Development And Characterization of Nystatin Ethosomal Drug Delivery System

***Corresponding Author**

1. Dr. B. Premkumar M. Pharm., Ph.D.,

Professor & HOD,

Sree Abirami college of Pharmacy,

L & T byepass Road, Eachanari Post, Coimbatore -641021.

Affiliated to The Tamil Nadu Dr. M.G.R. Medical University, Chennai.

Email id: prmmab@gmail.com, Mob no: 9160165004

Co-authors

2. Gayathri. T

Sree Abirami college of Pharmacy,

L & T byepass Road, Eachanari Post, Coimbatore -641021.

Affiliated to The Tamil Nadu Dr. M.G.R. Medical University, Chennai.

3. Dr. D. Dhachinamoorthi M. Pharm., Ph.D.,

Dean & Principal,

Sree Abirami college of Pharmacy,

L & T byepass Road, Eachanari Post, Coimbatore -641021.

Affiliated to The Tamil Nadu Dr. M.G.R. Medical University, Chennai.

Abstract:

Nystatin, a broad-spectrum polyene antifungal, suffers from negligible oral bioavailability and significant gastrointestinal degradation, limiting its systemic therapeutic applications. Ethosomal vesicles, ethanolic phospholipid-based carriers, enhance dermal and transdermal delivery by increasing stratum corneum lipid fluidity and enabling deeper skin penetration. The present work focuses on the development and characterization of Nystatin-loaded ethosomes prepared by cold method using phosphatidylcholine, ethanol, propylene glycol, and Carbopol 934, with optimization based on vesicle size, entrapment efficiency, in vitro release, release kinetics, and stability. Among eight formulations (F1-F8), formulation F5 demonstrated optimal particle size, highest entrapment efficiency (92.74%), and sustained zero-order release (94.16% at 8 h) with good stability over three months at different storage conditions, indicating its suitability as a promising topical/transdermal antifungal delivery system.

Keywords: *Ethosomes, Nystatin, Transdermal delivery, Drug delivery system, Vesicular carriers, In vitro release.*

Introduction:

The pharmaceutical industry worldwide caters to the specific health needs of patients suffering from various diseases by designing and supplying suitable dosage forms called formulations. An urge for preparing specialized drug delivery systems has emerged to enhance bioavailability and deliver drugs at desired specific sites within the human body. Various advanced drug delivery systems have been developed, exemplified by Phytosomes, Nanoparticles, Liposomes, Transferosomes, Ethosomes, Niosomes, Cubosomes, and Nano emulsions. Among these, Ethosomes are in the forefront for improving bioavailability and delivering drugs to the desired specific site. They offer excellent patient compliance and can be used in both pediatric and geriatric populations. Although oral administration is the most frequently used dosage form route due to its ease of production, administration, and dosage accuracy, it has significant limitations including first-pass metabolism and destruction in the gastrointestinal tract. To rectify these defects, innovative dosage forms have been contemplated. Recent research has demonstrated that the skin plays a pivotal role as a very important medium for drug delivery into the systemic circulation.

Definition and Structure of Ethosomes:

Ethosomes are ethanolic liposomes, defined as noninvasive delivery carriers that enable drugs to reach deep into the skin layers and/or the systemic circulation. These are soft, malleable vesicles tailored for enhanced delivery of active agents. Ethosomes are lipid vesicles containing phospholipids, high concentrations of alcohol (ethanol and isopropyl alcohol) and water. The size range of ethosomes varies from tens of nanometers (nm) to microns (Î¼m). Ethosomes permeate through the skin layers more rapidly and possess significantly higher transdermal flux compared to conventional liposomes.

Advantages of Ethosomal Drug Delivery:

1. Delivery of large molecules (peptides, protein molecules) is possible
2. Contains non-toxic raw materials in formulation
3. Enhanced permeation of drug through skin for transdermal drug delivery
4. Applicable in pharmaceutical, veterinary, and cosmetic fields
5. High patient compliance through semisolid dosage forms (gel or cream)
6. Simple method for drug delivery compared to Iontophoresis and Phonophoresis

7. Passive, non-invasive system available for immediate commercialization
8. Increased permeation of drug over conventional liposomes

Composition and Mechanism:
Ethosomes are composed mainly of phospholipids (phosphatidylcholine, phosphatidylserine, phosphatidic acid), high concentration of ethanol (22-70%), and water. The high concentration of ethanol makes ethosomes unique, as ethanol disturbs skin lipid bilayer organization. When integrated into a vesicle membrane, this gives the vesicle the ability to penetrate the stratum corneum. Due to high ethanol concentration, the lipid membrane is packed less tightly than conventional vesicles but maintains equivalent stability, allowing a more malleable structure and improved drug distribution ability in stratum corneum lipids.

The Mechanism of Drug Penetration Occurs in Two Phases:
Ethanol effect: Ethanol acts as a penetration enhancer, increasing the fluidity of cell membrane lipids and decreasing the density of lipid multilayers.
Ethosomal effect: The combination of ethanol and vesicular structure results in enhanced skin permeability and deep layer penetration.

Nystatin: Drug Profile:
Nystatin is a polyene antifungal antibiotic with broad-spectrum fungicidal and fungistatic activity against yeasts and fungi, most notably Candida species. It is synthesized by Streptomyces noursei and acts by binding to ergosterol in fungal cell membranes, disrupting membrane integrity and leading to cell lysis. However, systemic use is limited due to very low oral bioavailability and significant toxicity in parenteral forms. Its clinical applications are currently restricted to topical, oral cavity, and gastrointestinal infections.

Materials And Methods:

Materials:
Nystatin was obtained as a gift sample from Hetero Labs. Soya lecithin (phosphatidylcholine), ethanol, propylene glycol, and Carbopol 934 were procured from AR Chemicals, Hyderabad. The major excipients included:

Preformulation Studies:

API Characterization:
Nystatin appeared as a yellow, odorless, tasteless powder, very slightly soluble in water and sparingly soluble in organic solvents, with a melting point around 160°C. A calibration curve was constructed in phosphate buffer pH 7.4 at 230 nm after suitable dilution of a methanolic stock solution, showing linearity in the range 10-50 µg/ml with correlation coefficient $R^2 = 0.9992$, slope 0.0113, and intercept 0.0042.

FTIR Studies:
FTIR spectroscopy of pure Nystatin and its physical mixture with formulation excipients showed characteristic bands for:
- OH stretching (3900-3910 cm^{-1})
- OH bending (1200-1480 cm^{-1})
- C-H stretching (2630-2840 cm^{-1})
- C=O stretching (1595-1640 cm^{-1})

Shifts were within acceptable limits (±100 cm^{-1}), indicating absence of significant drug-excipient interaction.

Formulation of Nystatin Ethosomes:

Ethosomes were prepared by the cold method as follows:
1. Nystatin (200 mg), phosphatidylcholine (50-400 mg), and other lipid components were dissolved in ethanol (10 ml) under continuous stirring at room temperature in a covered vessel
2. Propylene glycol (5 ml) was added during stirring
3. The mixture was heated to 30°C in a water bath
4. Pre-warmed water (30°C) was added in a fine stream under constant stirring for 5 minutes
5. The suspension was sonicated using a probe sonicator to reduce vesicle size to the desired range
6. The formulation was stored under refrigeration at 4°C

Eight formulations (F1-F8) were prepared varying primarily in phosphatidylcholine content (50-400 mg) while maintaining constant ethanol (10 ml), propylene glycol (5 ml), Carbopol 934 (1% w/w), and Nystatin (200 mg).

Table 1: Composition of Nystatin Ethosomal Formulations (F1-F8)

Ingredients	F1	F2	F3	F4	F5	F6	F7	F8
Nystatin	200	200	200	200	200	200	200	200
Phosphatidylcholine(mg)	50	100	150	200	250	300	350	400
Ethanol (ml)	10	10	10	10	10	10	10	10
Propylene glycol (ml)	5	5	5	5	5	5	5	5
Carbopol 943 (g)	1	1	1	1	1	1	1	1

Evaluation of Ethosomes:

Particle Size and Morphology:
Particle size and morphology were assessed by Scanning Electron Microscopy (SEM) and dynamic light scattering (DLS). Formulations were analyzed for spherical vesicles with sizes in the submicron range, varying with lipid concentration.

Entrapment Efficiency:
Entrapment efficiency was determined by ultracentrifugation at 15,000 rpm for 60 minutes. The sedimented vesicles were lysed in ethanol, and the drug content was quantified by UV spectrophotometry at 230 nm using the equation:
Entrapment Efficiency (%) = (Amount of drug in vesicles / Total amount of drug) ×100

***In Vitro* Drug Release:**
In vitro drug release was studied using a Franz diffusion cell with a dialysis membrane and phosphate buffer pH 6.8 at 37 ± 2°C. Samples were withdrawn at regular intervals (0, 1, 2, 3, 4, 5, 6, 7, 8 hours) and replaced with fresh medium. The cumulative percentage drug release was calculated.

Release Kinetics:

Release data were fitted to the following models:
- Zero-order: % R = Kt
- First-order: Log % unreleased = Kt / 2.303
- Higuchi: % $R=Kt^{0.5}$
- Kerseymere Peppas: %R=Kt n

Stability Studies:

Stability testing of formulation F5 (optimized) was performed at three ICH conditions:
- 25°C / 60% RH (long-term)
- 30°C / 75% RH (intermediate)
- 40°C / 75% RH (accelerated)
Samples were analyzed at 0, 1, 2, and 3 months for drug release and physical appearance.

Results & Discussion:

Preformulation Studies:

API Evaluation:

a) Organoleptic Evaluation:

Table 2: Organoleptic Properties of Nystatin

Properties	Results
State	Powder
Taste	Tasteless
Odour	Odorless
Colour	Yellow powder

b) Solubility:
Nystatin is very slightly soluble in water and sparingly soluble in organic solvents.

c)Determination of melting point:
Melting point of Nystatin was found in the range of 160°C, which complied with the standard, indicating purity of the drug sample.

Calibration Curve:

Standard graph of 7.4pH:

Preparation of primary stock solution: Take 10mg of drug and add 5ml of ethanol and dissolve it, to this add 5ml of 7.4pH buffer.

Preparation of secondary stock solution: Take 1ml of primary stock solution and add 9ml of 7.4pH buffer to it. Standard solutions of different concentration were prepared and their absorbance were measured. Calibration curve was plotted against drug concentration versus absorbance as given below.

Table 3: Calibration Curve of Nystatin

S. No.	Concentration (µg/ml)	Absorbance (230nm)
1.	0	0
2.	10	0.125

3.	20	0.223
4.	30	0.343
5.	40	0.461
6.	50	0.563

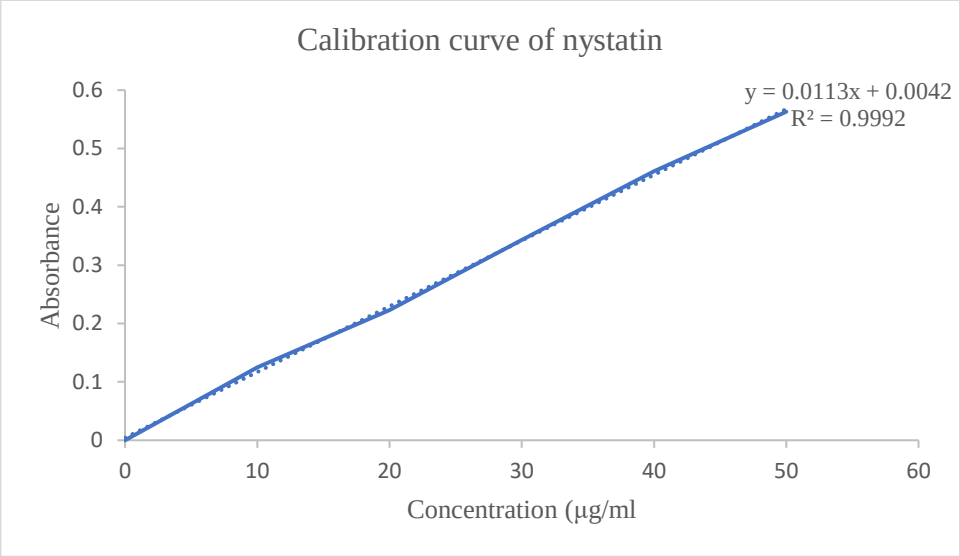

Figure 1: Calibration curve of Nystatin

Drug - Excipient Compatibility Studies (FT-IR):

Using the FTIR peak matching approach, the compatibility of the medicine with the chosen polymer and other excipients was assessed. The drug-polymer mixture showed no peaks that appeared or vanished, indicating that there was no chemical interaction between the medication, polymer and other molecules.

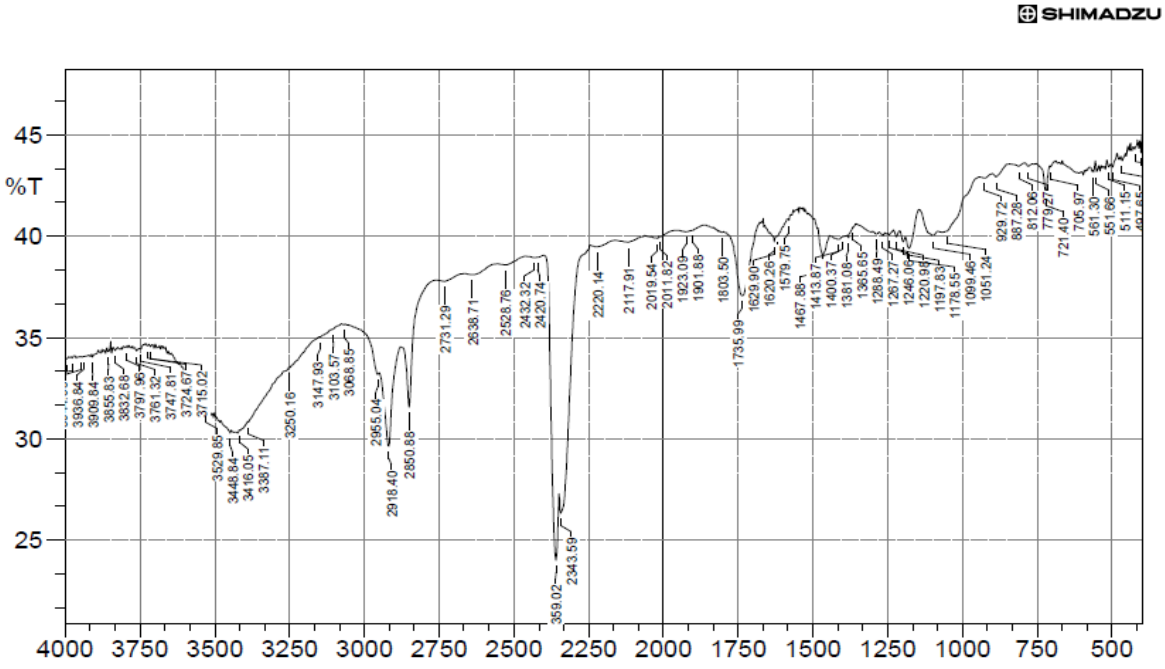

Figure 2: FTIR Spectra of Nystatin

Table 4: Characteristic Peaks and Frequency of Nystatin

S. No.	Characteristic Peaks	Frequency range (cm-1)	Frequency (cm-1)
1	OH stretching	4000-3500	3902.75
2	OH Bending	1500-1000	1204.65
3	C-H stretching	3000-2500	2839.31
4	C=O stretching	2000-1500	1599.93

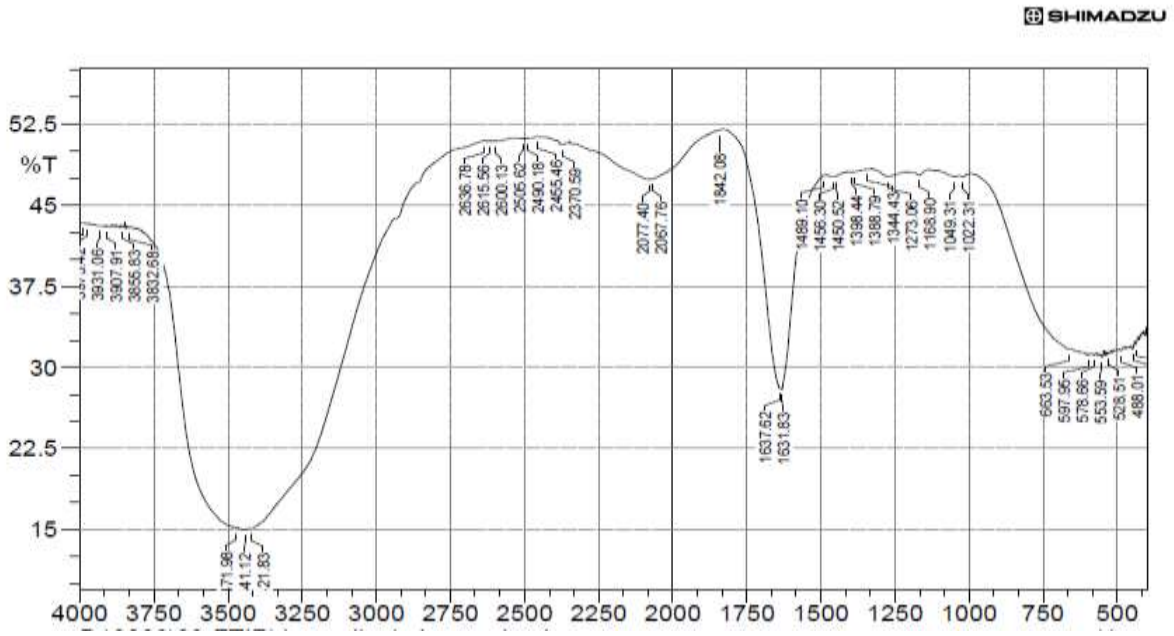

Figure 3: FTIR Spectra of Optimized Formulation

Table 5: Characteristic Peaks of Optimized Formulation

S. No.	Characteristic Peaks	Frequency range (cm-1)	Frequency (cm-1)
1	OH stretching	4000-3750	3907.65
2	OH Bending	1500-1000	1481.39
3	C-H stretching	3000-2500	2633.85
4	C=O stretching	2000-1500	1635.73

Compatibility studies were performed using IR spectrophotometer. The IR spectrum of Pure drug and physical mixture of drug and excipients were studied. The characteristic absorption of peaks was obtained as above and as they were in official limits (±100 cm-1) the drug is compatible with excipients.

Particle size:

Vesicle shape: Vesicle shape of the prepared formulation was found to be spherical from the SEM (scanning electron microscope) analysis at 15.00kV

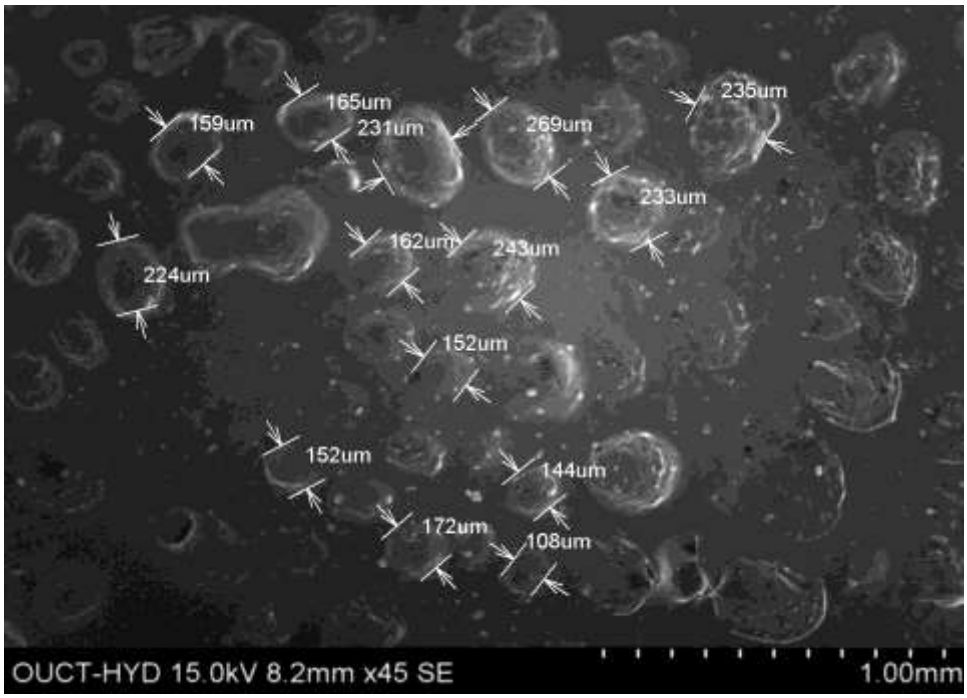

Figure 4: Particle size of optimized Formulation

Table 6: Mean Particle Size (Mps) Of Different Formulations Of Ethosomes

S. No	Formulation No.	Particle size(µm)
1	F1	165
2	F2	108
3	F3	231
4	F4	144
5	F5	224
6	F6	233
7	F7	269
8	F8	235

Drug entrapment efficiency:

Table 7: Different Batches of Ethosomes Made by Using Different Ratios of Lipids

S. No	Formulation no.	PDE
1	F1	83.98
2	F2	81.24
3	F3	72.45
4	F4	74.65
5	F5	92.74
6	F6	89.12
7	F7	74.22
8	F8	84.56

Drug release studies:

Table 8: Cumulative Percentage Drug Release from Various Formulations of Ethosomes

Time	Batch code							
	F1	F2	F3	F4	F5	F6	F7	F8
0	0	0	0	0	0	0	0	0
1	14.25	17.25	18.95	16.50	16.42	15.56	17.47	18.24
2	27.10	20.18	22.65	21.18	24.16	21.26	24.23	22.56
3	32.21	31.12	37.21	38.30	32.42	34.15	32.68	35.76
4	45.17	42.28	45.31	44.35	41.62	42.56	44.86	46.64
5	53.24	51.63	61.52	59.24	57.86	62.86	55.64	57.18
6	65.42	63.28	74.32	71.60	69.86	64.14	62.56	63.76
7	73.28	72.32	86.21	83.28	84.78	83.12	83.16	79.78
8	89.75	88.75	93.28	91.10	94.16	91.76	90.19	89.43

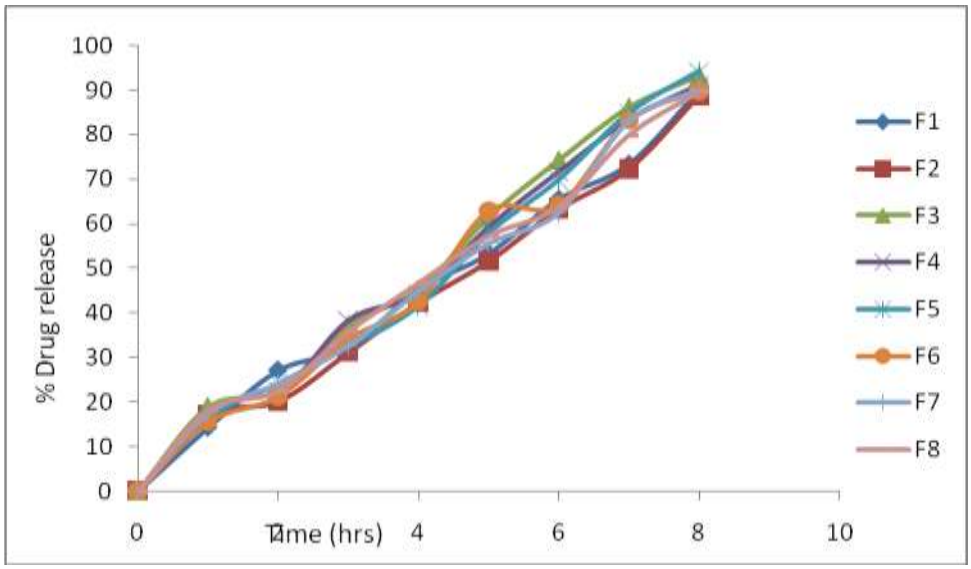

Figure 5: *In Vitro* Drug Release of Various Formulation

Table 9: Cumulative Percentage Drug Release of Formulation F5 Over 8 H

Time	%CDR	Log % CDR	Log T	Square T	ARA	Log % ARA
0	0	0	0	0	0	0
1	16.42	1.2153732	0	1	83.58	1.9221024
2	24.16	1.3830969	0.30103	1.414214	75.84	1.8798983
3	32.42	1.510813	0.477121	1.732051	67.58	1.8298182
4	41.62	1.6193021	0.60206	2	58.38	1.7662641
5	57.86	1.7623784	0.69897	2.236068	42.14	1.6246945
6	69.86	1.8442286	0.778151	2.44949	30.14	1.4791432
7	84.78	1.9282934	0.845098	2.645751	15.22	1.1824147
8	94.16	1.9738664	0.90309	2.828427	5.84	0.7664128

All the eight batches of formulation F5 were found to release the drug in 8 h. The cumulative percentage release was found to be 94.16 %.

Zero Order Kinetics:

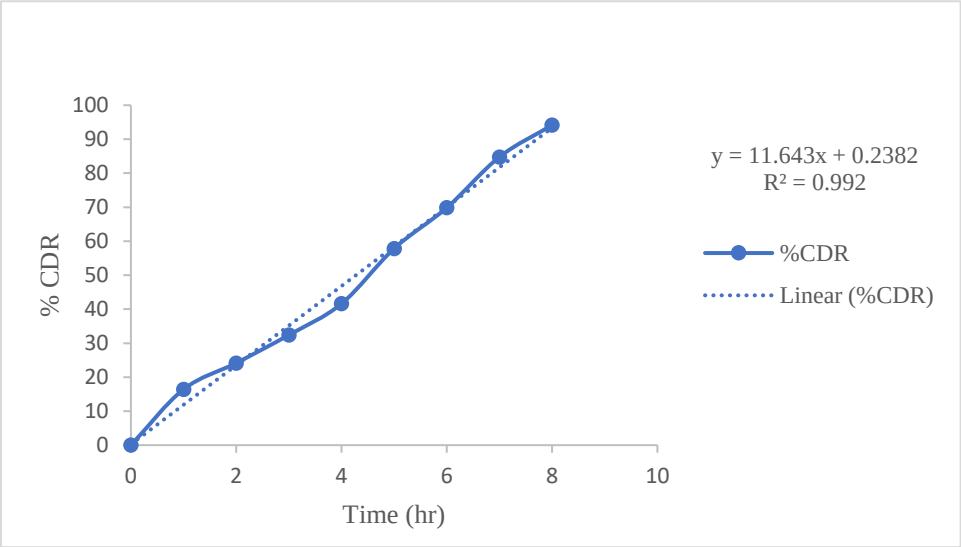

Figure 6: Zero Order Kinetics

First Order Kinetics:

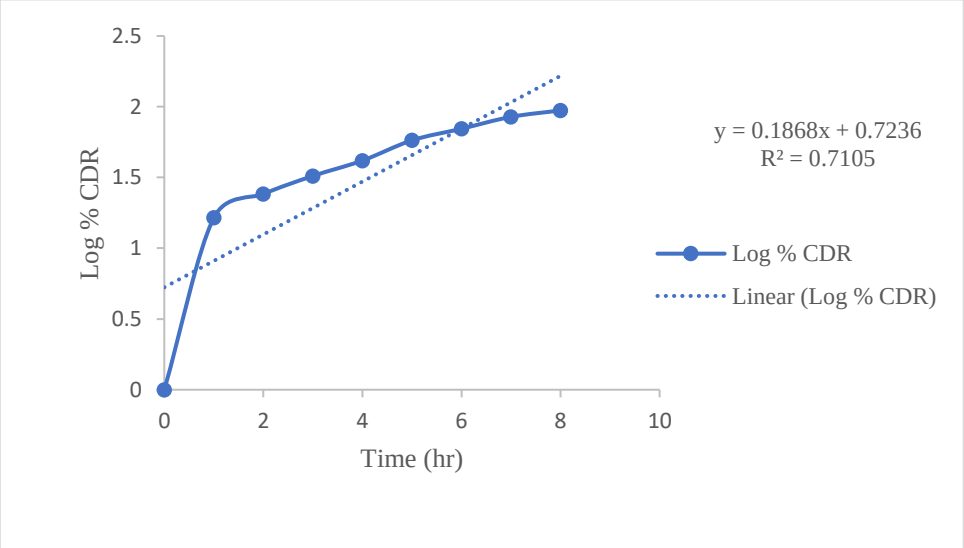

Figure 7: First Order Kinetics

Higuchi Model:

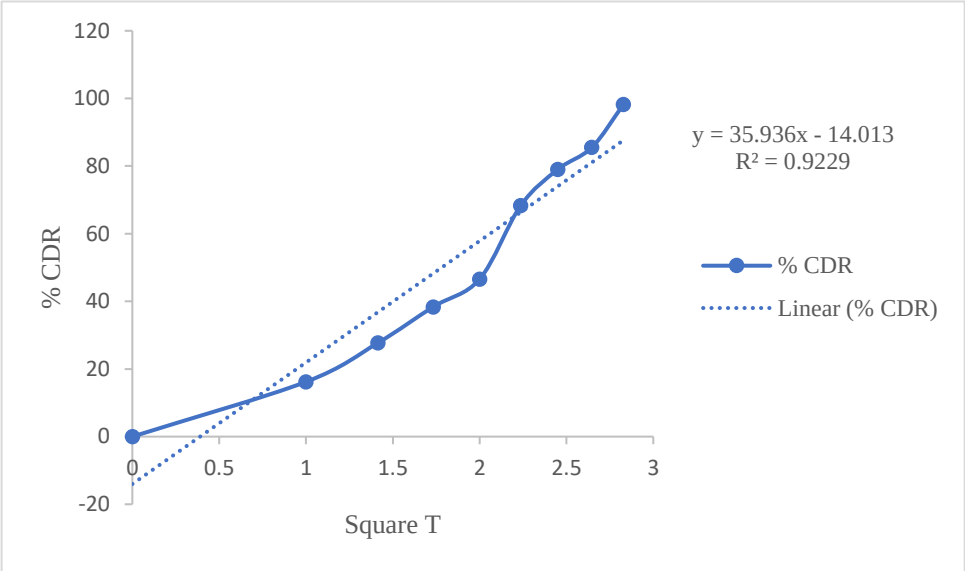

Figure 8: Higuchi Model

Korsmeyer – Peppas:

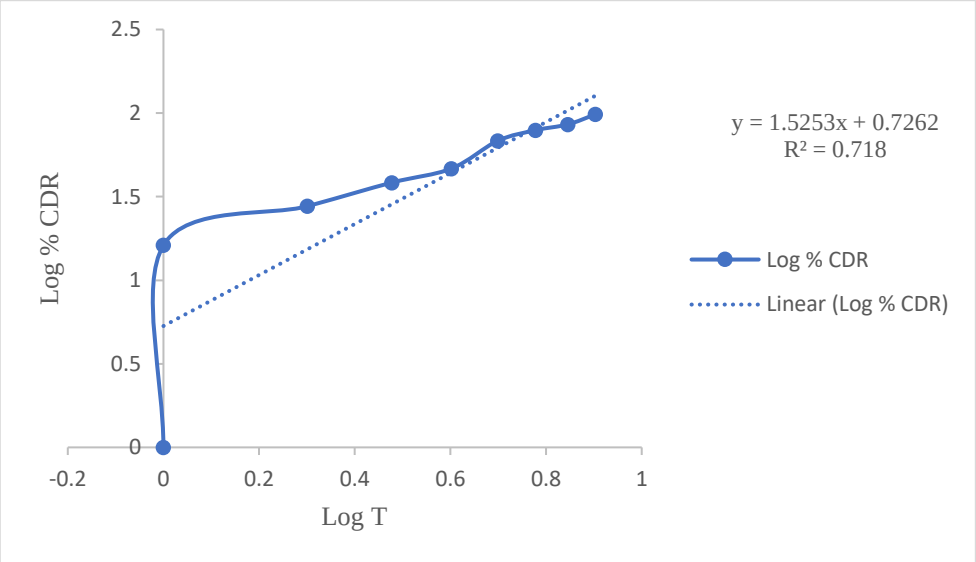

Figure 9: Korsmeyer – Peppas

The values of in vitro release were attempted to fit into various mathematical models. Plots of zero order, first order, Higuchi matrix and Peppas. Regression values are higher with Zero order release kinetics

Table 10: Regression Equations of Optimized Formulation

F. No	*In vitro* release in phosphate buffer pH 7.4 Regression values			
	Zero order	**First order**	**Higuchi Plot**	**Korsmeyer - Peppas**
F_5	0.992	0.710	0.929	0.718

The table indicates that r^2 values are higher for Zero order release kinetics. compared for all the formulations. Hence release from all the ethosomes followed diffusion rate-controlled mechanism.

Stability studies:

There was no significant change in physical and chemical properties of the tablets of formulation F-5 after 3 months. Parameters quantified at various time intervals were shown.

Table 11: Results of Stability Studies of Optimized Formulation F-5

Formulation Code	Parameters	Initial	1st Month	2nd Month	3rd Month	Limits as per Specifications
F-5	**25^0C/60%RH % Release**	94.16	93.20	93.17	93.11	**Not less than 85 %**
F-5	**30^0C/75% RH % Release**	94.16	93.21	93.18	93.12	**Not less than 85 %**
F-5	**40^0C/75% RH % Release**	94.16	93.18	93.11	93.08	**Not less than 85 %**

Discussion:

The preformulation studies confirmed that Nystatin is suitable for incorporation into a vesicular delivery system, with favorable spectroscopic and thermal characteristics and no evident incompatibility with selected excipients. The ethosomal system developed using the cold method successfully encapsulated Nystatin with high entrapment efficiency, particularly in F5, where optimized phosphatidylcholine content and constant ethanol level favored maximal drug loading and appropriate vesicle structure.

In vitro release studies showed controlled and extended drug release over 8 hours, with F5 achieving 94.16% cumulative release and exhibiting zero-order kinetics. This is desirable for maintaining relatively constant drug levels at the site of action or systemic circulation. Particle size and SEM data confirmed the formation of spherical, soft vesicles with size dependent on lipid content, consistent with ethosomal behavior reported for other drugs.

Stability studies demonstrated that the optimized formulation maintained its release characteristics over three months under various ICH-relevant storage conditions, indicating adequate physical and chemical stability for further development. The ethosomal system overcomes the limitations of conventional Nystatin

dosage forms by providing enhanced skin penetration, reduced systemic toxicity through localized delivery, and prolonged drug action.

Conclusion:

The present study successfully developed and characterized Nystatin-loaded ethosomes using the cold method. Eight formulations were prepared and evaluated for physicochemical and *in vitro* release characteristics. Formulation F5, containing 250 mg phosphatidylcholine, demonstrated optimal characteristics with: Maximum entrapment efficiency (92.74%), Appropriate particle size (224µm), Sustained zero-order release profile (94.16% at 8 h) & Good stability over three months at ICH conditions. The study establishes Nystatin-loaded ethosomes, particularly formulation F5, as a promising topical/transdermal antifungal delivery system capable of overcoming the low oral bioavailability and gastrointestinal degradation limitations of conventional Nystatin dosage forms. The sustained release and improved skin penetration properties of ethosomes make them suitable candidates for further in vivo studies and clinical development.

References:

1. Asbill, C. S., El-Kattan, A. F., & Michniak, B. (2000). Enhancement of transdermal drug delivery: Chemical and physical approaches. Critical Reviews in Therapeutic Drug Carrier Systems, 17, 621-650.
2. Gangwar, S., Singh, S., & Garg, G. (2010). Ethosomes: A novel tool for drug delivery through the skin. Journal of Pharmacy Research, 3(4), 688-691.
3. Heeremans, J. L. M., Gerristen, H. R., Meusen, S. P., Mijnheer, F. W., Gangaram, R. S., Panday, G., Prevost, R., Kluft, C., & Crommelin, D. J. A. (1995). The preparation of tissue type plasminogen activator (t-PA) containing liposomes: Entrapment efficacy and ultracentrifugation damage. Journal of Drug Targeting, 3, 301-308.
4. Jain, H., Patel, J., Joshi, K., Patel, P., & Upadhyay, U. M. (2011). Ethosomes: A novel drug carrier. International Journal of Clinical Practice, 7(1), 1-4.
5. Jain, S., Umamaheshwari, R. B., Bhadra, D., & Jain, N. K. (2004). Ethosomes: A novel vesicular carrier for enhanced transdermal delivery of an anti-HIV agent. Indian Journal of Pharmaceutical Sciences, 66, 72-81.
6. Kumar, K. P., Radhika, P. R., & Sivakumar, T. (2010). Ethosomes: A priority in transdermal drug delivery. International Journal of Advances in Pharmaceutical Sciences, 1, 111-121.
7. Kumar, R., Aslam, M. D., Tripathi, A., Prasad, D., Chaudhary, V., Jain, V., Mishra, S. K., & Singh, R. (2010). Ethosomes: Novel vesicular carriers in transdermal drug delivery. Journal of Global Pharma Technology, 2(6), 1-7.
8. Manosroi, A., Jantrawut, P., Khositsuntiwong, N., Manosroi, W., & Manosroi, J. (2009). Novel elastic nanovesicles for cosmeceutical and pharmaceutical applications. Chiang Mai Journal of Science, 36(2), 168-78.
9. Rakesh, R., & Anoop, K. R. (2012). Ethosome for transdermal and topical drug delivery. International Journal of Pharmaceutical Sciences and Research, 4(3), 17-24.
10. Rathore, A. R., Khambete, H., & Jain, S. (2013). Preparation and characterization of repaglinide loaded ethosomal gel for the treatment of NIDDM. International Journal of Pharmaceutical and Biological Archives, 4(2), 385-390.
11. Sachan, R., & Bajpai, M. (2013). Transdermal drug delivery system: A review. International Journal of Research and Development in Pharmacy and Life Sciences, 3(1), 748-765.
12. Shahwal, V., Samnani, A., Dubey, B., & Bhowmick, M. (2011). Ethosomes: An overview. International Journal of Biomedical and Advance Research, 2, 161-168.
13. Sivakranth, M., Anjuma Ara, P., Krishnaveni, C., & Venkatesh, E. (2012). Ethosomes: A novel vesicular drug delivery system. International Journal of Advances in Pharmaceutical Research, 2(1), 16-27.

14. Touitou, E. (2002). Drug delivery across skin. Expert Opinion on Biological Therapy, 2, 723-733.
15. Touitou, E., Dayan, N., Bergelson, L., Godin, B., & Eliaz, M. (2000). Ethosomes: Novel vesicular carriers for enhanced delivery. Journal of Control Release, 65, 403-418.
16.Touitou, E., Dayan, N., Levi-Schaffer, F., & Piliponsky, A. (1998). Novel lipid vesicular system for enhanced delivery. Journal of Lipid Research, 8, 113-120.
17. Touitou, E., Godin, B., Dayan, N., Weiss, C., Piliponsky, A., & Levi-Schaffer, F. (2001). Intracellular delivery mediated by an ethosomal carrier. Biomaterials, 22, 3053-3059.
18. Upadhyay, N., Mandal, S., Bhatia, L., Shailesh, S., & Chauhan, P. (2011). A review on ethosomes: An emerging approach for drug delivery through the skin. Recent Research in Science and Technology, 3(7), 19-24.
19. Verma, P., & Pathak, K. (2010). Therapeutic and cosmeceutical potential of ethosomes: An overview. Journal of Advanced Pharmaceutical Technology & Research, 1, 274-282.