formulation as a skin delivery mechanism is one of many methods designed to disrupt and weaken the highly structured intercellular lipids in an effort to improve medication transport across intact skin [5-8]. Niosomes are known to be effective delivery systems for topical medications [9].They operate as an organic solvent for the solubilization of poorly soluble medicines, such as corticosteroids, allowing for the application of higher local drug concentrations at maximum thermodynamic activity. They could function as a regulated transdermal delivery system or as a rate-limiting membrane barrier for the modification of systemic absorption. During in vitro experiments utilising human skin, there are primarily two types of vesicle skin interactions that can have a variety of impacts on dermal or transdermal drug delivery [10–12].Prior to

adhering to the cell surface, the vesicles in touch with the stratum corneum agglomerate and fuse. It is thought that this interaction creates a strong drug thermodynamic activity gradient at the vesicle-stratum corneum interface, which acts as the catalyst for lipophilic drug penetration through the stratum corneum. Second, freeze fracture electron microscopy (FFEM) and small angle X-ray scattering have shown that this form of interaction involves ultrastructural alterations in the intercellular lipid areas of the stratum corneum and its deeper layers at a maximum depth of around 10 m. (SAXS). A wide variety of fungus species, including Cryptococcus, Candida, Aspergillus, Blastomyces, and Histoplasma capsulatum var. Capsulatum, are susceptible to fluconazole, an oral triazole antimycotic drug [13]. The biopharmaceutics classification scheme (BCS) has designated fluconazole as a Class II medicine. Unformulated Fluconazole has a very low bioavailability. Low solubility and low permeability characterise fluconazole. Fluconazole can be specifically targeted to the site of action by being included in miniature niosomes, which improves the drug's therapeutic efficacy.

MATERIALS

The Mumbai-based Alkem Pharmaceuticals provided fluconazole. We bought Span 40, Span 60, and Tween 60 from SD Fine Chemicals Ltd. in Mumbai. From Mumbai's Loba Chemie Pvt Ltd., CHO was bought. We bought Carbopol 934 from Himedia in Mumbai. From SD Fine Chemicals Ltd. in Mumbai, sodium hydrogen orthophosphate and potassium dihydrogen orthophosphate were bought. From SD Fine Chemicals Ltd. in Mumbai, we bought chloroform, methanol, glycerol, and triethanolamine. The rest of the supplies were of an analytical calibre.

METHODOLOGY

Four sections made up the study:

A. Compatibility tests for drug excipients.

B. Niosome optimization using various non-ionic surfactant combinations after preparation, evaluation, and optimization (Spans and Tweens).

C. The creation, assessment, and optimization of gel.

D. The preparation, assessment, and optimization of niosomal gel

Preformulation Studies

Fourier Transform Infrared Spectroscopy Analysis of Fluconazole (pure drug)

In order to verify that the sample is a pure medicine, an investigation was carried out. Fluconazole (pure medication) was tested using Fourier transform infrared (FTIR 8400s, Shimadzu Japan) spectra. It was done using the potassium bromide disc method. By using pressure of 10tons/inch2 for 10 minutes, the pellet was created with the dry sample [14–15].

Preparation, Evaluation and Optimization of Niosome with Non-Ionic Surfactants (Tween 60 & Span 60)

Initial studies were carried out using the thin-film hydration approach and several non-ionic surfactants, such as span-60 and tween-60, in the ratios of 0.30:1:0.6, 0.6:1:0.6, and 0.9:1:0.6. In a 100 ml round bottom flask (RBF) containing 10 ml each of chloroform and methanol, surfactant (Span 60 and Tween 60) and CHO were dissolved in precisely weighed amounts. The solvent mixture received a weighed addition of medication. The solvent combination was then extracted from the liquid phase using flash evaporation at 60 °C and 150 rpm to create a thin layer on the flask walls. To completely remove any lingering solvent, vacuum was used. With 10 ml of pH 7.4 room temperature PBS, a dry lipid film was hydrated. Based on their % entrapment efficiency, Span 60 and Tween 60 were selected as the study's non-ionic surfactants (See Table). Choosing appropriate non-ionic surfactants at the optimal concentration was the focus of the preliminary work. The formulations' in-vitro dissolving profile was examined. For subsequent research, a non-ionic surfactant with a better in-vitro dissolving profile and requirements was chosen [16–19].

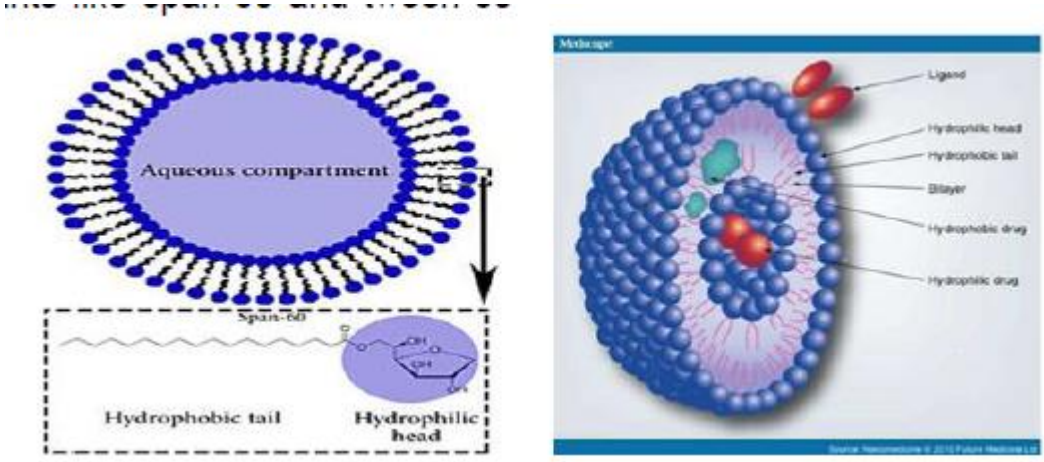


Fig1- Structure of Niosomes

Characterization of the Prepared Niosomes

The morphological characterisation of the manufactured niosomes (size, shape), % entrapment efficiency, and in-vitro release were evaluated.

Table 1. Preliminary trials of Fluconazole loaded Niosomes, cholesterol and non-ionic surfactant

Tween 60			
Cholesterol (mg)	**Surfactant (mg)**	**Drug (mg)**	**Ratio**
30	100	60	0.30:1:0.6
60	100	60	0.6:1:0.6
90	100	60	0.9:1:0.6

Span 60			
Cholesterol (mg)	**Surfactant (mg)**	**Drug (mg)**	**Ratio**
30	100	60	0.30:1:0.6
60	100	60	0.6:1:0.6
90	100	60	0.9:1:0.6

Entrapment Efficiency Study

To isolate the drug-containing niosomes for unentrapped drug, niosomal dispersion within the Ephendroff tubes was centrifuged at 7300 rpm at 40C for 20–30 min in two cycles. Spectrophotometric analysis of a clear fraction at 260 nm was used to identify free drugs. Three times with saline phosphate buffer pH 7.4, the vesicles pellet that was present in the precipitation was washed. After washing, 5ml of absolute alcohol:propylene glycol (1:1) were added to 5ml of 0.9% saline to break up the vesicles. At 260 nm, this was analysed spectrophotometrically [20–22]. Utilizing the formula, the percentage of drug entrapped was determined:

$$\text{Entrapment efficiency (\%)} = \frac{(\text{The total amount of drug} - \text{amount of free drug}) \times 100}{\text{The total amount of drug}}$$

Size, Shape and Morphological Characterization

To validate vesicle production, optical microscopy at a 45x magnification was used. Drying at room temperature allowed the niosomal suspension to be fixed to a glass slide, and the formation of vesicles was detected in the thin, dry layer of niosomal solution. Additionally, using a digital camera and a microscope, the niosomes' microphotography was captured. Using an electron microscope, the specific surface properties of a few different fluconazole niosome formulations were examined [23–25].

In-vitro Drug Release Study

Fluconazole release from niosomal formulations was determined using the membrane diffusion approach. A beaker with 100 ml of pH 7.4 PBS containing 10% v/v methanol (to preserve sink condition) was used to dialyze the remaining niosomes after the unentrapped medication was removed. A magnetic stirrer was used to agitate the receptor media, which was kept at a constant 37 0.5°C. Periodically, aliquots of 5ml were drawn and replenished with the same amount of medium. A UV spectrophotometer was used to analyse the sample that was taken at 260 nm. The tests were carried out three times [26–27].

Formulation of Gel

Different polymers were used to make gel at different concentrations, and it was found that carbopol 3% and guar gum 3% were the best combinations (see in table)

Niosomal Gel Formulation

An adequate gel was created by incorporating an improved formulation into a suitable gel foundation. Carbopol 3% w/w and guar gum 3% w/w in a water-glycerol mixture was the gel foundation chosen for integrating niosomes (7:3) By adding enough triethanolamine using a cold mechanical process, the resulting dispersion was neutralised [28–32] and made viscous..

S.No.	Ingredients	2%	3%	4%	5%
1	Carbopol 934	++	+++	++++	-
2	Guar gum	++	+++	++++	-
3	HPMC K15M	++	++	++	+++
4	Tragacanth gum	++	+++	++++	-

++ Gel not Formed, +++ Gel Formed, ++++ Very Hard Gel Formed

Table 2- Formulation of gel with different polymers

S.No	Ingredients	%
1	Carbopol	3%
2	Niosome Formulation	10%
3.	Triethanolamine	Q.S.
4.	Water	QS to 100%

Table 3- Formulation design for Niosomal gel

NIOSOMAL GEL EVALUATION

Physical appearance

The prepared gel was checked for clarity, colour, homogeneity, and the presence of foreign particles.

pH

A precise 2.5gm of gel was weighed out and dissolved in 25 ml of purified water. A digital pH metre was used to determine the dispersion's pH [33].

Rheological study

To measure viscosity, a Brookfield programmed DV III ultra-viscometer was used. To evaluate viscosity, spindle number CP 52 was run at the ideal speed of 0.01 rpm [34–36].

Zeta potential, particle size and size distribution

Based on dynamic light scattering, the zeta sizer (HORIBA-SZ-100Z) was used to measure particle size. The diluted suspension of nanoparticles was made in double-distilled water and sonicated for 30 seconds on an ice bath. At 25 °C and a scattering angle of 1730, the material was examined. At 25oC, zeta potential was determined using a zeta sizer based on electrophoretic mobility. To ascertain the particle size distribution, the Polydispersity index (PI) was also estimated [28].

In vitro drug diffusion study

An open-ended glass cylinder serves as the study equipment in this instance. One side of the cylinder had a dialysis membrane attached to it with the aid of an adhesive solution that had been soaked in distilled water for 24 hours before to usage. The cell (i.e., the donor compartment) is filled with gel equal to 10 mg of fluconazole, and the receptor compartment is a beaker containing 100 ml of pH 7.4 PBS that contains 10% v/v methanol. The complete assembly was set up such that the lower end of the cell-holding gel is only a few millimetres deep (1-2mm) below the surface of the diffusion medium. At 37 0.5°C, the mixture was stirred. Five millilitre portions were removed from the receptor compartment and replaced with equivalent volumes of brand-new buffer. The obtained sample was examined using a UV-visible spectrophotometer at 260 nm [37–38].

Calculation of Release Kinetics for FNG

Tables include the mathematical formulas for calculating release kinetics and interpreting diffusion mechanisms. For all formulations, the release coefficient (r2) was computed. The Korsemeyer Peppas equation [39] was used to compute the release component "n."

RESULT AND DISCUSSION

Preparation and Optimization of Niosomes by Thin-Film Hydration Method

Initially, vesicles were made with different amounts of cholesterol [39, 40–41]. Based on vesicle production with outstanding clarity by photomicrographic analysis as reported in Table, the optimal formulations were chosen for future studies.

Table 4- Preliminary Trial of Fluconazole loaded Niosomes, Cholesterol and Non Ionic Surfactant

Tween 60				
Cholesterol (mg)	**Surfactant (mg)**	**Drug (mg)**	**Ratio**	**Observation**
30	100	60	0.30:1:0.6	++
60	100	60	0.6:1:0.6	+++
90	100	60	0.9:1:0.6	++++

Span 60				
Cholesterol (mg)	**Surfactant (mg)**	**Drug (mg)**	**Ratio**	**Observation**
30	100	60	0.30:1:0.6	++
60	100	60	0.6:1:0.6	+++
90	100	60	0.9:1:0.6	++++

++ Good, +++Very Good, ++++Excellent

DRUG-EXCIPIENTS COMPATIBILITY STUDIES

Fourier Transforms Infrared Spectroscopy Analysis

To find out if there are any interactions between the excipient used and the pure medicine (fluconazole), an FTIR investigation was undertaken. The KBr pellet approach was used to carry it out, and it was scanned [42–43]. Figures 2 and 3 show the FTIR spectra of the pure medication and the blends of polymer and physical mixtures. The peaks illustrating fluconazole were comparable in the pure drug and the mixture of fluconazole with other excipients employed, which suggests there are no interactions. It was also found that the functional peaks persisted after excipients were added to the drug. Therefore, it indicates that the medicine was stable and compatible with the excipients utilised throughout the process

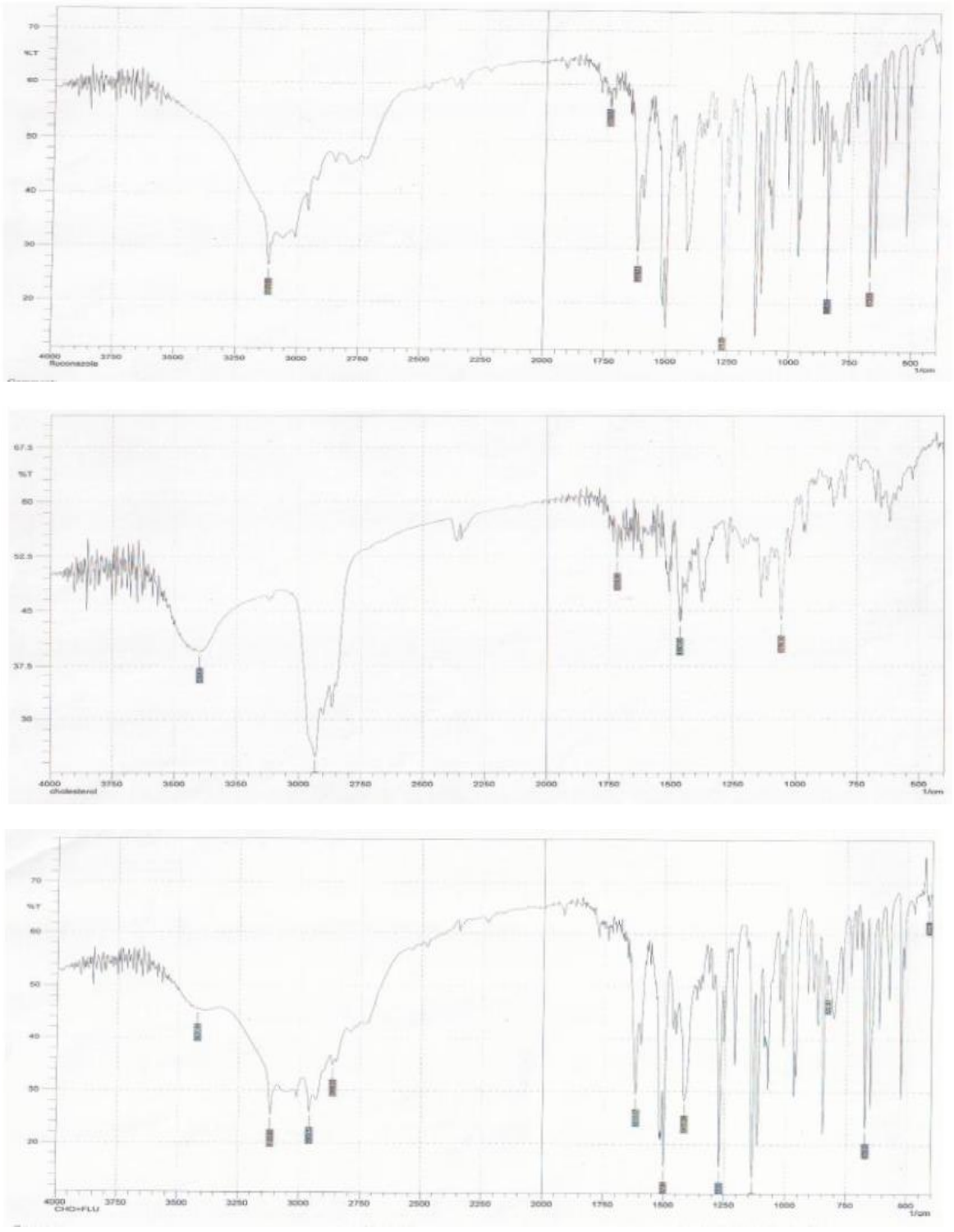


Fig. 2. FTIR spectra of (i) Fluconazole (ii) Cholesterol (iii)Fluconazole + Cholesterol

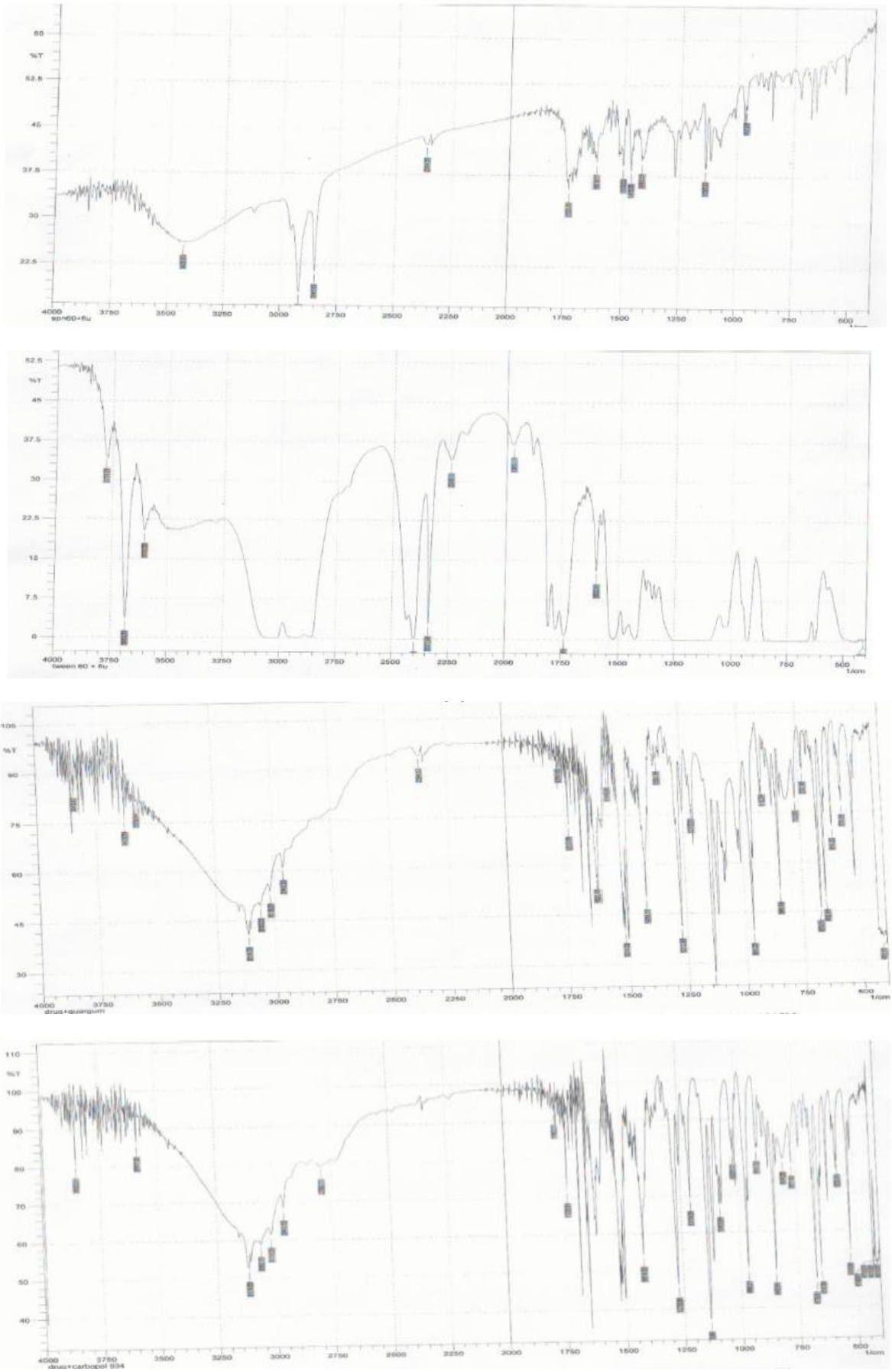


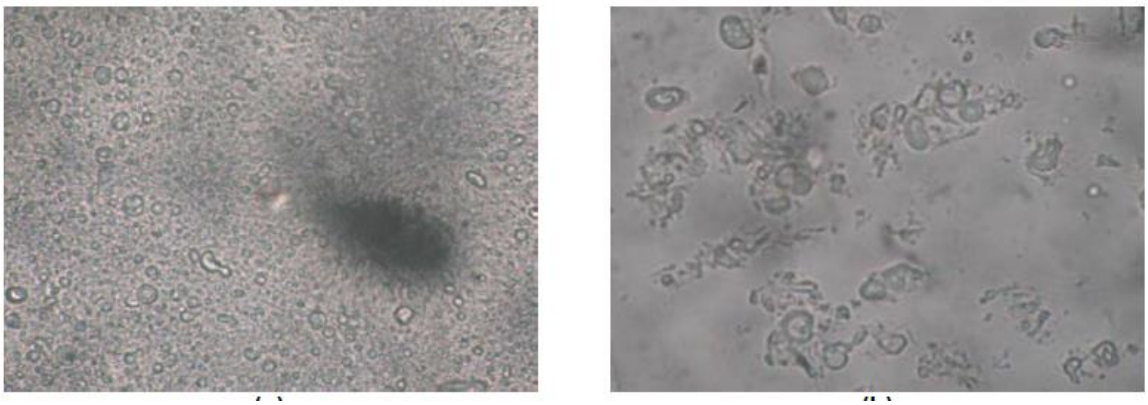
Fig. 3. FTIR spectra of (i) Fluconazole + Span 60 (ii) Fluconazole + Tween 60 (iii) Fluconazole + Guar gum and (iv) Fluconazole+ Carbopal 934

Table 5- Interpretation of Fluconazole IR Spectra

Region in cm^3	Functional Grous	Types of Vibrations
3029.51	Alcohols Groups	O-H stretching
2950.43	Alkane Groups	C-H stretching
1618.17	Alkynylyne Groups	C=C
1270.00	Alkane Groups	C-H stretching
1415.70	Alkane Groups	C-H Bending
1519.80	Alkane Groups	C=N stretching
1115.70	Acid Groups	C-0 stretching

MICROSCOPIC CHARACTERIZATION OF SPAN 60 AND TWEEN 60

The production of vesicles was verified using optical microscopy at a 45x magnification. Niosomal suspension was fixed to a glass slide by drying at room temperature, and vesicles were seen forming on the dry, thin film of the suspension. Niosome microphotography was done with a digital camera (Fig.). An electron microscope was used to examine the specific surface properties of certain fluconazole niosome formulations [25]. Examining the surface morphology of niosomes with a triangular research microscope and a Fuji film digital camera revealed that the vesicles had a spherical appearance, as seen in Fig.



ENTRAPMENT EFFICIENCY OF NIOSOMES

The major criterion, the entrapment of drug within a vesicular carrier, must be established in order to assess the delivery capability of the system. As shown in Table, the amount and type of non-ionic surfactants (Spans and Tweens) used for the preparation of niosomes as well as the method of preparation all formulations were evaluated for their entrapment efficiency in an effort to examine the impact of niosome composition on drug loading capacity [19, 22].The range of findings for all formulations is between 77.650.25 and 94.120.48. A maximum entrapment efficiency of 94.120.48 was shown for Formulation FS5, which contains Span 60, and 93.900.70 was shown for Formulation FT4, which contains Tween 60.

Table 6. Entrapment efficiency of Niosomes

Formulation Code	Surfactant	Ratio (SUF:CHO:DRUG)	%Entrapment efficiency
FS1	Span60	1.5:1:0.6	77.65+_0.25
FS2	Span60	2:1:0.6	92.90+_0.30
FS3	Span60	2.5:1:0.6	84.95+_0.14
FS4	Span60	3:1:0.6	93.51+_0.60
FS5	Span60	3.5:1:0.6	94.12+_0.48
FT1	Tween 60	1.5:1:0.6	82.30+_0.38
FT2	Tween 60	2:1:0.6	90.2+_0.27
FT3	Tween 60	2.5:1:0.6	87.64+_0.65
FT4	Tween 60	3:1:0.6	93.90+_0.70
FT5	Tween 60	3.5:1:0.6	93.30+_0.50

EVALUATION OF FLUCONAZOLE NIOSOMES AND NIOSOMAL GEL

Particle Size and Morphology

SEM, vesicle size, and zeta potential were measured using the HORIBA-SZ-100Z analyzer to assess the generated niosomes and niosomal gel. The vesicular properties shown in Figs. 4 and 5 were confirmed by scanning electron microscopy, which was used to assess the surface morphology, vesicle structure [44], and shape utilising Span 60 and Tween 60.

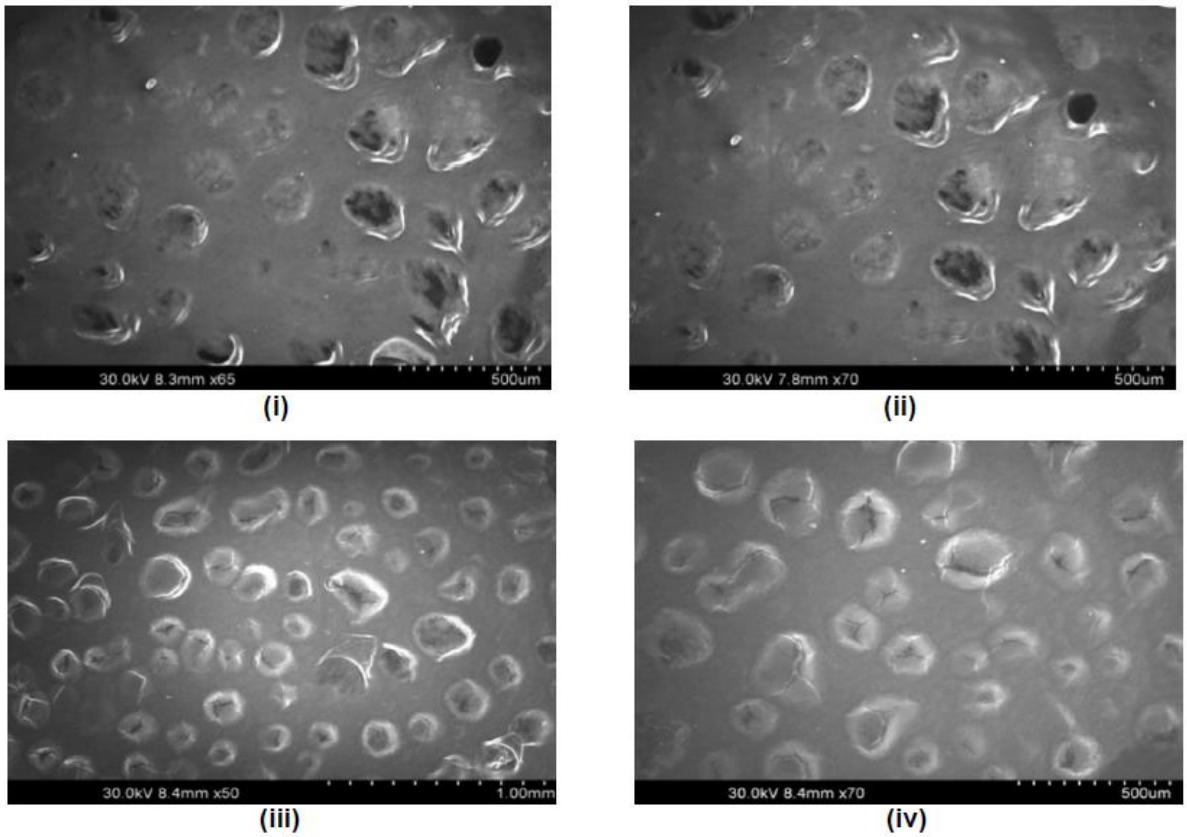


Fig. 4. Surface morphology of Fluconazole Niosomes (i) and (ii) FNS5 Fluconazole Niosome using Span 60, (iii) and (iv) FNT4 Fluconazole Niosome using Tween 60

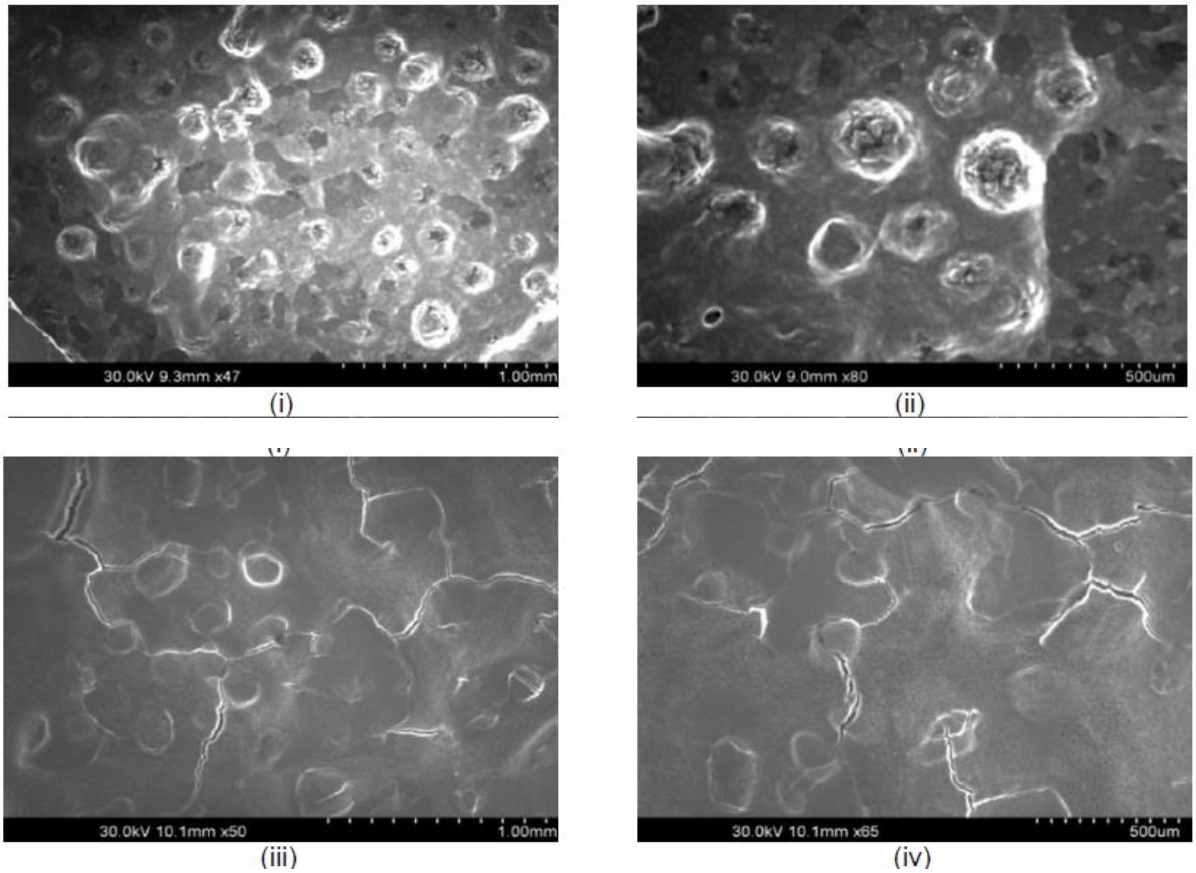


Fig. 5. Surface morphology of Fluconazole Niosomes (i) and (ii) FCS niosomal gel using Span 60, (iii) and (iv) FCT niosomal gel using Tween 60

ZETA POTENTIAL OF NIOSOMAL GEL

Zeta potential is a variable that indicates whether a vesicular system is thermodynamically stable. As shown in Figs. 6 and 7, the optimised niosomal gels FCS and FCT had zeta potentials of -10.2 mV and -46.4 mV, respectively. The formulation appears to be reasonably stable based on the literature's reported zeta potential range of -50 mV to +50 mV. The amount of zeta potential indicates the possible stability of a colloidal system. All gel particles that have greater zeta potentials repel one another and lose their propensity to approach one another. Positive charge serves as a marker for the production of cationic vesicles and increased zeta potential. A system with strong electrostatic repelling forces caused by high zeta potential, either positive or negative, is more stable [45,46].

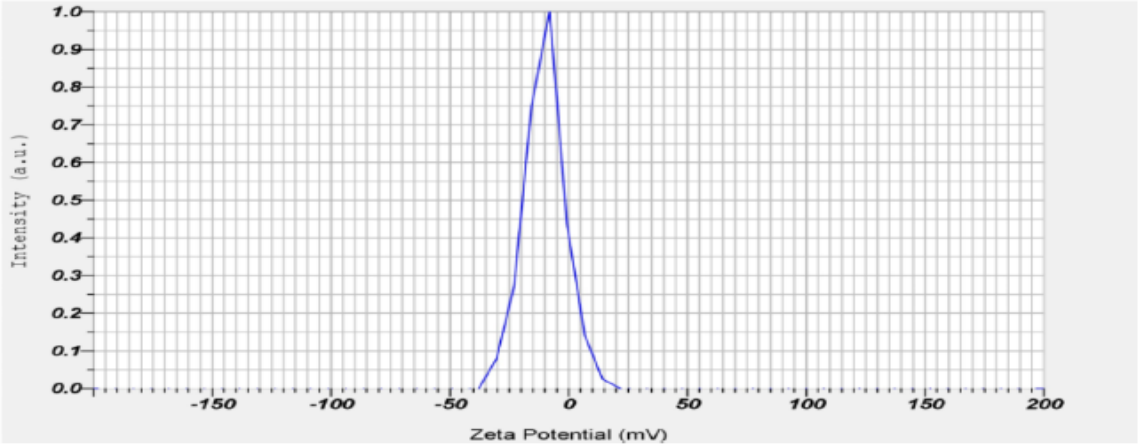


Fig. 6. Zeta potential of Niosomal gel Span 60

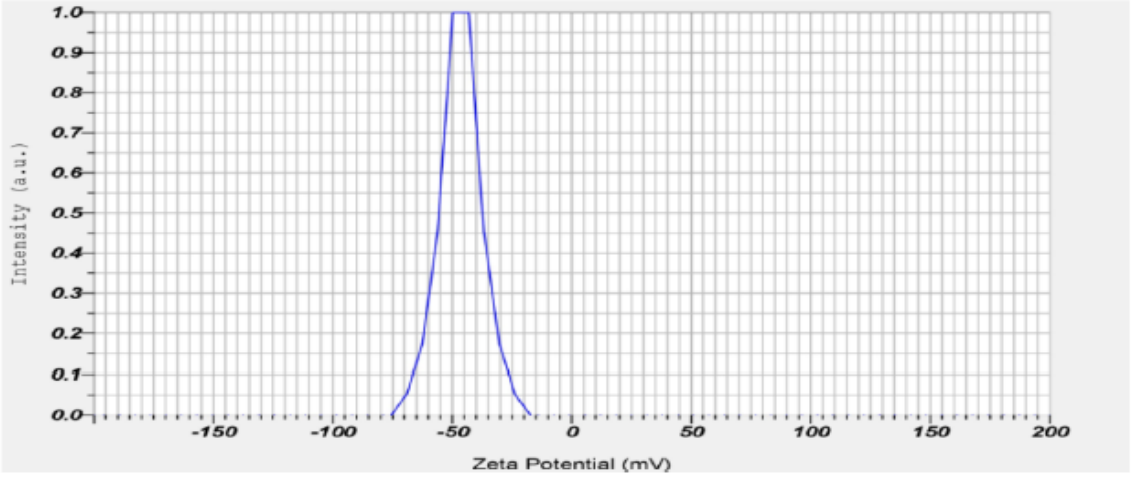


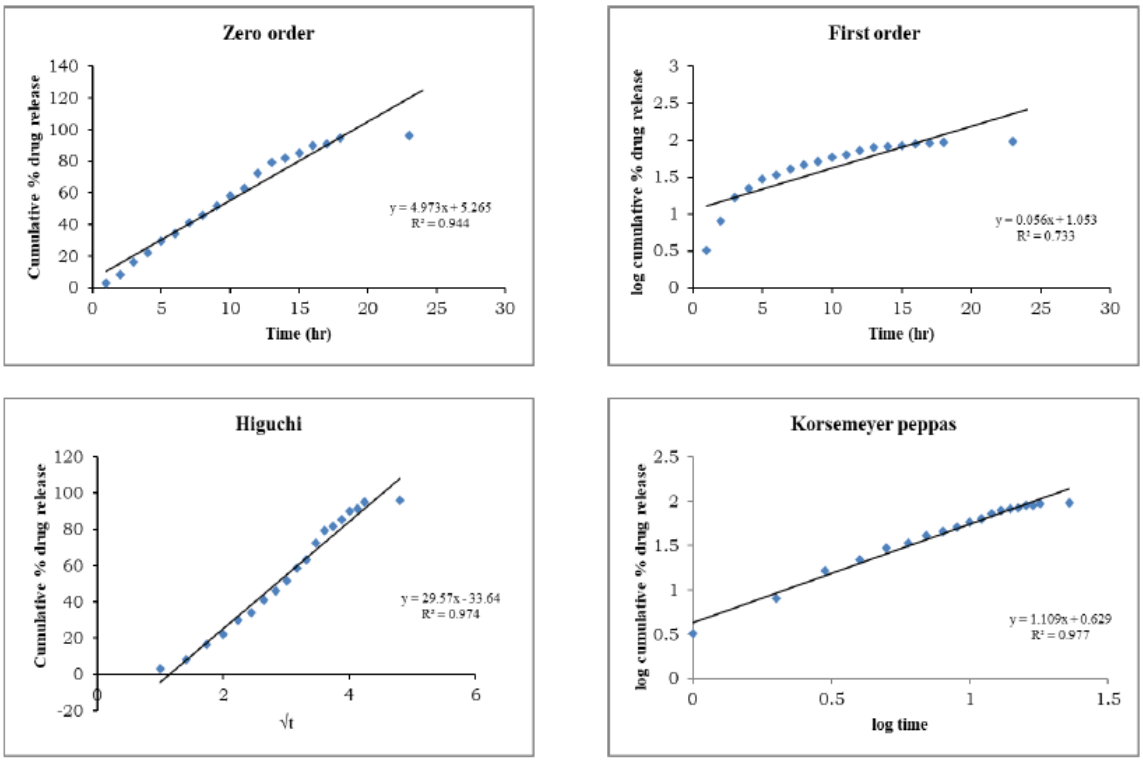
Fig. 7. Zeta potential of Niosomal gel Tween 60

MODEL-DEPENDENT KINETICS

Model-dependent kinetics, such as zero, first-order, Higuchi, and Peppas models, were fitted to the formulations that had been created. Release kinetics were shown against time for each formulation to fit the zero order, first order, Higuchi kinetic model, and Korsmeyer Peppas equations. The plots were used to obtain the regression coefficient and "n" values. For all formulations, the 'n' value was used to analyse the drug release mechanism. FNS5 and FNT4 were the formulas that performed best overall. Formulation showed zero-order kinetics and Korsmeyer Peppas after fickian diffusion for FNS5. [45]

Table 7- Model Dependent Kinetics

Formulations	**Zero**	**First Order**	**Higuchi Order**	**Pepas**	
	r	r	r	r	n
FNS5	0.944	0.732	0.975	0.976	0.042
FNT4	0.955	0.787	0.986	0.990	0.815



.**Fig. 8.** Model dependent kinetics of formulation FNS5

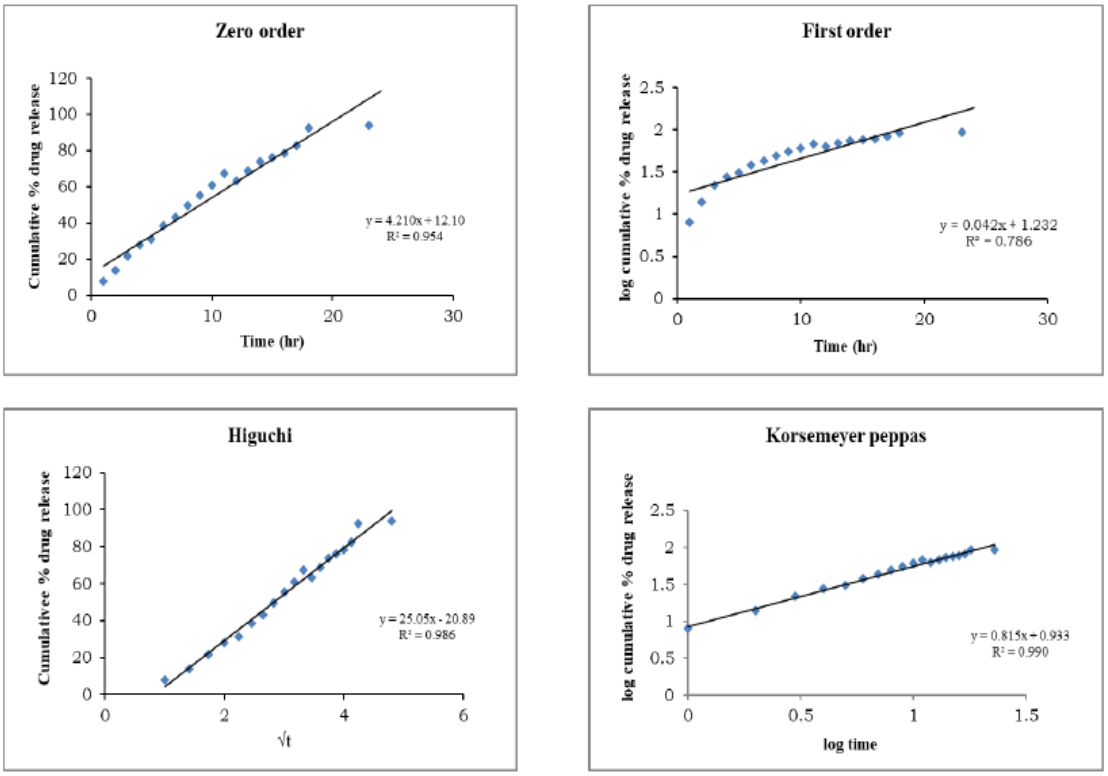


Fig. 9. Model dependent kinetics of formulation FNT4

In-vitro drug release studies

The fluconazole niosomal gel was tested in-vitro using Franz diffusion cells in pH 7.4 PBS. Franz diffusion cells were used for each formulation during the in-vitro diffusion investigations on dialysis membrane. Every hour, samples were taken and analysed with a UV-Visible double beam spectrophotometer at 260 nm [46–47].

Table 8*In vitro* drug release study of prepared gel formulation

TIME(hr)	% Cumulative Drug Release	
	Plain Gel	Niosomal Gel
0	0	0
1	6.00	2.37
3	29.04	29.32
4	45.90	44.65
5	60.34	62.76
6	75.32	65.45
7		66.98
8		70.87
9		72.87
10		75.56
12		80.76

MODEL DEPENDENT KINETICS FOR NIOSOMAL GEL

Plotting the release kinetics for each formulation versus time allowed the zero-order, first-order, Higuchi kinetic model, and Korsmeyer Peppas equations to be fitted. The plots were used to determine the regression coefficient and the values for "n". For all formulations, the 'n' value was used to analyse the drug release mechanism. The formulas with the best results were FCT and FCS. With regard to formulation, FCT showed zero-order kinetics and a Higuchi model that was based on fickian diffusion [46–47]. Formulations, zero-order kinetics in FCS, and transport in Korsmeyer Peppas after super case II.

Table 9- Evaluation of gel

Formulations	pH	Viscosity (cps)	Spread ability (gm/cm^2)
FNCT	6.7	66,888.58	17.19
FNCS	6.4	70.464.09	20.73
FNGT	6.1	63,250.02	24.95
FNGS	6.6	73,170.14	35.20

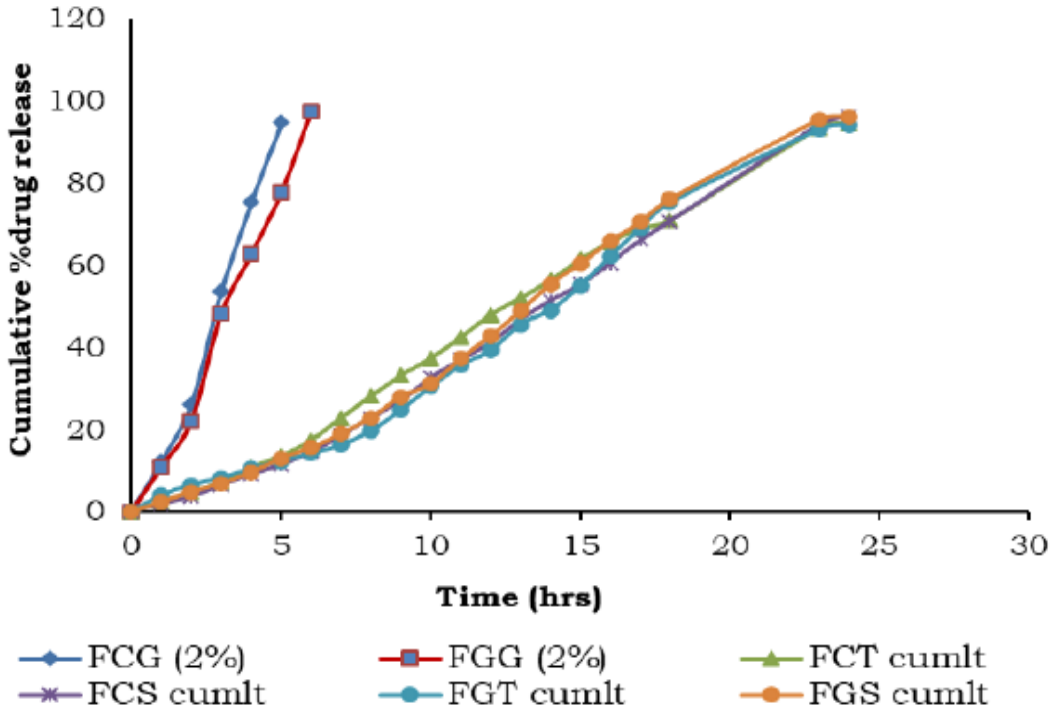


Fig. 10. Diffusion profile of Niosomal gel

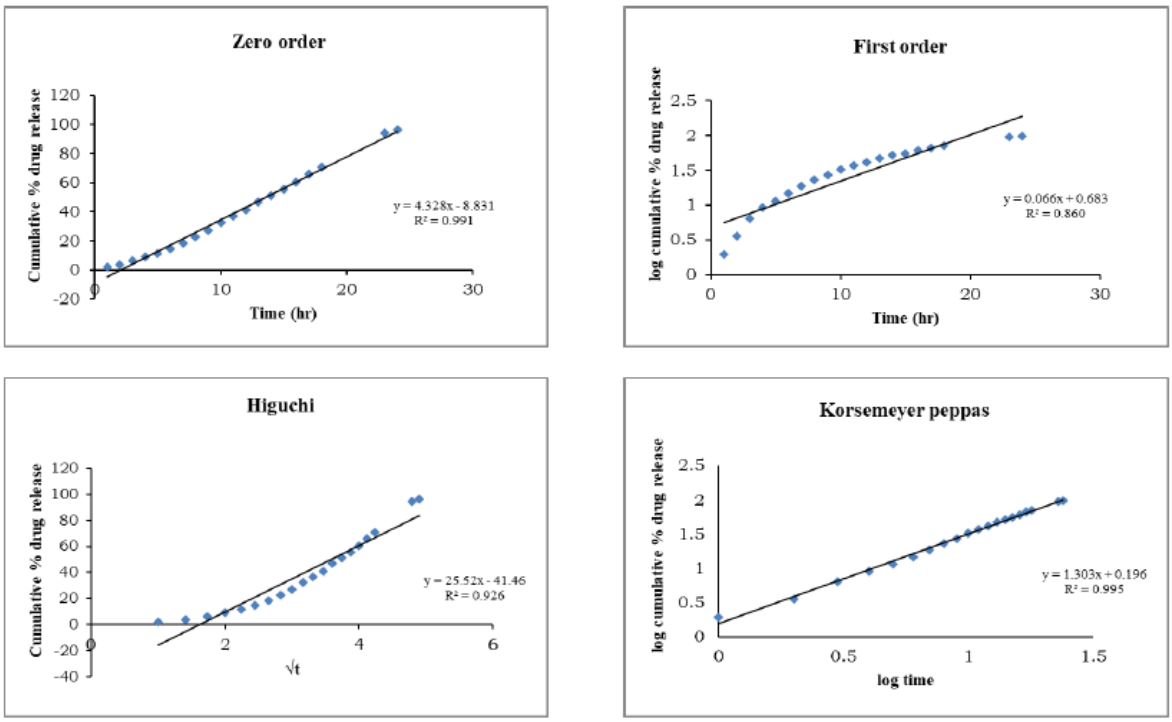


Fig. 11. Model dependent kinetics of formulation FCT

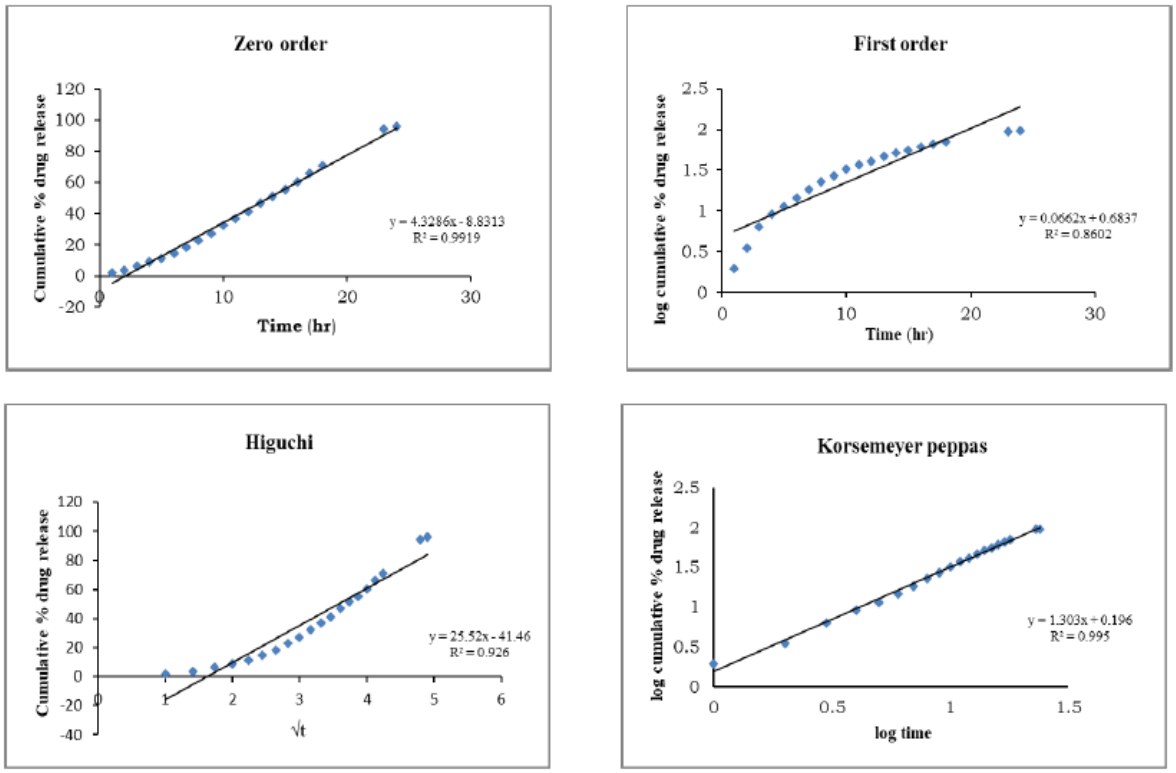


Fig. 12. Model dependent kinetics of formulation FCS

CONCLUSION

In the current research, a fluconazole niosomal gel was created and evaluated using a thin-film hydration technique that optimised the amounts of non-ionic surfactants. Furthermore, the findings of the FT-IR analysis experiments demonstrate that there is no interaction between the medication, cholesterol, and non-ionic surfactants. Here, cholesterol serves as a fluidity buffer, membrane stabiliser, and addition to the membrane, which helps to stabilise the vesicles. The formulations were created utilising various non-ionic surfactants while maintaining a constant cholesterol concentration. The surfactant concentrations used were Span 20, 40, and Span 60. Both FS5 with Span 60 and FT4 with Tween 60 had entrapment efficiencies ranging from 77.650.25 to 94.120.48. For several formulations, in-vitro diffusion experiments were carried out. The longer release of the medicine from the niosome suggests that the frequency of administration and

side effects dramatically decrease, consequently increasing patient compliance. The drug's penetration and release are improved when it is administered as a gel-type formulation. As a result, niosomal gel rather than standard topical gel loaded with pure medication could be a recommended carrier to deliver fluconazole.

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