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DEVELOPMENT AND IN-VITRO EVALUATION OF TERBINAFINE-LOADED TRANSFEROSOMAL GEL FOR DEEP DERMAL FUNGAL INFECTION

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Abstract:

The present study aimed to develop and evaluate a Terbinafine-loaded transferosomal gel for enhanced topical delivery in the treatment of deep dermal fungal infections. Transferosomes were prepared by the thin-film hydration method using soya lecithin, cholesterol, and Span 80 in varying concentrations. The prepared transferosomes were incorporated into Carbopol 934 gel base to obtain transferosomal gel formulations (F1–F8). Preformulation studies, including organoleptic evaluation, solubility analysis, melting point determination, UV spectrophotometric analysis, and FTIR studies, were carried out to confirm the identity and compatibility of the drug. Particle size analysis revealed vesicle sizes in the nanometric range (218–324 nm), with formulation F7 exhibiting the smallest particle size of 218 nm and highest entrapment efficiency of 79.86%. SEM studies confirmed spherical and smooth vesicles without aggregation. Zeta potential values ranged from -20 mV to -28 mV, indicating good physical stability of the transferosomes. The transferosomal gels showed acceptable pH, viscosity, and spread ability suitable for topical application. In vitro drug release studies demonstrated sustained release of Terbinafine over 12 hours, with formulation F7 showing maximum drug release of 97.86%. Drug release kinetic analysis indicated that the optimized formulation followed the Higuchi diffusion model with concentration-dependent release characteristics. Stability studies performed according to ICH guidelines confirmed that the optimized formulation remained stable for three months under different storage conditions without significant changes in drug release or physical appearance. The results concluded that Terbinafine-loaded transferosomal gel is a promising carrier system for enhanced topical delivery, improved permeation, sustained drug release, and effective treatment of deep dermal fungal infections.

Keywords: *Soya lecithin, FTIR studies, Thin film hydration method, Transferosomes, Gel, In vitro drug release studies*

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INTRODUCTION:

Fungal infections of the skin are among the most common dermatological disorders affecting millions of people worldwide. Deep dermal fungal infections, caused mainly by dermatophytes and opportunistic fungi, involve the invasion of fungi into deeper layers of the skin and surrounding tissues. These infections are often associated with symptoms such as itching, redness, inflammation, scaling, and discomfort, significantly affecting the quality of life of patients.[1] Terbinafine hydrochloride is a broad-spectrum antifungal agent belonging to the allylamine class. It acts by inhibiting the enzyme squalene epoxidase, thereby blocking ergosterol biosynthesis and causing accumulation of squalene within fungal cells, ultimately leading to fungal cell death.[2] To overcome these limitations, novel vesicular drug delivery systems have gained considerable attention. Among them, transferosomes are ultra-deformable lipid vesicles composed mainly of phospholipids and edge activators.[3] These vesicles possess excellent elasticity and deformability, enabling them to penetrate through narrow pores of the skin barrier more efficiently than conventional liposomes.[4] Transferosomes enhance drug permeation, improve drug deposition in deeper skin layers, provide controlled drug release, and reduce systemic side effects. Due to these advantages, transferosomes have emerged as promising carriers for transdermal and topical drug delivery applications.[5] Incorporation of terbinafine into transferosomal vesicles can improve the penetration of the drug into deep dermal tissues, thereby enhancing antifungal activity and reducing dosing frequency.[5] Furthermore, incorporation of transferosomes into a suitable gel base improves patient compliance by providing better spreadability, ease of application, prolonged residence time on the skin, and enhanced stability of the formulation.[7] Therefore, the present study focuses on the development and in-vitro evaluation of terbinafine-loaded transferosomal gel for the treatment of deep dermal fungal infections.[8]

MATERIALS AND METHODS:

MATERIALS

Terbinafine was procured from Hetero Labs, HYD. PLGA and polyvinyl alcohol were obtained from SD Fine Chemical, Mumbai. Other chemicals and the reagents used were of analytical grade.

METODOLOGY

Fourier transform infrared spectroscopy:

Fourier transform IR spectra were obtained on Shimadzu FT-IR spectrometer. Samples were prepared in KBr disks (2mg sample in 200mg KBr). The scanning range was 450-4000 $cm^{-1,}$ and the resolution was 4 cm^{-1}.[9]

Formulation development

Table-3: Formulation table

Ingredients	F1	F2	F3	F4	F5	F6	F7	F8
Terbinafine(mg)	20	20	20	20	20	20	20	20
Soya lecithin(mg)	100	200	300	400	500	600	700	800
Cholesterol(mg)	20	20	20	20	20	20	20	20
Span 80(mg)	10	10	10	10	10	10	10	10
Chloroform(ml)	10	10	10	10	10	10	10	10
Methanol(ml)	5	5	5	5	5	5	5	5

Preparation method transferosomes

Transferosomes containing Terbinafine were prepared by the thin film hydration method using different concentrations of soya lecithin as shown in formulations F1–F8. Accurately weighed quantities of Terbinafine, soya lecithin, cholesterol, and Span 80 were dissolved in a mixture of chloroform and methanol (2:1 ratio) taken in a clean, dry round-bottom flask. The organic solvents were evaporated under reduced pressure using a rotary vacuum evaporator at 40–45°C until a thin lipid film was formed on the inner wall of the flask. The formed film was further dried to remove traces of solvent completely.

The dried lipid film was then hydrated with phosphate buffer pH 7.4 while rotating the flask gently to obtain vesicular dispersion. Hydration was continued for about 1 hour to allow complete swelling of the lipid film. The resulting suspension was kept for maturation at room temperature for a suitable period. The obtained transferosomal dispersion was then sonicated using a probe sonicator or bath sonicator for a few minutes to reduce vesicle size and obtain uniform transferosomes. Finally, the prepared transferosomal formulations (F1–F8) were collected and stored in sealed containers under refrigerated conditions for further evaluation studies.[10]

Incorporation into gel base (Carbopol gel)

Table-1: Formulation development of transferosomal gel

Ingredients	F1	F2	F3	F4	F5	F6	F7	F8
Transferosomes (ml)	5	5	5	5	5	5	5	5
Carbopol 934 (mg)	100	200	300	400	500	600	700	800
Methyl paraben (ml)	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01
Glycerine (ml)	1	1	1	1	1	1	1	1
Water	q. s	q. s	q. s	q. s	q. s	q. s	q. s	q. s

Prepare Carbopol gel base

Transferosomal gel formulations of Terbinafine were prepared using Carbopol 934 as the gelling polymer. Required quantities of Carbopol 934 for formulations F1–F8 were accurately weighed and dispersed slowly in distilled water with continuous stirring to obtain a uniform dispersion. The polymer dispersion was allowed to hydrate and swell completely for about 24 hours to form a clear gel base. Methyl paraben was added as a preservative, and glycerine was incorporated into the hydrated Carbopol dispersion to improve smoothness and consistency of the gel. The mixture was stirred continuously until a homogeneous gel base was formed. Separately prepared transferosomal suspension (5 mL) was then added slowly into the gel base with gentle stirring to ensure uniform distribution of transferosomes throughout the formulation. The pH of the gel was adjusted to skin-compatible pH using triethanolamine until a smooth and transparent gel was obtained. The prepared transferosomal gel formulations were packed in suitable airtight containers and stored for further evaluation studies.[11]

CHARACTERIZATION[12,13,14]

Particle Size

The particle size of the Transferosomes were determined using Particle Size Analyzer (PSA) with the dynamic light scattering (DLS) method. The measurements were performed using Horiba Scientific SZ-100, with the sample diluted 10 times in aqueous medium at room temperature.

Zeta-potential:

The sample was diluted with distilled water (1:100 (V/V)) and zeta potential was determined using Malvern zetasizer (Nano ZS, Malvern Instruments, United Kingdom). Measurement was based on the electrophoretic mobility of the particles, which was converted to the zeta potential by inbuilt software based on the Helmholtz-Smoluchowski equation.

SEM analysis

The shape, surface characteristics, and size of the Transferosomes were observed by scanning electron microscopy. Once again, 0.2 g of the Transferosomes in a glass tube was diluted with 10 ml of pH 7.4 phosphate buffer. The Transferosomes were mounted on an aluminium stub using double-sided adhesive carbon tape. Then the vesicles were sputter-coated with gold palladium (Au/Pd) using a vacuum evaporator (Edwards) and examined using a scanning electron microscope (Hitachi 3700N, Germany) equipped with a digital camera, at 10 kV accelerating voltage.

EVALUATION PARAMETERS OF TRANSFEROSOMAL GEL

Physical evaluation:

The formulation was manually examined to check any variations in the color, odor, and texture.

Measurement of pH:

PH of each formulation was determined by using pH meter. This was calibrated before with buffer solutions.

Determination of viscosity:

The viscosity measurement of Transferosomal gels was determined by using a Brookfield viscometer. 30gm of gel preparation was kept in 50ml beaker, set at room temperature and spindle at 5, 10, 20, 50, and 100rpm.

In vitro release studies:

Transferosomal gel sample (0.5g) was placed on the membrane and diffusion study was carried out at 37^{o}C using 250ml phosphate buffer (pH 7.4) as receptor medium. 5ml of each sample was withdrawn periodically at 12hrs. Each sample was replaced with equal volume of fresh receptor medium. Samples were analyzed by UV-spectrophotometer for drug content using phosphate buffer.

Spread ability

Two sets of glass slides of standard dimensions were taken. The transferosomal gel formulation was placed over one of the slides. The other slide was placed on the top of the gel, such that the gel was sandwiched between the two slides in an area occupied by a distance of 7.5 cm along the slides. 1g weight of gel was placed on the upper slides so that the gel was between the two slides was pressed uniformly to form a thin layer. The weight was removed and the excess of gel adhering to the slides was scrapped off. The two slides in position were fixed to a stand without slightest disturbance and in such a way that only upper slides to slip off freely by the force of weight tied on it. A 200mg weight was tied to the upper slide carefully. The time taken for the upper slide to travel the distance of 7.5 cm and separated away from the lower slide under the influence of the weight was noted. The experiment

was repeated for three times and the mean time was taken for calculation.

Spread ability was calculated by using the following formula: S= m × l/t

where,

S= spread ability, m-weight tied to upper slides (1 g),

l- Length of the glass slide (7.5 cm), t- time taken in sec.

Drug entrapment efficiency

Each formulation (1 g) was taken in a 50 mL volumetric flask and made up to volume with 7.4 phosphate buffer and shaken well to dissolve the active constituents in 7.4 phosphate buffer. The solution was filtered through Whatman filter paper and 0.1 mL of the filtrate was pipetted out and diluted to 10 mL with 7.4 phosphate buffer. The content of active constituents was estimated spectro photometrically by using standard curve plotted.

In vitro diffusion profile

In vitro release study of the formulated Transferosomal gel was carried out by using diffusion cell through membrane as a dialysis membrane. Diffusion cell with inner diameter 24mm was used for the study. 1 mL formulation was placed in donor compartment and freshly prepared 7.4 phosphate buffer was placed in receptor compartment. Dialysis membrane was mounted in between donor and receptor compartment. The position of the donor compartment was adjusted so that the membrane just touches the diffusion medium. The whole assembly was placed on the thermostatically controlled magnetic stirrer. The temperature of the medium was maintained at 37°C ± 0.5°C. 1mL of sample was withdrawn from receiver compartment after 1, 2, 3, 4, 5, 6, 8,10 & 12 hrs and same volume of fresh medium was replaced. The withdrawn samples were diluted to 10mL in a volumetric flask with distilled water and analysed by UV spectrophotometer.

Drug release kinetics[15]

The models used were zero order (equation 1) First order (equation 2) and Higuchi model (equation 3) and Korsmeyer Peppas model (equation 4).

i) Zero order kinetics:

$R = K_o t$ -- (1)

R=cumulative percent drug

Ko=zero order rate constant

ii) First order kinetics

$\log C = \log C_o - K_1 t / 2.303$ -- (2)

where C = cumulative percent drug

K_1 = first order rate constant

iii) Higuchi model

$R = K_H t^{0.5}$ -- (3)

Where R = cumulative percent drug

K_H = higuchi model rate constant

iv) Korsmeyer peppas model:

$M t / M \alpha = K_k t^n$

$\log M t / M \alpha = \log K_k + n \log t$ -- (4)

where K_k = korsermeyer peppas rate constant

'M t / M α' is the fractional drug , n = diffusional exponent, which characterizes the mechanism of drug.

The obtained regression co-efficient (which neared 0.999) was used to understand the pattern of the drug from the Transferosomal gel.

Stability studies [16]

The main objective of the stability testing is to provide evidence on how the quality of the drug product varies with time under the influence of temperature and humidity. The stability study for the Transferosomal gel formulation was done as per ICH guidelines in a stability chamber for a period of 3 months.

RESULTS AND DISCUSSION:

FTIR Studies

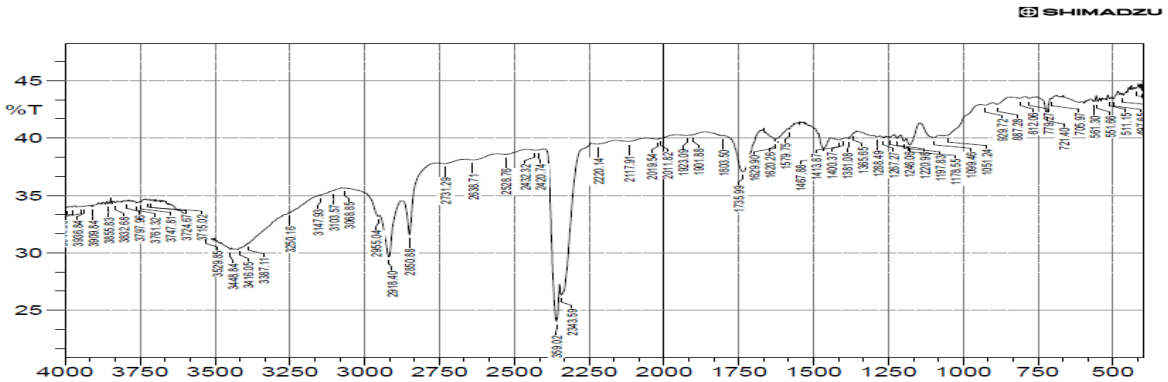

Fig-1: FTIR Spectra of Terbinafine

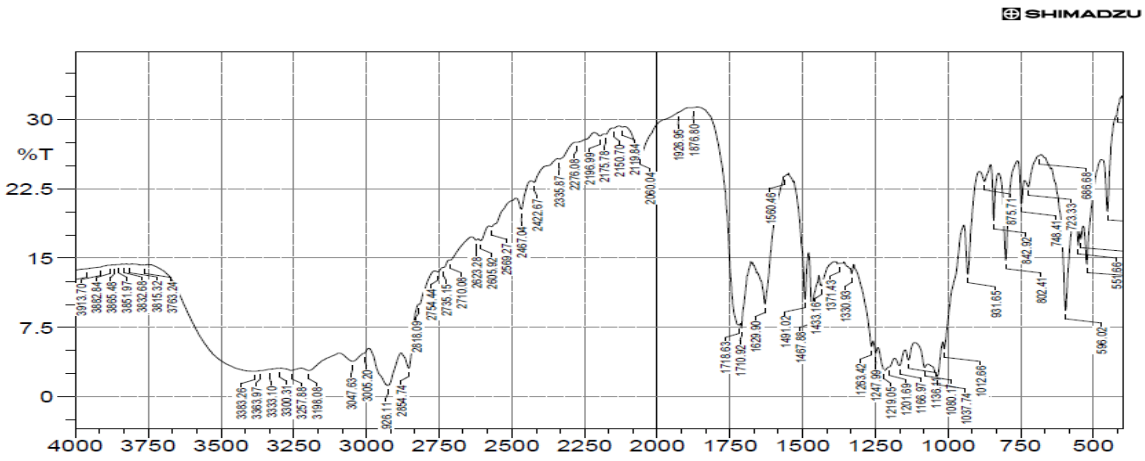

Fig-2 : FT-IR graph for optimise formulation

Determination of Vesicle morphology and Size

Particle size Analysis

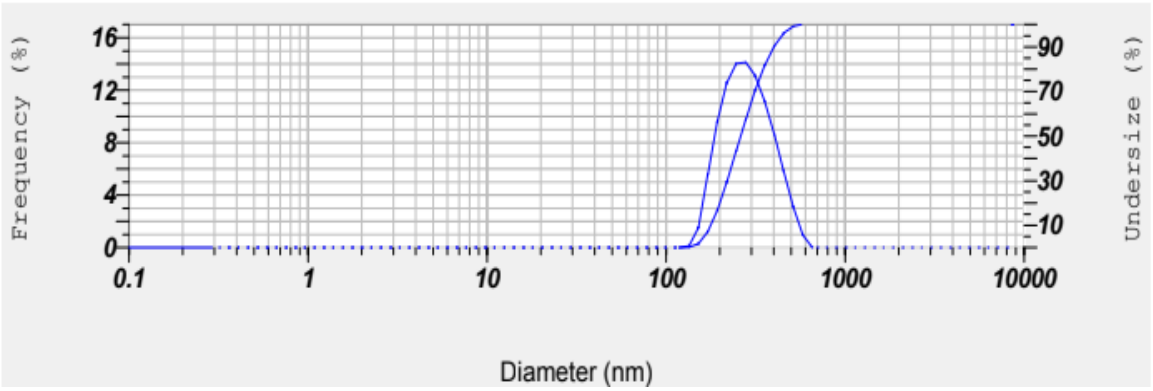

Fig-3: Particle size Analysis of Transferosomes

Discussion: The particle size analysis of the prepared Terbinafine transferosomes showed that all formulations possessed nanometric vesicle sizes ranging from 218 nm to 324 nm. Among all formulations, F7 exhibited the smallest particle size of 218 nm, which may be attributed to the optimum concentration of phospholipid and surfactant combination. Smaller vesicle size is advantageous for enhanced skin permeation and improved drug delivery through the stratum corneum. The narrow particle size distribution indicated uniform vesicle formation and stability of the transferosomal system. The optimized formulation F7 was selected for further studies due to its desirable nanosize and better entrapment efficiency.

SEM Analysis

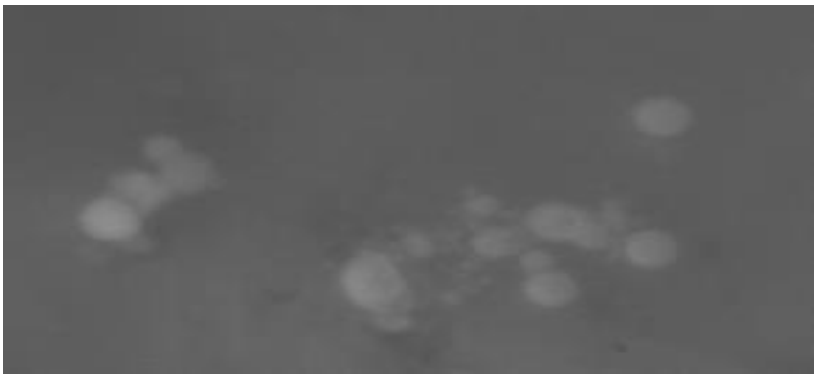

Fig-4: SEM analysis of transferosomes

Discussion: Scanning Electron Microscopy (SEM) was employed to study the surface morphology of the transferosomes. SEM images revealed that the vesicles were nearly spherical in shape, discrete, and uniformly distributed. The surface appeared smooth without any visible aggregation or crystal formation, indicating efficient vesicle formation and successful encapsulation of Terbinafine . The absence of drug crystals on the vesicle surface further confirms that Terbinafine was effectively entrapped within the lipid bilayer rather than remaining adsorbed on the surface. These morphological characteristics are favorable for stability and enhanced permeation of the transferosomal system.

Zeta potential

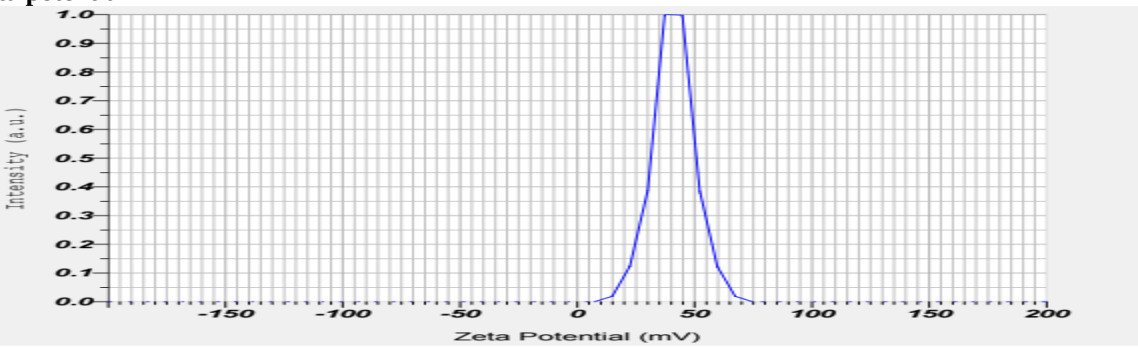

Fig-5: Zeta potential of Transferosomes

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

F. No	Particle size (nm)	Zeta potential(mV)
F1	248	-24
F2	236	-27
F3	269	-28
F4	273	-23
F5	286	-27
F6	324	-25
F7	218	-22
F8	316	-20

Discussion: The zeta potential values of all formulations ranged from -20 mV to -28 mV, indicating moderate stability of the prepared transferosomes. The negative surface charge may be due to the presence of phospholipids and surfactants in the vesicular system. Formulations with higher negative zeta potential values showed better physical stability because electrostatic repulsion prevents vesicle aggregation. Formulation F3 showed the highest zeta potential value (-28 mV), whereas F7 exhibited -22 mV, which was sufficient to maintain stability and dispersion uniformity during storage.

Yield of Transferosomes

Table-3: Yield of Transferosomes

F. No	Yield (%)
F1	69.89
F2	72.52
F3	70.15
F4	68.55
F5	73.72
F6	69.83
F7	80.21
F8	78.82

Discussion: The percentage yield of transferosomes was found in the range of 68.55% to 80.21%. Formulation F7 showed the highest yield of 80.21%, indicating efficient preparation methodology and minimal loss of materials during formulation processing. The high percentage yield confirmed the reproducibility and suitability of the thin-film hydration method for preparation of transferosomes. The variation in yield among formulations may be due to differences in lipid composition and surfactant concentration.

EVALUATION PARAMETERS:

P^H and Viscosity

Table-4: PH and Viscosity values of all formulations

F. code	pH	Viscosity (cps)
F1	5.6	170
F2	5.9	159
F3	6.2	163
F4	6.7	168
F5	5.6	171
F6	6.5	165
F7	6.8	163
F8	6.3	161

Discussion: The pH values of all gel formulations were found between 5.6 and 6.8, which are within the acceptable range for topical application and compatible with skin pH. This indicates that the prepared gels are less likely to cause irritation upon application. The viscosity values ranged from 159 cps to 171 cps, demonstrating good consistency and spreadability characteristics. The optimized formulation F7 showed a pH of 6.8 and viscosity of 163 cps, indicating suitable rheological behavior for easy application and retention on the skin surface.

Spread ability

Table-5: Spread ability values of all formulations

F. code	Spread ability (g.cm/sec)
F1	5.23
F2	5.42
F3	6.21
F4	5.36
F5	5.22
F6	5.20
F7	5.19
F8	5.36

Discussion: The spreadability values of the formulations ranged from 5.19 to 6.21 g.cm/sec. Adequate spreadability is an important property for topical formulations as it ensures uniform application over the skin surface. Formulation F3 exhibited the highest spreadability value, whereas F7 showed satisfactory spreadability of 5.19 g.cm/sec. The results indicated that all formulations possessed acceptable spreading characteristics due to optimum viscosity and gel consistency.

Entrapment Efficiency:

Table-6: Drug entrapment efficiency

F. code	Drug entrapment efficiency
F1	69.37
F2	70.25
F3	72.36
F4	73.68
F5	68.98
F6	75.16
F7	79.86
F8	75.15

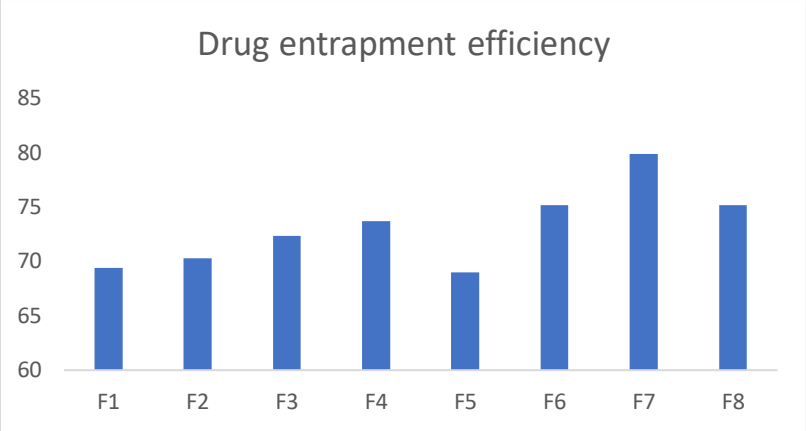

Fig-6: Drug entrapment efficiency of all formulation

Discussion: The entrapment efficiency of transferosomal formulations was observed between 68.98% and 79.86%. Formulation F7 showed the highest entrapment efficiency of 79.86%, indicating efficient incorporation of Terbinafine within the lipid bilayer vesicles. Higher entrapment efficiency may be due to the optimum ratio of lipid and surfactant, which enhanced drug accommodation within the vesicular structure. The high drug entrapment confirms the suitability of transferosomes as an effective carrier system for topical drug delivery.

***In vitro* release study:**

Table-7: *In vitro* drug release profiles of Transferosomal gel (F1-F8)

Time (hr)	F_1	F_2	F_3	F_4	F_5	F_6	F_7	F_8
0	0	0	0	0	0	0	0	0
1	17.63	18.25	17.63	18.15	16.89	17.46	19.58	18.42
2	29.86	27.69	28.12	26.98	27.46	28.95	29.86	27.46
3	41.25	42.36	39.86	43.26	44.58	45.58	49.58	47.63
4	55.67	56.39	54.71	55.98	56.93	59.36	58.45	57.48
6	65.98	66.38	67.13	68.19	67.73	66.58	69.36	65.42
8	75.81	74.59	72.59	73.56	75.45	74.53	75.81	76.39
10	82.25	83.15	81.98	82.15	83.69	82.36	86.58	84.58
12	91.17	93.36	94.56	95.25	96.28	95.12	97.86	96.37

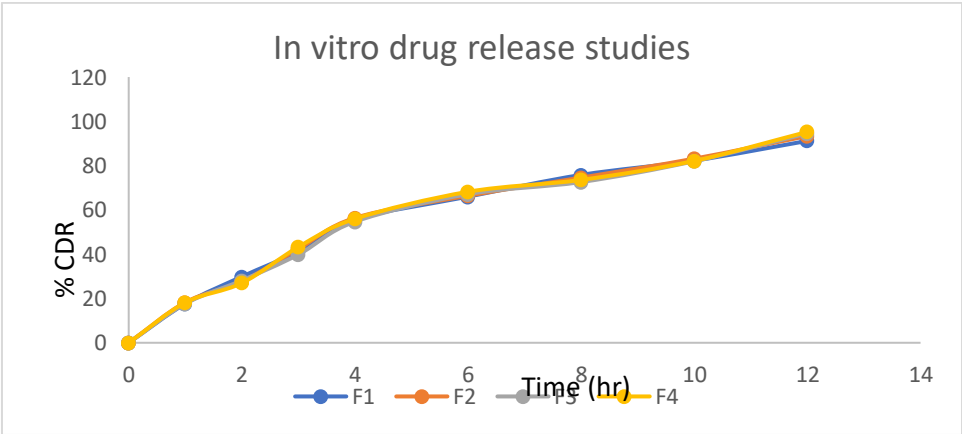

Fig-7: In vitro drug release studies of F1-F4 formulations

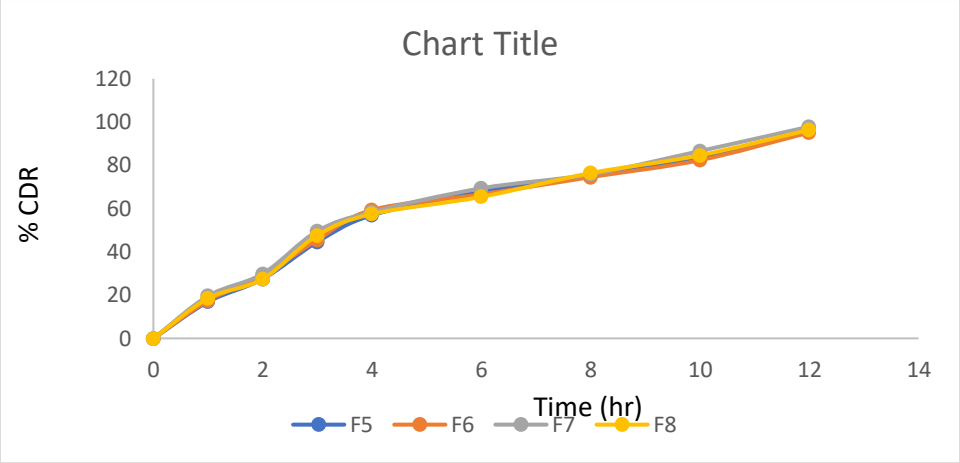

Fig-8: In vitro drug release studies of F5-F8 formulations

Discussion: The in vitro drug release study demonstrated sustained release behavior of Terbinafine from all transferosomal gel formulations over a period of 12 hours. Among all formulations, F7 showed the highest cumulative drug release of 97.86% at the end of 12 hours. The enhanced release profile of F7 may be due to smaller vesicle size, better entrapment efficiency, and optimum composition of edge activator and lipid. The transferosomal gel exhibited controlled and prolonged drug release, which is beneficial for maintaining therapeutic drug concentration for an extended period and improving patient compliance.

Kinetic modelling of drug release

All the 8 formulation of prepared transferosomal gel of Terbinafine were subjected to in vitro release studies these studies were carried out using diffusion apparatus.

Zero order kinetics

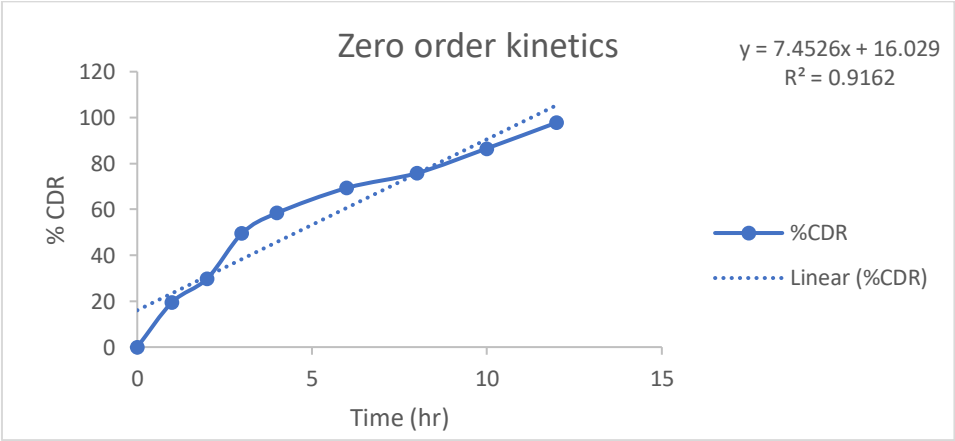

Fig-9: Zero order kinetics of optimized formulation

First order kinetics

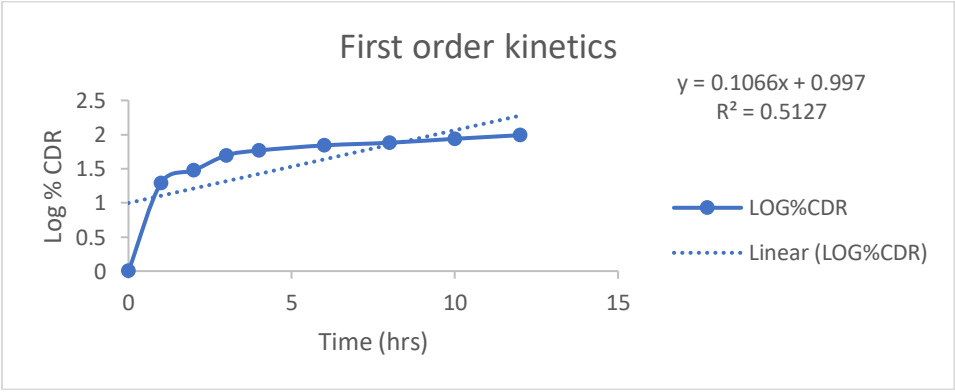

Fig-1: First order kinetics of optimized formulation

Higuchi model

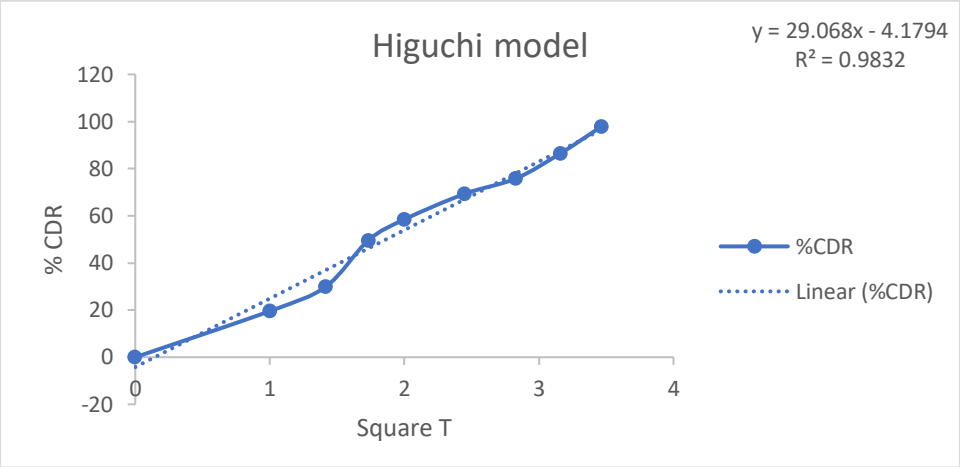

Fig-11: Higuchi model of optimized formulation

Korsmeyer peppas

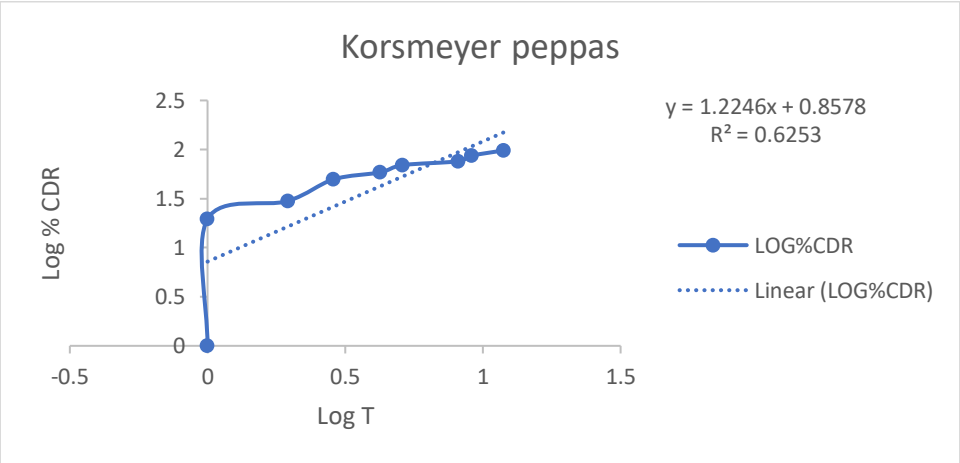

Fig-12: Korsmeyer peppas of optimized formulation

Discussion: Kinetic modelling of the release data for the optimized batch (F7) showed that the release profile fitted First-order kinetics with a good correlation coefficient, indicating that the release rate was concentration-dependent. The release mechanism followed the Higuchi diffusion model, suggesting that the predominant mechanism of drug liberation from the gel was diffusion through the hydrated matrix.

Stability studies

Table-12: Stability studies of optimized formulations at 40 ± 2 ^{0}C and 75 ± 5% RH for 3 months

Table-8: Stability studies of all formulations

F.no	Parameters	Initial	1st Month	2nd Month	3rd Month	Limits as per Specifications
F-7	25^{0}C/60%RH % Release	97.86	96.83	95.69	94.72	Not less than 85 %
F-7	30^{0}C/75% RH % Release	97.86	96.19	95.53	94.49	Not less than 85 %
F-7	40^{0}C/75% RH % Release	97.86	96.25	95.76	94.15	Not less than 85 %

Discussion: Stability studies of the optimized formulation F7 were carried out under different storage conditions for a period of three months. The formulation retained satisfactory drug release characteristics with only slight reduction in percentage drug release over the study period. The cumulative drug release remained above 94% under all storage conditions, which was within acceptable specification limits. No significant changes in physical appearance, drug release, or formulation performance were observed, indicating that the optimized transferosomal gel possessed good stability and suitability for long-term storage.

CONCLUSION:

The present study successfully developed Terbinafine-loaded transferosomal gel using the thin-film hydration technique and Carbopol gel base for enhanced topical drug delivery. The prepared transferosomal formulations exhibited desirable physicochemical characteristics, nanosized vesicles, good entrapment efficiency, satisfactory stability, and prolonged drug release behavior. Among all formulations, F7 was identified as the optimized formulation due to its smaller particle size, higher entrapment efficiency, excellent drug release profile, and acceptable rheological properties. The transferosomal gel provided sustained release of Terbinafine over an extended period, which may enhance drug penetration through the skin and improve therapeutic effectiveness in deep dermal fungal infections. Overall, the developed transferosomal gel system can be considered a promising and effective carrier for topical delivery of Terbinafine with improved stability, enhanced permeation, controlled drug release, and better patient compliance compared to conventional topical formulations.

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