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DEVELOPMENT AND CHARACTERIZATION OF NYSTATIN ETHOSOMAL DRUG DELIVERY SYSTEM

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Abstract:

The present study aimed to formulate and evaluate Nystatin-loaded ethosomes for improved topical drug delivery and sustained drug release. Nystatin is an antifungal drug used in the treatment of fungal infections. Ethosomes are lipid-based vesicular carriers containing phospholipids and alcohol, which may enhance drug delivery through the skin. In the present investigation, eight different ethosomal formulations (F1–F8) were prepared by the cold method using varying concentrations of phosphatidylcholine. The prepared formulations were characterized for particle size, zeta potential, surface morphology, entrapment efficiency, in-vitro drug release, release kinetics, and stability. The particle size ranged from 236 to 301 nm, while the zeta potential ranged from -22 to -30 mV. Among all formulations, F7 exhibited the smallest particle size of 236 nm, the highest negative zeta potential of -30 mV, and maximum entrapment efficiency of 92.20%. In-vitro drug release studies conducted over eight hours showed that F7 achieved the highest cumulative drug release of 95.22%. Drug release kinetic analysis demonstrated the best fit with the zero-order model (R^2 = 0.983), followed by the Higuchi model (R^2 = 0.963). Stability studies conducted for three months under different storage conditions showed that the optimized formulation maintained drug release above the specified limit of 85%. The study concluded that formulation F7 was the optimized Nystatin-loaded ethosomal formulation based on its physicochemical characteristics, high entrapment efficiency, and satisfactory drug release profile. Nystatin-loaded ethosomes may represent a promising approach for topical antifungal drug delivery.

Keywords: *Nystatin, Ethosomes, Phosphatidylcholine, Topical drug delivery, Entrapment efficiency, Particle size, Sustained drug release.*

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INTRODUCTION:

Nanotechnology-based drug delivery systems have gained considerable attention in pharmaceutical research because of their ability to improve drug solubilization, enhance drug permeation, provide controlled drug release, and improve drug stability.[1] Among these systems, ethosomes have emerged as promising vesicular carriers for the delivery of both hydrophilic and lipophilic drugs. Ethosomes are soft, flexible lipid vesicles primarily composed of phospholipids, relatively high concentrations of ethanol, and water.[2] The presence of ethanol provides flexibility to the vesicular membrane and improves the interaction of vesicles with biological membranes. Ethosomes are particularly useful for topical and transdermal drug delivery because ethanol acts as a penetration enhancer by modifying the organization of stratum corneum lipids.[3] The phospholipid vesicles can interact with skin lipids, facilitating drug deposition and penetration into deeper skin layers. The formulation of ethosomes generally involves phospholipids such as soya lecithin or phosphatidylcholine, ethanol, and an aqueous phase. Nystatin-loaded ethosomes offer a promising approach for improving topical antifungal drug delivery.[4] Encapsulation of nystatin into ethosomal vesicles may improve its dispersion within the formulation, enhance drug deposition in the skin, and provide sustained drug release at the application site.[5] The development of a nystatin ethosomal drug delivery system involves the preparation of vesicles using a suitable phospholipid concentration and ethanol content. Different formulations can be prepared by varying the concentrations of formulation components to identify the optimized formulation. The prepared ethosomes can be characterized for vesicle size, polydispersity index, zeta potential, entrapment efficiency, morphology, pH, drug content, and in vitro drug release. [6]Nystatin is a polyene macrolide antifungal antibiotic widely used for the treatment of infections caused by Candida species. [7] The optimized nystatin ethosomal formulation is expected to provide improved drug incorporation, desirable vesicle characteristics, controlled drug release, and enhanced topical drug delivery compared with conventional formulations. The study may provide useful information regarding the potential application of ethosomal vesicular systems in the treatment of superficial fungal infections.

MATERIALS:

Nystatin was procured from Hetero Labs, Hyderabad. Phosphotidylcholine was obtained from Synpharma Research Labs, Hyderabad. Other chemicals and the reagents used were of analytical grade.

METHODOLOGY:

Formulation development

Table-1: Composition of Nystatin Ethosomes (F1 to F8)

S.No.	Ingredients (mg)	F1	F2	F3	F4	F5	F6	F7	F8
1	Nystatin	100	100	100	100	100	100	100	100
2	Phosphotidyl choline	100	200	300	400	500	600	700	800
3	Ethanol	5	5	5	5	5	5	5	5
4	Water	10	10	10	10	10	10	10	10

Cold Method Procedure [8]

Nystatin-loaded ethosomes were prepared by the cold method, which is one of the most commonly employed techniques for the preparation of ethosomal vesicles. Initially, the required quantity of phosphatidylcholine was accurately weighed and dissolved in the specified amount of ethanol under continuous magnetic stirring at room temperature (25 ± 2°C). The weighed quantity of Nystatin (100 mg) was then added to the ethanolic phospholipid solution and stirred until a clear solution was obtained. Since Nystatin is highly soluble in ethanol, it dissolved completely in the ethanolic phase. Separately, the required quantity of distilled water was maintained at the same temperature as the ethanolic solution. The aqueous phase was then added dropwise to the ethanolic lipid solution under continuous magnetic stirring at 700–1000 rpm. Stirring was continued for 30–45 minutes to facilitate the spontaneous formation of ethosomal vesicles. The resulting dispersion was further homogenized using a high-speed homogenizer at 10,000–15,000 rpm for 5–10 minutes to reduce vesicle size and obtain a uniform dispersion. If required, the formulation was subjected to probe sonication for 5–10 minutes to further decrease the particle size and improve vesicle uniformity. The prepared ethosomal suspension was transferred into

airtight amber-coloured glass containers and stored under refrigerated conditions (4 ± 2°C) until further evaluation.

CHARACTERIZATION

Particle size:

The average particle size of the prepared Nystatin-loaded ethosomes was determined using Dynamic Light Scattering (DLS) with a particle size analyzer (Malvern Zetasizer or equivalent). Before analysis, the ethosomal suspension was diluted appropriately with filtered distilled water (1:100) to avoid multiple scattering effects. The diluted sample was gently mixed and transferred into a disposable polystyrene cuvette. Measurements were performed at 25 ± 2°C with a scattering angle of 90°. The average vesicle size and polydispersity index (PDI) were recorded. Each measurement was carried out in triplicate, and the mean ± standard deviation was calculated.[9]

SEM analysis

The surface morphology of the Nystatin-loaded ethosomes was examined using Scanning Electron Microscopy (SEM). A small amount of the ethosomal suspension was placed on a clean aluminum specimen stub and allowed to dry at room temperature or under vacuum. The dried sample was coated with a thin layer of gold using a sputter coater to improve electrical conductivity. The coated sample was then placed in the SEM chamber and observed under suitable accelerating voltage (typically 10–20 kV). Images were captured at different magnifications to evaluate the shape, surface characteristics, and morphology of the ethosomal vesicles.[10]

Zeta Potential

The zeta potential of the prepared ethosomal formulations was determined using a Zeta Potential Analyzer (Malvern Zetasizer or equivalent) based on the principle of electrophoretic light scattering. The ethosomal suspension was diluted with filtered distilled water to obtain an appropriate concentration. The diluted sample was transferred into a folded capillary cell without introducing air bubbles. Measurements were performed at 25 ± 2°C, and the zeta potential values were recorded in millivolts (mV). Each formulation was analyzed in triplicate, and the average value was reported. The zeta potential was used to assess the surface charge and physical stability of the ethosomal vesicles..[11]

Drug entrapment efficiency

The entrapment efficiency of Nystatin-loaded ethosomes was determined by the ultracentrifugation method. Approximately 2 mL of the prepared ethosomal suspension was transferred into centrifuge tubes and centrifuged at 20,000 rpm for 60 minutes at 4°C using a refrigerated ultracentrifuge. After centrifugation, the clear supernatant containing the free (unentrapped) drug was carefully separated from the sedimented ethosomal vesicles. The amount of free Nystatin present in the supernatant was determined by suitable dilution with ethanol or methanol, followed by spectrophotometric analysis at the λmax of Nystatin (approximately 305 nm) using a UV–Visible spectrophotometer.[12] The sedimented ethosomal vesicles represented the entrapped drug. The percentage entrapment efficiency (%EE) was calculated using the following equation:

$$\text{EE}\ (\%) = \frac{\text{Total drug added} - \text{Free (unencapsulated) drug}}{\text{Total drug added}} \times 100$$

In-vitro Drug release studies

The in vitro drug release study of the prepared Nystatin-loaded ethosomal formulations was carried out using the dialysis membrane diffusion method with a Franz diffusion cell. An accurately measured quantity of the ethosomal formulation equivalent to 100 mg of Nystatin was placed in the donor compartment of the Franz diffusion cell. The hydrated dialysis membrane was carefully mounted between the donor and receptor compartments, ensuring that no air bubbles were trapped beneath the membrane. The receptor compartment was filled with phosphate buffer (pH 7.4) containing 20–30% ethanol to maintain sink conditions, as Nystatin is poorly soluble in aqueous media. The receptor medium was maintained at 37 ± 0.5°C and stirred continuously using a magnetic stirrer at 300–500 rpm throughout the experiment. At predetermined time intervals, 5 mL of the receptor medium was withdrawn and immediately replaced with an equal volume of fresh prewarmed dissolution medium to maintain a constant volume and sink conditions. The collected samples were suitably diluted, if necessary, and analyzed using a UV–Visible spectrophotometer at the λmax of Nystatin (approximately 305 nm). The cumulative percentage of drug released was calculated and plotted against time.[13]

Percentage of drug release was determined using the following formula.

$$\text{Perentage drug release} = \frac{\text{Da}}{\text{Dt}} \times 100$$

Where, Dt = Total amount of the drug in the patch

Da = The amount of drug released

Stability studies

The stability study of the prepared Nystatin-loaded ethosomal formulations was carried out according to the recommendations of the International Council for Harmonisation (ICH) guidelines to evaluate the physical and chemical stability of the formulations during storage.[14]

RESULTS AND DISCUSSION:

Determination of Size

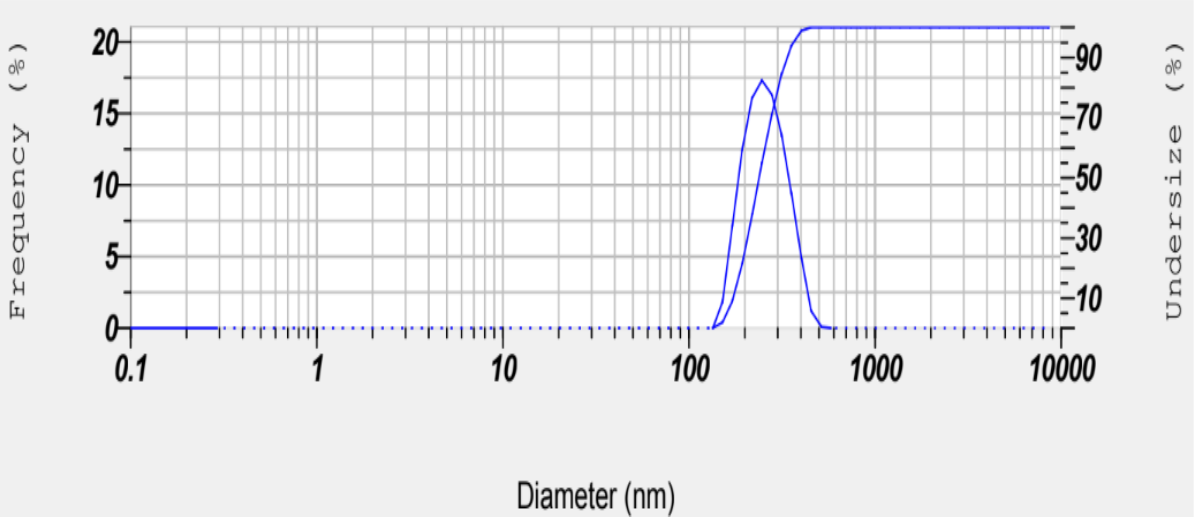

Fig-1: Particle size analysis of Optimized formulation of Ethosomes

Zeta potential

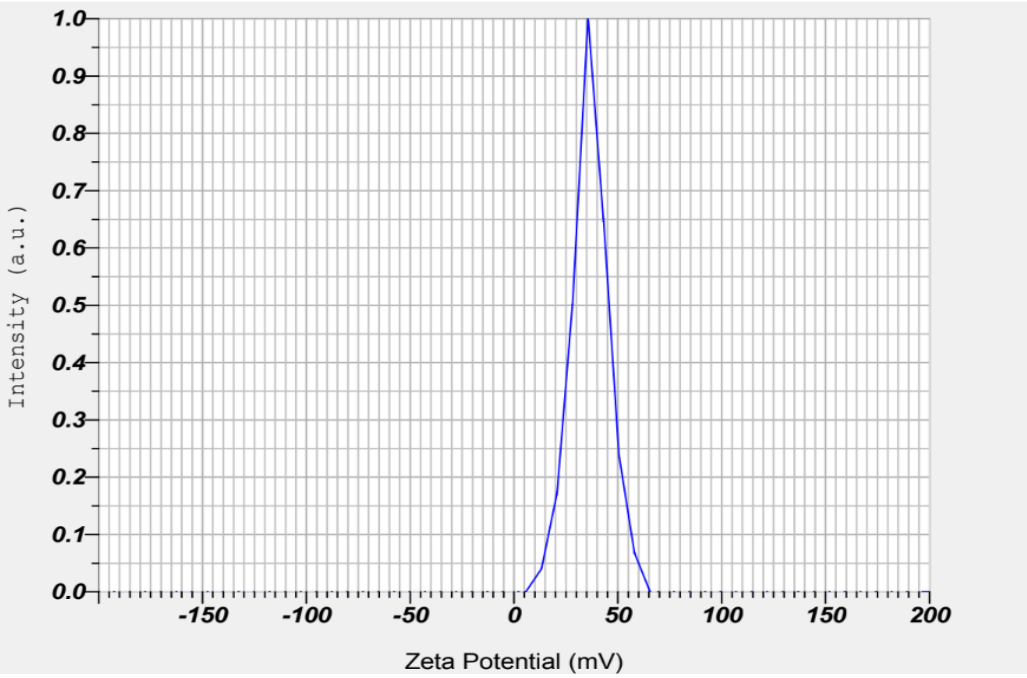

Fig-2: Zeta potential of Ethosomes

Table-2: Evaluation Studies of particle size and Zeta potential Ethosomes

F. No	Particle size (nm)	Zeta potential (mV)
F1	278	-26
F2	301	-27
F3	270	-25
F4	292	-27
F5	299	-28
F6	265	-27
F7	236	-30
F8	248	-22

SEM Analysis

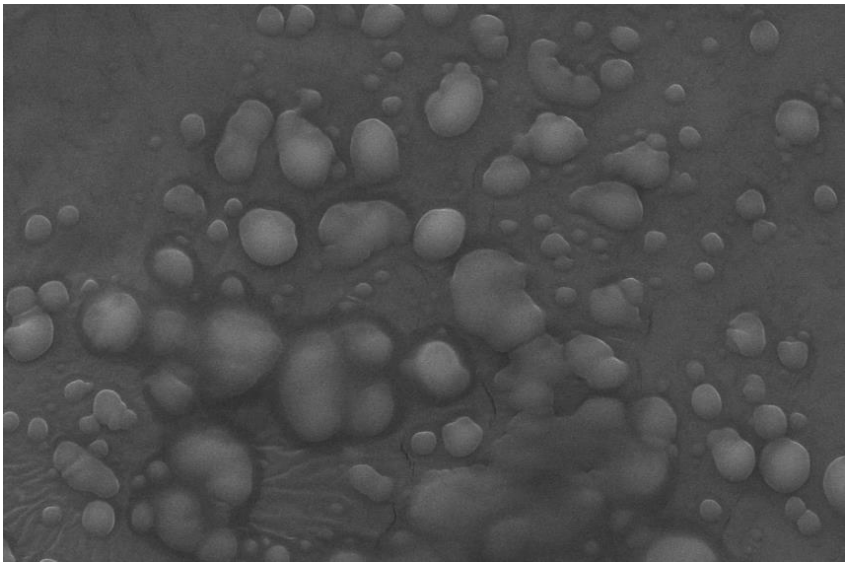

Fig-3: SEM Analysis of Ethosomes

Discussion

Particle Size Analysis

The particle size of the prepared ethosomal formulations (F1–F8) was evaluated to determine the vesicle size and uniformity of the formulations. The particle size was found to be in the range of 236–301 nm. Among all the formulations, F7 exhibited the smallest particle size of 236 nm, followed by F8 (248 nm), whereas F2 showed the largest particle size of 301 nm. The smaller particle size indicates better vesicle dispersion and may contribute to improved skin penetration and drug release.

Zeta Potential Analysis

Zeta potential analysis was performed to determine the surface charge and physical stability of the prepared ethosomal formulations. The zeta potential values ranged from -22 to -30 mV. F7 showed the highest negative zeta potential of -30 mV, indicating better electrostatic repulsion between vesicles and potentially improved resistance to aggregation. F5 showed a zeta potential of -28 mV, while F8 exhibited the lowest negative zeta potential of -22 mV. The negative zeta potential values indicate that the ethosomal vesicles possess a negatively charged surface. Formulations with higher absolute zeta potential values generally exhibit better electrostatic stability.

SEM Analysis

Scanning Electron Microscopy (SEM) was performed to study the surface morphology and shape of the prepared ethosomal vesicles. SEM analysis helps to evaluate the surface characteristics, vesicle shape and aggregation behaviour of the formulation. The SEM imageswere used to observe the morphological characteristics of the prepared ethosomes. The analysis provides supporting evidence regarding the formation and physical characteristics of the ethosomal vesicles.

Drug entrapment Efficiency

Table-3: Drug entrapment Efficiency of all formulations

F. No	Drug entrapment efficiency (%)
F1	78.9
F2	80.66
F3	87.76
F4	82.21
F5	85.45
F6	88.6
F7	92.2
F8	91.22

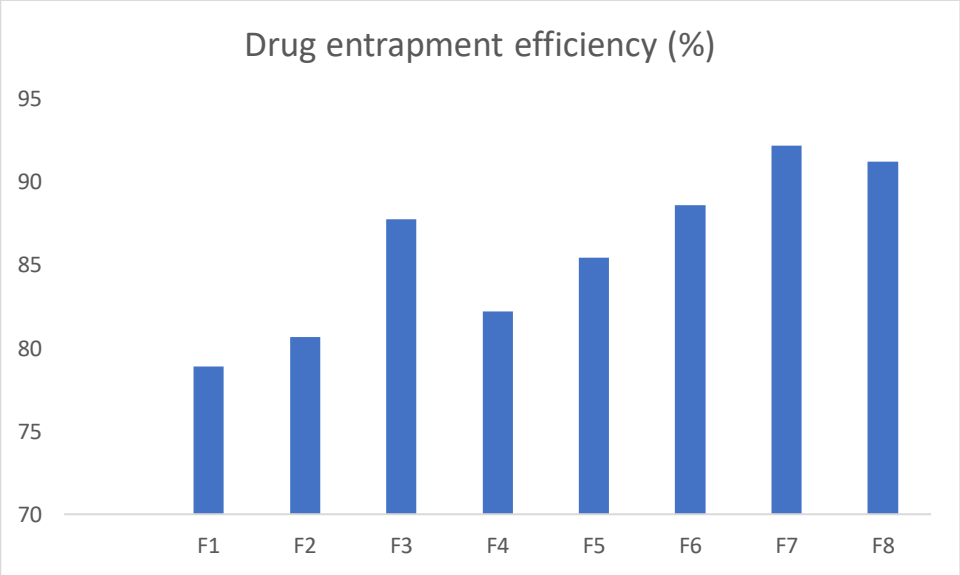

Fig-4: Drug entrapment Efficiency of all formulations

Discussion

The entrapment efficiency results demonstrated that increasing the phospholipid concentration influenced drug encapsulation. Among the eight formulations, F7 showed the highest entrapment efficiency of 92.20%. This may be attributed to the suitable phospholipid concentration, which could provide a favourable vesicular structure for drug incorporation.

***In vitro* release study**

Table-4: *In vitro* drug release profiles of Nystatin Ethosomes (F1-F8)

Time (hr)	F1	F2	F3	F4	F5	F6	F7	F8
0	0	0	0	0	0	0	0	0
1	12.65	15.21	11.89	14.76	13.22	16.55	18.12	11.17
2	23.2	27.21	20.12	25.65	24.9	29.65	32.8	21.45
3	35.38	39.33	31.37	37.19	36.55	42.35	45.26	33.68
4	46.56	51.9	42.56	49.31	48.12	53.12	58.31	44.28
5	57.1	63.87	53.67	60.33	59.66	66.09	70.32	55.21
6	67.58	73.12	63.12	70.56	69.22	74.59	80.37	65.36
7	76.09	82.68	72.4	79.41	78.8	83.55	88.44	74.22
8	88.8	89.9	81.31	87.1	90.12	91.47	95.22	92.55

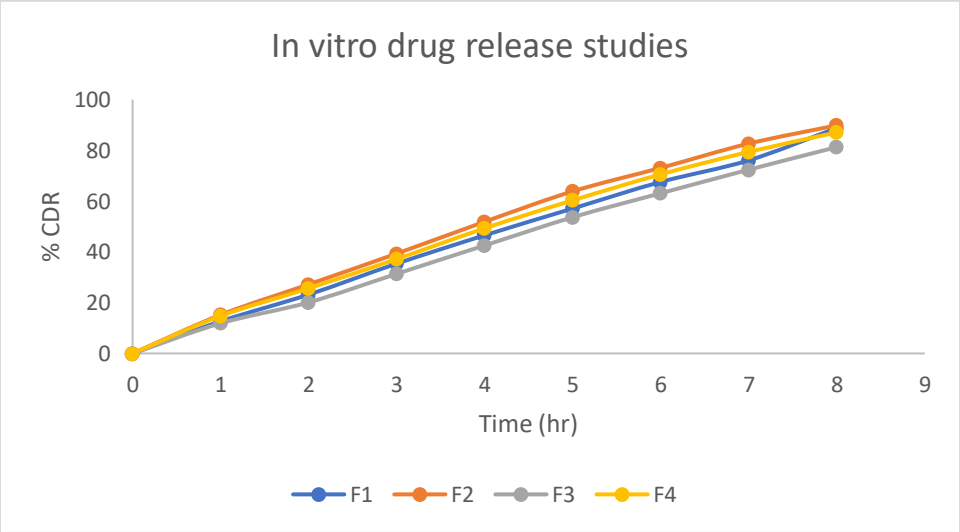

Fig-5: In vitro drug release studies of F1-F4 formulations

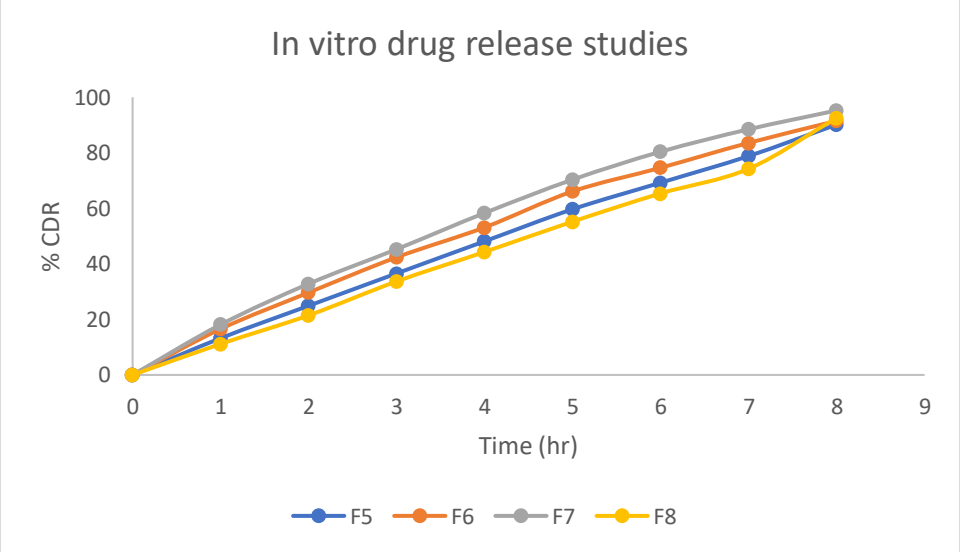

Fig-6 In vitro drug release studies of F1-F4 formulations

Discussion

The in-vitro drug release studies of Nystatin-loaded ethosomal formulations (F1–F8) were carried out over a period of 8 hours to evaluate their drug release characteristics. The cumulative percentage of drug release was determined at different time intervals from 0 to 8 hours.

The results indicated a gradual increase in drug release with increasing time for all eight formulations. At the initial time interval of 1 hour, the drug release ranged from 11.17% to 18.12%. At 4 hours, the drug release increased to 42.56–58.31%, indicating sustained drug release from the ethosomal vesicles.

At the end of 8 hours, formulation F7 showed a maximum drug release of 95.22%, followed by F8 (92.55%), F6 (91.47%), F5 (90.12%), F2 (89.90%), F1 (88.80%), F4 (87.10%) and F3 (81.31%).

Among all the formulations, F7 exhibited the highest cumulative drug release (95.22%), indicating better drug release performance compared with the other formulations. F3 showed the lowest drug release (81.31%) at 8 hours.

The improved drug release from F7 may be associated with its optimized vesicle characteristics, including the smaller particle size (236 nm) and higher absolute zeta potential (-30 mV). The nanosized vesicles may provide favourable surface area for drug release, while the negative zeta potential may contribute to better colloidal stability.

The drug release profile demonstrated that the ethosomal formulations provided gradual and sustained release of Nystatin over the studied period. The differences in drug release among the formulations may be attributed to variations in formulation composition, vesicle size, phospholipid concentration and ethanol content.

Kinetic modelling of drug release

Zero order kinetics

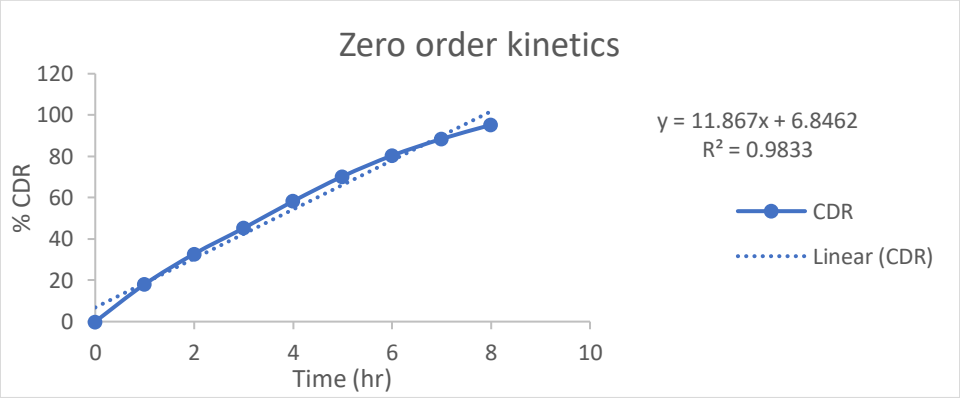

Fig-7: Zero order kinetics of optimized formulation

First order kinetics

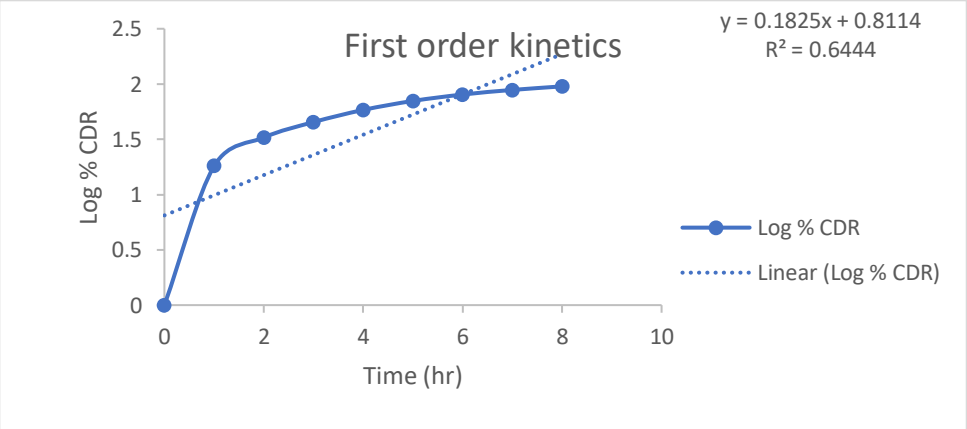

Fig-8: First order kinetics of optimized formulation

Higuchi model

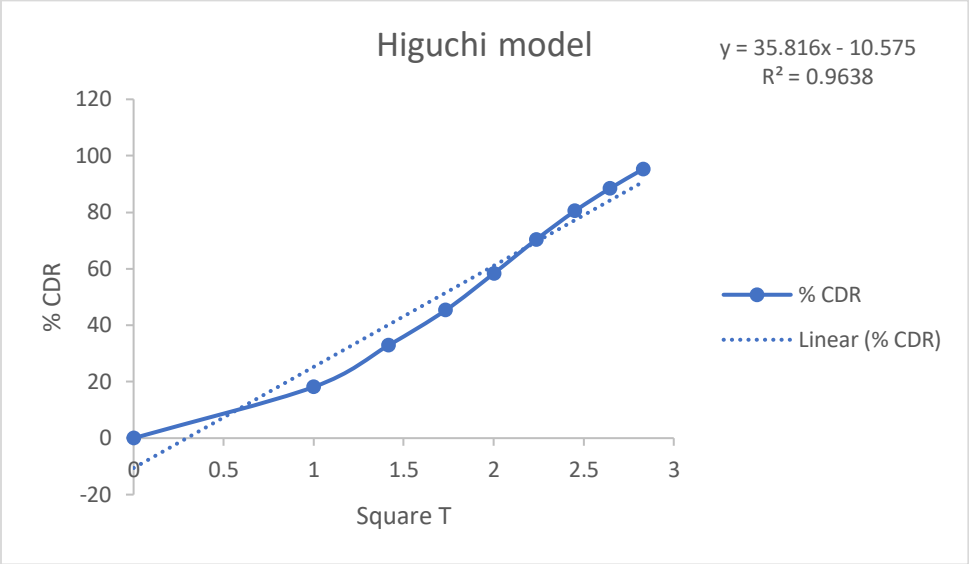

Fig-9: Higuchi model of optimized formulation

Korsmeyer peppas

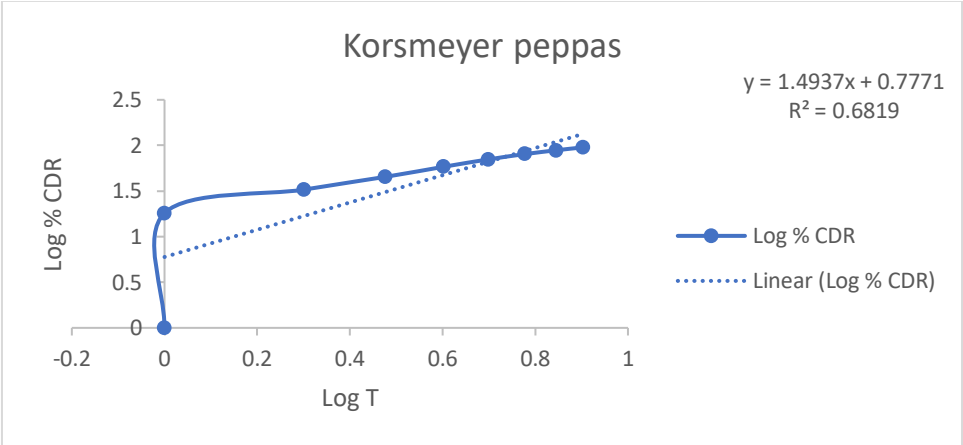

Fig-10: Korsmeyer peppas of optimized formulation

Based on the regression coefficient values, the optimized Nystatin ethosomal formulation F7 showed the best fit with the zero-order kinetic model, followed by the Higuchi model. The findings indicate that the optimized ethosomal formulation provides a promising sustained drug release profile over 8 hours.

Stability studies

Table-5: Results of stability studies of optimized formulation

F. Code	Parameters	Initial	1st Month	2nd Month	3rd Month	Limits as per Specifications
F-7	25^{0}C/60%RH % Release	95.22	94.39	93.20	92.19	Not less than 85 %
F-7	30^{0}C/75% RH % Release	95.22	94.12	93.19	92.16	Not less than 85 %
F-7	40^{0}C/75% RH % Release	95.22	94.09	93.29	92.12	Not less than 85 %

Discussion

Based on the stability study results, the optimized Nystatin ethosomal formulation (F7) demonstrated satisfactory stability over three months at 25°C/60% RH, 30°C/75% RH and 40°C/75% RH. All the tested formulations maintained drug release above the specified limit of 85%. Therefore, F7 can be considered a promising and relatively stable ethosomal formulation for further development. However, longer-term stability studies, along with evaluation of particle size, zeta potential, drug content and vesicle morphology, are necessary to establish its overall stability and determine its appropriate shelf life.

CONCLUSION:

The present study successfully developed and evaluated Nystatin-loaded ethosomal formulations using different concentrations of phosphatidylcholine. The prepared formulations demonstrated satisfactory physicochemical characteristics, good drug entrapment efficiency, and sustained in-vitro drug release. Among the eight formulations, F7 was identified as the optimized formulation based on its overall evaluation results. It exhibited a particle size of 236 nm, zeta potential of -30 mV, and maximum drug entrapment efficiency of 92.20%. Furthermore, F7 showed the highest cumulative drug release of 95.22% at the end of eight hours. The drug release kinetics demonstrated the best fit with the zero-order model, suggesting a comparatively consistent drug release profile. Stability studies also indicated that the optimized formulation retained more than 85% drug release after three months under the tested storage conditions. Thus, Nystatin-loaded ethosomes offer a promising approach for topical drug delivery, with the potential to improve drug encapsulation and provide sustained drug release. The optimized formulation F7 may be considered a suitable candidate for further pharmaceutical development. However, additional studies, including ex-vivo skin permeation, skin deposition, antifungal activity, skin irritation, long-term stability are required to establish its safety, efficacy, and clinical applicability.

REFERENCES:

1. Touitou E, Dayan N, Bergelson L, Godin B, Eliaz M. Ethosomes—novel vesicular carriers for enhanced delivery: characterization and skin penetration properties. J Control Release. 2000;65(3):403-18.
2. Barry BW. Novel mechanisms and devices to enable successful transdermal drug delivery. Eur J Pharm Sci. 2001;14(2):101-14.
3. Jain S, Tiwary AK, Sapra B, Jain NK. Formulation and evaluation of ethosomes for transdermal delivery of lamivudine. AAPS PharmSciTech. 2007;8(4):E111.
4. Dayan N, Touitou E. Carriers for skin delivery of trihexyphenidyl HCl: ethosomes vs. liposomes. Biomaterials. 2000;21(18):1879-85.
5. El Maghraby GM, Barry BW, Williams AC. Liposomes and skin: from drug delivery to model membranes. Eur J Pharm Sci. 2008;34(4-5):203-22.
6. Touitou E, Godin B, Dayan N, Weiss C, Piliponsky A, Levi-Schaffer F. Intracellular delivery mediated by an ethosomal carrier. Biomaterials. 2001;22(22):3053-9.
7. Uppadhyay Nirved, Vyash Lokesh, Joshi H.M. Formulation and evaluation of ethosomes of Sesbania grandiflora Linn. Seeds. Novel Science International Journal of Pharmaceutical Science. (2012); 1(6)
8. Abdul A, Mohammad R, Al MAM, Al JFI, Alam MA. Enhanced anti-inflammatory activity of carbopol loaded meloxicam nanoethosomal gels. Int J Biol Macromol., 2014;67:99-104.
9. Faisal W, Soliman GM, Hamdan AM. Enhanced skin deposition and delivery of voriconazole using ethosomal preparations. J Liposome Res. 2018;28(1):14-21.
10. Gupta P, Hafeez A, Kushwaha P. Development and evaluation of topical ethosomal gel for fungal infections. Drug Res (Stuttg). 2023;73(1):46-53.
11. Jafari A, Daneshamouz S, Ghasemiyeh P, Mohammadi-Samani S. Ethosomes as dermal/transdermal drug delivery systems: applications, preparation and characterization. J Liposome Res. 2023;33(1):34-52.
12. Mahajan K, Sharma P, Abbot V, Chauhan K. Ethosomes as a carrier for transdermal drug delivery system: methodology and recent developments. J Liposome Res. 2024;34(4):697-714.
13. Gupta P, Kushwaha P, Hafeez A. Development and characterization of topical ethosomal gel for improved antifungal therapeutics. J Mol Liq. 2024;405:125111.
14. Verma P, Pathak K. Nanosized ethanolic vesicles loaded with econazole nitrate for the treatment of deep fungal infections through topical gel formulation. Nanomedicine. 2012;8(4):489-96.