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FORMULATION AND EVALUATION OF DIFAMILAST LOADED TRANSFEROSOMES FOR ENHANCED TOPICAL SKIN THERAPY

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Abstract:

The present study was undertaken to formulate and evaluate Difamilast-loaded transferosomes for enhanced transdermal drug delivery. Difamilast, a phosphodiesterase-4 (PDE-4) inhibitor used in the management of atopic dermatitis, possesses limited skin permeation when administered conventionally. FTIR studies demonstrated the compatibility of the drug with selected excipients, indicating the absence of any significant chemical interaction. The prepared transferosomal formulations exhibited particle sizes ranging from 122 to 135 nm and zeta potential values between −19 mV and −29 mV, indicating nanosized vesicles with good physical stability. Entrapment efficiency was found to range from 79.63% to 84.20%, confirming efficient drug incorporation within the vesicular system. In vitro diffusion studies revealed sustained drug release from all formulations over a period of 8 hours. Among the developed formulations, F4 exhibited the highest cumulative drug release (98.84%) and maximum entrapment efficiency (84.20%), indicating superior formulation performance. SEM analysis confirmed the formation of spherical, smooth, and non-aggregated transferosomal vesicles. Stability studies conducted on the optimized formulation (F4) for three months under different storage conditions showed no significant changes in drug release characteristics, confirming the stability of the formulation. The results suggest that Difamilast-loaded transferosomes are a promising vesicular drug delivery system capable of enhancing drug entrapment, improving stability, and providing sustained transdermal drug release, thereby offering potential therapeutic advantages for topical treatment.

Keywords: *Difamilast, Transferosomes, Transdermal Drug Delivery, Vesicular System, Entrapment Efficiency, Zeta Potential, In Vitro Drug Release, Stability Studies.*

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INTRODUCTION:

At present, the most common form of delivery of drugs is the oral route. While this has the notable advantage of easy administration, it also has significant drawbacks namely poor bioavailability due to hepatic first pass metabolism and the tendency to produce rapid blood level spikes (both high and low), leading to a need for high and/or frequent dosing, which can be both cost prohibitive and inconvenient. To overcome these difficulties, there is a need for the development of new drug delivery system; which will improve the therapeutic efficacy and safety of drugs by more precise (i.e. site specific), spatial and temporal placement within the body thereby reducing both the size and number of doses. New drug delivery system are also essential for the delivery of novel, genetically engineered pharmaceuticals (i.e. peptides, proteins) to their site of action, without incurring significant immunogenicity or biological inactivation. Apart from these advantages the pharmaceutical companies recognize the possibility of repattening successful drugs by applying the concepts and techniques of controlled drug delivery system coupled with the increased expense in bringing new drug moiety to the market1. One of the methods most often utilized has been transdermal delivery - meaning transport of therapeutic substances through the skin for systemic effect. Closely related is percutaneous delivery, which is transport into target tissues, with an attempt to avoid systemic effects.Transferosomes are such novel vesicular drug delivery systems whose uniqueness is an ultra deformable vesicle. It can squeeze itself through a pore, many times smaller than its size owing to its elasticity, designed to enhance the skin penetration and deliver the drug non-invasively through the skin barrier without measurable loss. Transferosomes have been widely used as carrier for the controlled and targeted delivery of proteins, peptides, hormones and several drugs. The oral delivery of peptides such as insulin and interferons, is impossible due to their instability and rapid degradation in the harsh environment of gastro intestinal tract.

MATERIALS:

Difamilast was procured from Synpharma Research Labs, Hyd, Cholesterol, Span 80, Lecithin, Chloroform and Methanol were obtained from Synpharma Research Labs, Hyderabad. Other chemicals and the reagents used were of analytical grade.

METHODOLOGY:

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}.

Formulation development

Table-1: Formulation table Trasferosomes

Ingredients	F1	F2	F3	F4
Difamilast(mg)	10	10	10	10
Soya lecithin(mg)	100	150	200	250
Cholesterol(mg)	20	20	20	20
Span 80(ml)	0.5	1.0	1.5	2.0
Chloroform (ml)	7.5	7.5	7.5	7.5
Methanol (ml)	2.5	2.5	2.5	2.5

Preparation method Trasferosomes

A thin-film hydration technique was employed to form transferosomes. Specific amounts of lipids were dissolved in a mixture of organic solvents consisting of methanol and chloroform (1:3) in a dry round-bottom flask. The evaporation of organic solvents was performed under vacuum using a rota evaporator at 80 rpm, at 48-50 °C. The thin lipid film thus obtained was then hydrated using water containing surfactant and drug by rotation for 1 hour at 50 °C. Each formulation was placed in an ultrasonicator bath at 150 W for 20 seconds. A dialysis bag was used to remove the free drug, and the final formulation was stored at 4 °C for further use. Blank transferosomes were prepared using the same method for each formulation.

Characterization

Vesicle size

Transferosomes vesicles can be visualized by SEM and optical microscope. The Morphological characterization of transfersome vesicle such as shape and surface feature were projected by using optical microscope and SEM.

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.

Determination of entrapment efficiency percentage:

The amount of Difamilast entrapped in transferosomes was estimated by centrifugation method. 1gm of Transferosomes was taken and diluted with 10ml phosphate buffer (pH 7.4). This suspension was sonicated using bath sonicator for 20 minutes. Later this solution was placed in centrifugation tube and centrifuged at 14000 rpm for 30 minutes. 0.5ml of supernatant was withdrawn and diluted before going for absorbance measurement using UV spectrophotometer (UV-2960 Lab India). This gives us the total amount of unentrapped drug. Entrapment efficiency is expressed as the percent of drug trapped.

$$\% \text{ Entrapment} = \frac{\text{Total drug} - \text{Diffused drug}}{\text{Total drug}} \times 100$$

In-vitro drug release studies

Modified Franz diffusion cell with a receiver compartment volume of 18ml and effective diffusion area of $2cm^2$ was used for this study. In-vitro drug study was performed by using dialysis membrane in phosphate buffer solution (pH 7.4). To perform in-vitro drug release study, dialysis membrane was mounted horizontally on the receptor compartment of Franz diffusion cell. The effective permeation area of donor compartment exposed to receptor compartment was $2cm^2$ and capacity of receptor compartment was 10ml. The receptor compartment was filled with 10ml of phosphate buffer (pH 7.4) maintained at 37± 0.5^0C and stirred by a magnetic bar at 100rpm. Transfersomal formulation equivalent to 80mg drug was placed on the cellophane membrane and the top of the diffusion cell was covered. At appropriate time intervals 5 ml aliquots of the receptor medium were withdrawn and immediately replaced by an equal volume of fresh phosphate buffer (pH 7.4) to maintain sink conditions. The samples were analyzed spectrophotometrically at λ max 254 nm.

Stability Studies of transferosomes

After measuring the initial percentage entrapment of the drug in the optimized formulation, the three batches of the same formulation were stored in sealed glass ampoules (one each) at refrigeration temperature (4±2ºC), room temperature (25±2ºC) and body temperature (37±2ºC) for a period of at least 3 months. The percentage entrapment of the drug and % drug content was determined in the formulations after 90 days to know the amount of drug leaked out. The percent drug lost was calculated taking the initial entrapment of drug as 100%.

RESULTS & DISCUSSION:

COMPATABILITY STUDIES:

FT-IR Spectrum of Difamilast

FT-IR Spectra of Difamilast and F6 formulation were recorded. All these peaks have appeared in formulation and physical mixture, indicating no chemical interaction between drug and excipients.

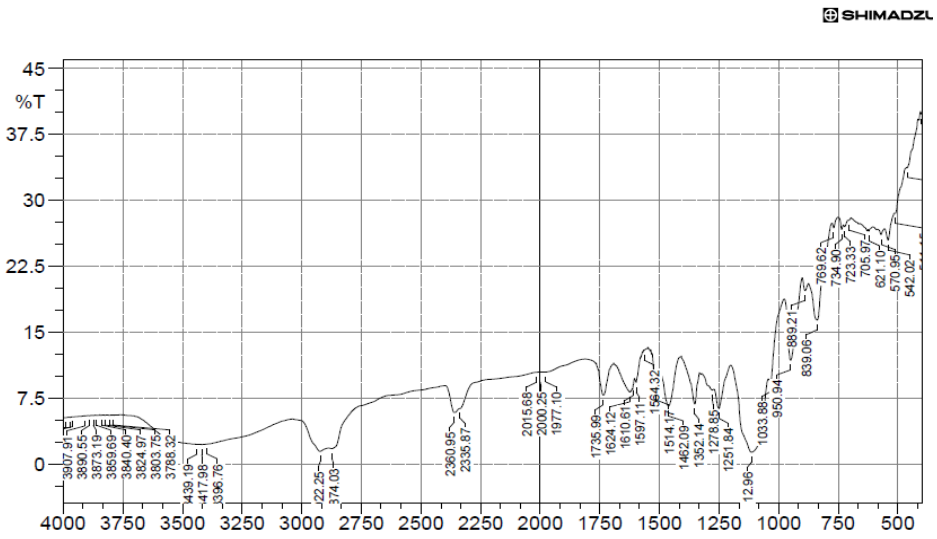


Fig1: FTIR Studies of Difamilast

Table-2: Characteristic Peaks for Difamilast

S.No.	Characteristic Peaks	Frequency range (cm-1)	Frequency (cm-1)
1	OH stretching	3200-3500	3342.55
2	OH Bending	3000-3100	3065.96
3	C-H stretching	2850-2950	2924.87
4	C=O stretching	1650-1750	1687.85

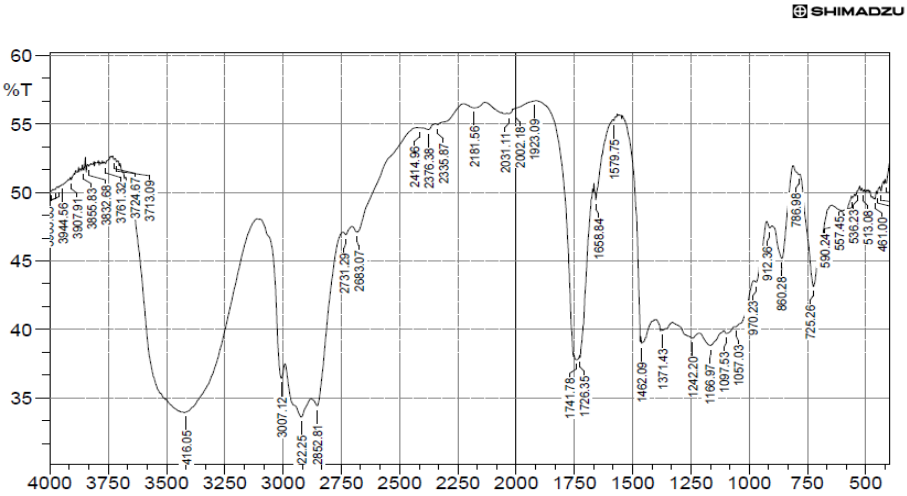


Fig-2: FTIR Studies of physical mixture of drug and excipients

Table-3: Characteristic Peaks for drug and excipients

S.No.	Characteristic Peaks	Frequency range (cm-1)	Frequency (cm-1)
1	OH stretching	3200-3600	3399.91
2	OH Bending	1350-1450	1450.05
3	C=O stretching	1700-1750	1736.20

FTIR spectra of drug in KBr pellets at moderate scanning speed be 4000-400 cm-1 was made. 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 of Transferosomes

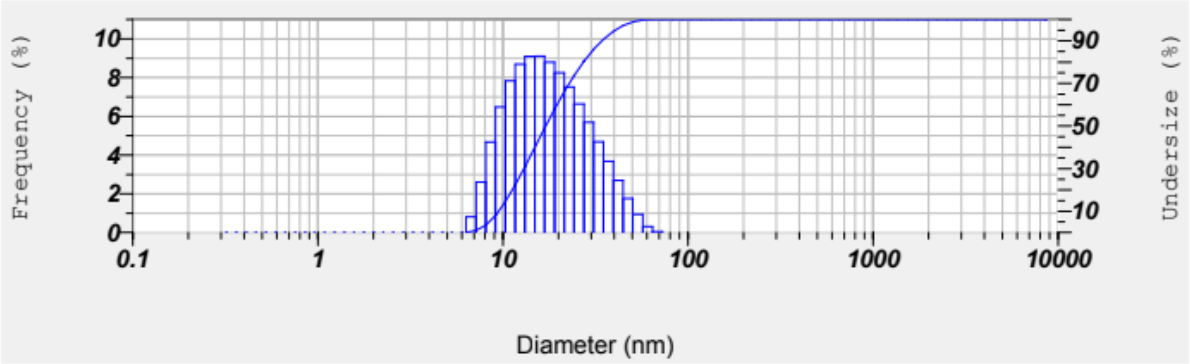


Fig-3: Particle size of Optimized formulation

The mean particle size of optimized transferosomes was found to be 122 nm

Table-4: Particle size

F. No	Particle size	Zeta potential(mV)
F1	131	-23
F2	135	-19
F3	122	-29
F4	125	-25

Zeta potential

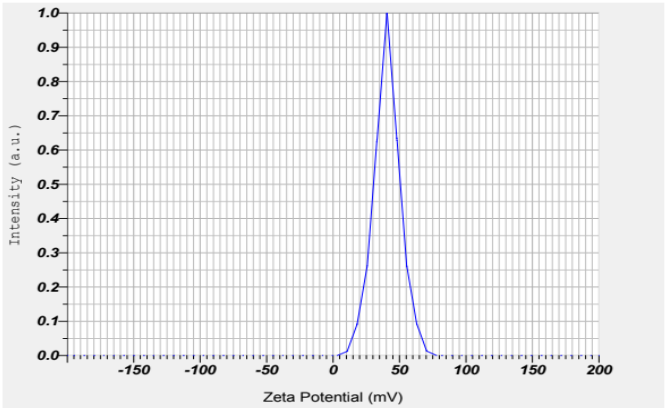


Fig-4: Zeta potential of Transferosomes

Discussion: The zeta potential values varied from –19 mV to –29 mV, indicating a sufficiently high negative surface charge to confer electrostatic repulsion between vesicles and thereby good physical stability of the dispersions. Among the batches, F3 (–29 mV) exhibited the highest absolute zeta potential, supporting better stability against aggregation.

SEM Analysis

Scanning electron microscopy (SEM) SEM revealed that the Difamilast transferosome's were smooth and spherical without any aggregation.

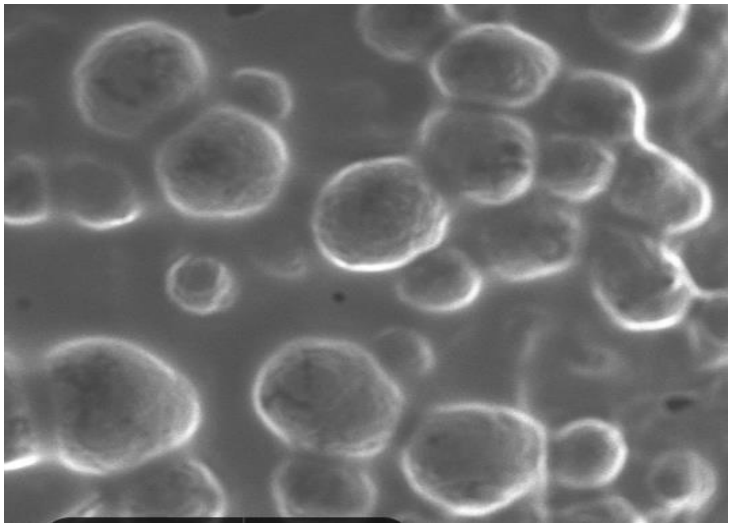


Fig-5: SEM Analysis of Transferosomes

Entrapment efficiency

Table-5: Percentage entrapment efficiency

F. No	% EE
F1	79.63
F2	81.23
F3	82.39
F4	84.20

The optimized formulation (F4) was selected based on its highest entrapment efficiency (84.20%), indicating superior drug loading capacity.

In vitro drug release studies

The diffusion medium used was phosphate buffer solution pH 7.4. In the Franz diffusion cell, the Dialysis membrane was mounted between donor and receptor compartment of diffusion cell. Area of membrane separating two compartments was 1.7662 cm^2. Transferosomes vesicle (2 ml) volume was accurately pipetted into donor compartment which was then covered with aluminum foil to avoid any evaporation. Diffusion medium (10 ml) was maintained at 37 ±1 ° C, so that the membrane just touches the receptor medium surface. A magnetic bar continuously stirred in the diffusion medium at 100 rpm. Each of 1 ml volume was withdrawn at required time intervals and replaced by 1 ml volume of receptor medium (phosphate buffer pH 7.4) to maintain the sink condition. These samples were analyzed by UV spectrophotometer at maximum wavelength 254 nm.

Table-6: Diffusion studies of Transferosomes

Time	F1	F2	F3	F4
0	0	0	0	0
1	27.10	24.60	26.39	30.25
2	35.13	32.99	38.56	42.16
3	45.32	55.37	56.83	49.80
4	56.35	65.10	67.89	69.86
6	78.10	77.52	78.15	79.54
8	94.50	95.69	92.38	98.84

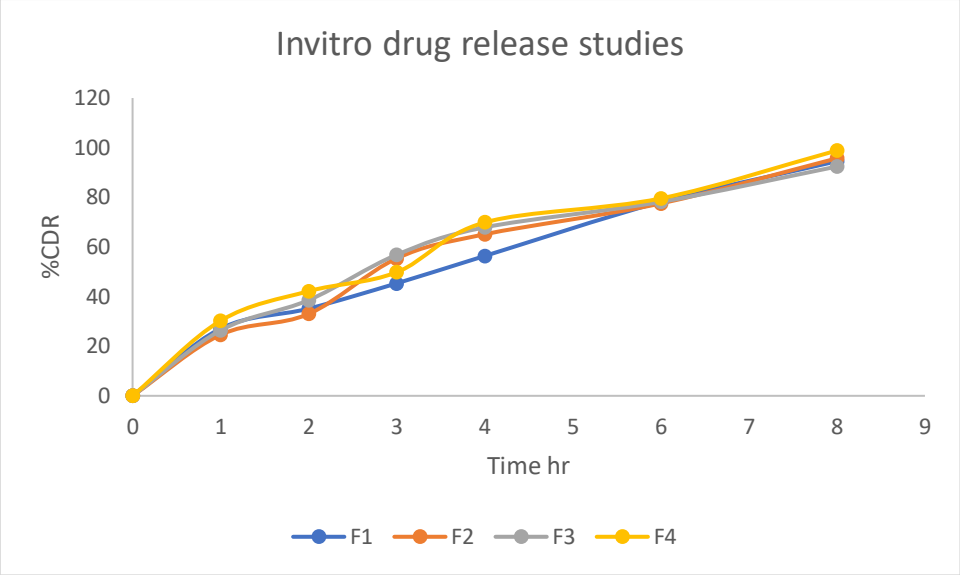


Fig-6: Diffusion studies of Transferosomes (F1-F4)

Stability studies:

There was no significant change in physical and chemical properties of the transferosomes formulation F4 after 3 months. Parameters quantified at various time intervals were shown.

Table-7: Results of stability studies of optimized formulation F-4

F. Code	Parameters	Initial	1st Month	2nd Month	3rd Month	Limits as per Specifications
F-4	25^{0}C/60%RH % Release	98.84	97.57	96.60	95.55	Not less than 85 %
F-4	30^{0}C/75% RH % Release	98.84	97.69	96.57	95.50	Not less than 85 %
F-4	40^{0}C/75% RH % Release	98.84	97.25	96.16	95.12	Not less than 85 %

CONCLUSION:

The present study successfully formulated and evaluated Difamilast-loaded transferosomes for enhanced transdermal drug delivery. FTIR studies confirmed the compatibility of Difamilast with the selected excipients, while SEM analysis revealed spherical, nanosized vesicles with good stability. Among all formulations, F4 was identified as the optimized formulation, exhibiting a particle size of 125 nm, entrapment efficiency of 84.20%, and maximum cumulative drug release of 98.84% over 8 hours. Stability studies demonstrated that the optimized formulation remained stable for three months without significant changes in drug release. These findings suggest that Difamilast-loaded transferosomes are a promising carrier system for sustained transdermal drug delivery and may be suitable for further in vivo studies.

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