Ashdin Publishing
Journal of Drug and Alcohol Research
Vol. 13 (2024), Article ID 236296, 13 pages
DOI: 10.4303/JDAR/236296

Research Article

Formulation and Optimization of Transferosomal Gel of Drug Fusdic acid using Central Composite Design

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Received: 01 May 2024; Manuscript No: JDAR-24-139330; **Editor assigned:** 03 May 2024; PreQC No: JDAR-24-139330 (PQ); **Reviewed:** 17 May 2024; QC No: JDAR-24-139330; **Revised:** 22 May 2024; Manuscript No: JDAR-24-139330 (R); **Published:** 29 May 2024; **DOI:** 10.4303/JDAR/236296

Abstract

Introduction: Impetigo can be treated by using Fusdic acid (FA), in the present study transferosomes of FA were prepared and incorporated into gel and studied for drug release, extent of permeability into the skin.

Methods: in the process of preparation of fusdic acid transferosomes variouslipids,edge activators with varying concentrations were tested and found that it had good solubility in Dimyristolyphosphatidylcholine and Tween 80. Rotary thin film hydration method was selected as a method of choice for preparation of Transferosomes. Various ranges of lipid: surfactant, stirring speed, %entrapment efficiency, %drug content, zeta potential, drug release etc parameters were studied for prepared transferosomes.

Results: Central composite design was used for optimizing the best formulation. Extrudability, viscosity, spreadability tests were performed for the prepared gels. CLSM studies were performed to identify the extent of drug permeability in to the skin layers.

Conclusion: From the above methods used and the results obtained, it was found that the Fusdic acid gel prepared by using FF7 formulation was concluded as the best.

Keywords: Fusdic acid; Transferosomes; Central composite design

Introduction

Fusidic acid is bacteriostatic agent obtained from the fungus Fusidium coccineum in 1962. It belongs from class fusidanes [1]. It is a steroid antibiotic of narrow spectrum, which is predominantly active against gram-positive bacteria. It is mainly active against Staphylococcus aureus, S. epidermis, Clostridium spp., and corynebacterial [2]. S. aureus is one of the species that is a leading threat to public health and causes morbidity or mortality [3]. Fusidic acid inhibits protein synthesis of bacteria by interfering with its elongation factor G (translocase) and may be by other mechanisms. Fusidic acid acts through 4 phases, i.e., Initiation, elongation, translocation, and release.

Fusidic acid is mainly used in skin and soft tissue infections. The common skin infections in which fusidic acid is used are impetigo, erythrasma, bullous impetigo, psoriasis, folliculitis, furuncles, carbuncles, contagiosa, infected wounds, and burns [4-6].

Transfersome consisting of a Phosphatidyl Choline (PC) and a non-ionic Edge Activator (EA). Phosphatidyl choline is the main component of the biomembrane, consisting of a hydrophilic polar head group of a phosphate group and 2 hydrophobic fatty acid chains. EA is a structure having both hydrophilicity and hydrophobicity, and a single chain surfactant with a large curvature is generally used, which destabilizes the lipid bilayer of the vesicles and increases the ultra-deformability of the bilayer by lowering its interfacial tension. It also relatively affects the physical properties of the transfersome [7,8]. Therefore, it easily penetrates through skin pores much smaller than itself to achieve transdermal penetration and prolong the release and increase the activity of the drug [9,10].

The oral and parenteral administration of fusidic acid poses adverse effects such as phlebitis, rhabdomyolysis, hepatotoxicity, diarrhea, and gastrointestinal discomfort. These adverse events occur because of the wide systemic non-specific distribution of fusidic acid, which decreases its therapeutic efficacy at the site of action, i.e skin [11,12]. The topical route for drug administration has many benefits over other routes of administration. It has lesser side effects and better patient compliance.

Hence, in the present study, we planned to prepare to prepare

transferosomes of Fusdic acid for better permeability and improved bioavailability.

Materials and Methods

Fusdic acid procured from Glenmark Pharmaeuticals-Mumbai, Capmul, Captex 200 purchased from MCM Abitec Group (USA), Tween 20, Span 20, Tween 80, Egg lecithin, PEG 200, PEG 400, Propylene glycol procured from Merck (Mumbai), Labrafac Lipophile WL1349, Labrasol Gattefosse, Cremophor EL, Labrafil purchased from Gattefosse, France.

Determination of wavelength (λmax)

Accurately measured quantity (100 mg) of Fusidic Acid was dissolved in 100 ml of ethanol, which produces the stock solution of 1000 µg/ml [13]. 10 ml of prepared stock solution was diluted with 100 ml phosphate buffer pH 5.5 to produce drug concentration of 100 µg/ml. From which 1 ml test solution was again diluted with 10 ml 6.4 pH phosphate buffer to produce 10 µg/ml. This test solution was further scanned in between 200 nm to400 nm against the 6.4 pH phosphate buffer as blank by using UV spectrophotometer.

Calibration curve of fusidic acid

Fusidic acid concentrations ranging from (2 µg/ml-10 µg/ml) in phosphate buffer (pH 5.5) were prepared and the absorbance was measured at 228 nm. For the standard graph, 100 mg of FA was accurately weighed and dissolved in 100 ml of ethanol, which produces stock solution (1000 µg/ml). 10 ml of previous stock solutions were diluted in 100 ml phosphate buffer of pH 5.5 to produce drug concentration of 100 µg/ml. Then 0.2 ml, 0.4 ml, 0.6 ml, 0.8 ml and 1 ml of these solutions were further diluted to 10 ml with phosphate buffer of pH 5.5 and the absorbance was taken at 228 nm by using UV spectrophotometer.

Selection of oils, surfactants and cosurfactants for fusdic acid

The selection of oil, surfactant and cosurfactant was determined by their ability to solubilize drug [14]. Solubility of Fusdic acid in various phospholipids were studied and found good solubility in Dimyristolyphosphatidylcholine (DMPC) and different surfactants were also studied and found high solubility in Tween 80. Hence the composition of these were used for further studies (Table 1).

Table 1: Solubility of fusdic acid in various phospholipids and surfactants

Phospholipids/surfactants	Solubility of FSC in mg/ml Mean ± S.D.*
Phospholipids	
Olive oil	1.9 ± 0.52
Peanut oil	4.6 ± 0.99
Sesame oil	6.7 ± 0.44
Capmul MCM	8.2 ± 0.19
Arachis oil	9.6 ± 0.47
Castor oil	10.3 ± 0.29
Linseed oil	12.6 ± 0.21
Soyabean Oil	15.7 ± 0.25
Labrafac LipophileWL1349	23.6 ± 0.43
Soya phosphatidylcholine (SPC)	39.8 ± 0.65
Hydrogenated soya phosphatidylcholine (HSPC)	43.2 ± 0.29
Dimyristolyphosphatidylcholine (DMPC)	59.7 ± 0.67
Surfactants	
Tween 20	69.4 ± 0.63
Span 20	84.9 ± 0.66
Labrasol	86.7 ± 0.19
Cremophor EL	93.5 ± 0.15
Labrafil	96.7 ± 0.19
Cetomagragol	125 ± 0.19
Tween 80	135.3 ± 0.66
(DMPC)+Tween 80	257.7 ± 0.44

Selection of solvents based on solubility: The lipids that were selected had shown good solubility in Ethanol, Methanol and Chloroform. Out of these Ethanol and chloroform had best solubility. Hence, these were selected for further studies.

Optimization of transfersomes

Formulation optimization of the transfersomal system consists of following components:

1. Screening of compatible edge activator, lipid for good deformability of transfersome.
2. Maintaining of lipid: Edge activator ratio for good deformability of transfersome.
3. Selection of optimum concentration of drug which can be incorporated in the transfersomal formulation.

Optimization of edge activator: In the development of transfersome, selection of best edge activator plays a major role. It possesses an aqueous core enclosed in a complex lipidic bilayer. This bilayer composition makes the vesicle self-regulating and self-optimising. This also allows transfersome to cross various transport barriers more efficiently.

In the development of formulation, Lipid (85%), drug (0.01 gm) organic solvent (10 ml), water for hydration (10 ml) were kept constant and different edge activators were used in 15% concentration. The optimization of edge activator was done on the basis of good penetration ability through dialysis membrane of molecular weight 12000 kDa-14000 kDa.

From the above studies, it was observed Fusdic acid, had good solubility in Tween 80.

Optimization of lipid: In the development of formulation, selected edge activator, drug, organic solvent, water for hydration were kept constant and different lipids were used in 85% concentration. The optimization of lipid was done on the basis of good penetration ability through dialysis membrane of molecular weight 12000 kDa-14000 kDa.

From the studies performed, it was observed that Fusdic

acid has good solubility in Dimyristolyphosphatidylcholine.

Optimization of lipid-edge activator ratio: After selection of lipid and edge activator, optimum percentage of lipid and edge activator was adjusted so as to form mixed vesicle, with good fluidity through the olfactory mucosa. Furthermore, the formulation was developed with constant quantity of drug (0.01gm), organic solvent (10 ml) and water for hydration (10 ml) and various ratio of selected lipid and edge activator were used (95%:5%, 90%:10%, 85%:15%, 80%:20%) (Table 2). The best possible ratio of lipid and edge activator was selected depending upon their penetration ability through dialysis membrane of molecular weight 12000 kDa-14000 kDa.

Table 2: Formulation of fusdic acid transfersomes using different lipid: Edge activator ratio

Lipid: Edge activator (%)	Drug (g)	Ethanol (ml)	Water (ml)
95:05:00	0.02	10	10
90:10:00	0.02	10	10
85:15:00	0.02	10	10
80:20:00	0.02	10	10

Optimization of drug concentration: This study was done to know the actual and maximum quantity of drug can be included in the transfersomal formulation.

In this study the quantity of organic solvent (10 ml), water for hydration (10 ml), lipid: Edge activator ratio (85%:15%) were kept constant but different concentration of drug (0.01 g, 0.02 g, 0.03 g, 0.04 g) were used (Table 3). The selection of drug concentration was done by entrapment efficiency.

Table 3: Fusdic acid transfersomes preparation with variation in drug quantities

Lipid: Edge activator (%)	Drug (g)	Ethanol/ methanol (ml)	Water (ml)
85:15:00	0.01	10	10
85:15:00	0.02	10	10
85:15:00	0.03	10	10
85:15:00	0.04	10	10

Compatability studies

FTIR studies: The study was performed using FTIR. IR graphs were obtained for both pure drug and optimized batch of transfersomes.

Differential scanning calorimetry: The study was executed using Differential calorimeter (DSC60 Shimadzu). Thermograms for optimized batch of transfersome and drug were individually obtained. Drugs and formulated transfersomal suspensions with drug were put in aluminum pan separately and scanned at the speed of 10°C/min over the temperature ranges of -30°C to 200°C were employed.

XRD: X-ray scattering measurements of Fusdic acid and Transferosomes of Fusdic acid were carried out with X-ray diffractometer (Rigaku Miniflex-600 X-Ray Diffractometer) at 30 mA current and 30 kV voltage. Samples were positioned on sample stage and irradiated with Cu Kα radiation source (1.742 Aº), scanning rate (2 h/ min) of 5°C/min at 30 mA current and 30 kV voltage. X-ray diffraction angle (2θ) between 5° and 50° was measured and analyzed to determine the crystalline properties of samples.

Method of preparation

Various methods were used in the preparation of transferosomes which include thin film hydration technique, reverse phase evaporation method and vortexing-sonication method. Of these, a most common method is thin film hydration method [15].

The Fusdic acid (FA) loaded transfersomes were prepared by rotary thin film hydration method. Hydrogenated soya phosphatidylcholine, Cetomagragol, and FA were dissolved in a chlororoform and ethanol mixture (in the ration of 2:1 v/v) in a round bottom flask. The solvent mixture was removed by using rotary film evaporator under reduced pressure at 60°C ± °C and 60 rpm to get a homogeneous lipid film. The flask was kept under vacuum to remove residual solvent. The thin lipid film was hydrated with PBS pH 5.5 above the transition temperature of lipid at 60 rpm for 60 min to obtain large Multilamellar Vesicles (MLVs). The resulting MLVs were kept overnight at 4°C to allow the complete hydration of the vesicles. The MLVs were then subjected to probe sonication at 4°C for 2 min using ultrasonic sonicator to get Small Unilamellar Vesicles (SUVs). The SUVs were then passed 10 times through 0.45 µm and 0.20 µm hydrophilic syringe filters to get uniform sized nanovesicles which were kept at 4°C for further

Table 4: Formulation of fusdic acid transfersomes using different lipid: Edge activator ratio

Formulation Code	E A	Lipid	Lipid:EA (%)	Drug (gm)	Ethanol (ml)	Water (ml)
FF1	CML	DMPC	95:05:00	0.01	10	10
FF2	CML	DMPC	90:10:00	0.02	10	10
FF3	CML	DMPC	85:15:00	0.02	10	10
FF4	CML	DMPC	80:20:00	0.02	10	10
FF5	T 80	DMPC	95:05:00	0.02	10	10
FF6	T 80	DMPC	90:10:00	0.02	10	10
FF7	T 80	DMPC	85:15:00	0.02	10	10
FF8	T 80	DMPC	80:20:00	0.02	10	10
FF9	S-80	DMPC	95:05:00	0.02	10	10

FF10	S-80	DMPC	90:10:00	0.02	10	10
FF11	S-80	DMPC	85:15:00	0.02	10	10
FF12	S-80	DMPC	80:20:00	0.02	10	10

characterization. A similar methodology was used for the preparing blank transfersomes without Fusdic acid. The formulation is shown in Table 4.

Composite design: A central composite design (spherical type, single center point, and α=1.414) was employed for the optimization of transferosomes containing Fusdic acid with Amount of lipids (A) and Amount of surfactants (B) as 2 prime selected independent variables (factors). CCD was used to explore the influence of formulation variables on (Y1)% drug loading (%w/w), (Y2) entrapment efficiency (%w/w) and (Y3) Particle size (nm) of the prepared transferosomes. The matrix of the design including investigated factors and responses are also shown in Tables 5 and 6. Design-Expert 12 software (Stat-Ease Inc., USA) was used for generation and evaluation of experimental design. Each independent variable had 3 level which were coded as -1, 0, +1, -alpha and +alpha i.e., DMPC (320, 350, 380, 307.5 and 392.4), Tween 80 (10, 15, 20, 3.78 and 46.21). Total 13 runs were performed with an aim to obtain

Table 5: Variables and their levels in composite design for formulation of fusdic acid loaded transfersomes

Formulation code	Dimyristoly phosphatidyl Choline (X1)	Tween 80 (X2)	Entrapment efficiency (%EE)	Particle size (nm)
FA1	1(380)	0(15)	86.05 ± 1.09	169.98 ± 2.09
FA2	1(380)	-1(10)	87.33 ± 0.94	168.99 ± 1.08
FA3	0(350)	1(20)	69.09 ± 1.29	256.41 ± 2.89
FA4	0(350)	0(15)	99.5 ± 0.58	270.1 ± 1.27
FA5	1(380)	1(20)	72.54 ± 1.28	249.31 ± 2.61
FA6	-1(320)	0(15)	72.65 ± 0.97	370.67 ± 1.37
FA7	0(350)	-1(10)	72.9 ± 0.87	369.4 ± 2.06
FA8	-1(320)	1(20)	87.32 ± 1.04	172.8 ± 1.38
FA9	-1(320)	-1(10)	62.55 ± 1.65	370.44 ± 2.72
FA10	0(350)	3.78	69.09 ± 0.85	216.5 ± 1.28
FA11	307.5	0(15)	86.87 ± 0.93	168.9 ± 2.72
FA12	392.4	0(15)	70.11 ± 1.34	368.05 ± 1.25
FA13	0(350)	46.2	68.25 ± 1.25	264.66 ± 2.93

Table 6: formulation of fusdic acid transferosomal gel

Ingredients	FA7 (F1)	FA7 (F2)	FA7 (F3)	FA7 (F4)
Fusdic acid Transferosomes equivalent to mg	20	20	20	20
Carbopol (%w/w)	0.5	1	1.5	2
Propylene Glycol(%)	0.1	0.1	0.1	0.1
Methyl Paraben	0.02%	0.02%	0.02%	0.02%
Trietanolamaine	0.10%	0.10%	0.10%	0.10%
Distilled water	QS	QS	QS	QS

a desired effect of formulation. Evaluation of the quality of fit and response surface modelling were performed using Design Expert-DX 13.0 software.

Quadratic Model: $\beta 0+\beta 1X1+\beta 12X1X2+\beta 11X12+\beta 22X22$. (Eq 1)

Where Y-Dependent variable,

β0-Arithmetic mean response of the 13 runs

βi (β1, β2, β12, β11 and β22)-estimated coefficient for the corresponding factor Xi (X1,

X2, X1X2, X1X1and X2X2).

The main effect (X1 and X2) symbolizes the average result of altering one factor from its low to high value. The interaction terms (X1 X2) depict change in the response when 2 factors are concurrently changed.

The equations permit the study of the effect of each factor along with their interaction over the various responses.

Evaluation of transfersome

Entrapment efficiency: For determination of entrapment efficiency transferosomes were separated from free unentrapped drug by ultracentrifugation method. Separated vesicles were resuspended in 5 ml ethanol for FA and disruption was further carried out by sonication (PciAnalyticsJIJ 158) for 15min to get clear solution and then filtered out. 1 ml of filtrate was further diluted with phosphate buffer (pH 5.5) up to 10 ml. Further

dilution was made if needed and drug concentration was analysed spectrophotometrically for FA at 228 nm. All spectrophotometric analysis was conducted in triplicate and the values were averaged.

$$\text{Entrapment efficiency} = \frac{\text{Amount entrapped} \times 100}{\text{Total amount}}$$

Particle size analysis: Particle size measurement was made with 50 µl of sample using Horiba scientific SZ-100 Size Analyzer at 90° sizing.

***In vitro* drug release:** *In vitro* drug release from transfersome was carried out using Franz diffusion cell with effective surface area 3.14 cm^2 and 15 ml capacity. Dialysis membrane (Hi media molecular weight 12000 kDa-14000 kDa) was used for study. Dialysis membrane was activated by soaking in phosphate buffer of pH 5.5 for 24 h prior to the experiment and was mounted in between donor and receptor compartments. The receptor medium was 15 ml phosphate buffer (pH 5.5) and donor medium consisted of 0.1 ml transfersomal suspension. The receptor content was stirred with magneticstirrer at 34°C temperature. 1 ml of samples was periodically withdrawn at specific time interval 1 h, 2 h, 4 h, 6 h, 8 h, 10 h, 12 h, 14 h, 16 h, 18 h, 20 h, 22 h, 24 h and replaced with 1 ml of fresh phosphate buffer solution, and assayed by spectrophotometer for FA at 228 nm respectively. This experiment was conducted triplicate.

Evaluation study of optimized formulation: Transfersomal formulations for FA were selected for further characterisation depending upon its optimum permeation ability in minimum time.

Zeta potential and polydispersity index: Zeta potential, polydispersity index of optimized formulation was measured by dynamic light scattering (Malvern ZetasizerVer). 6.32 MAL1065515 (Malvern Ltd, Malvern, UK) by diluting 1 ml of vesicular suspension with 10 ml distilled water. Polydispersity index was used for the measurement for even distribution of particle size in vesicular suspension.

Visualization of vesicles by Transmission Electronmicroscopy (TEM): All the prepared batches were observed under phase contrast microscopy with the magnification power of 100X (Olympus). Photographs of vesicles were taken using Olympus camera (Olympus MJU 9010). The optimized batch was visualized using Jeol/JM 2100, Source LaB6 electron microscope (TEM) with an accelerating voltage of 200 kV for surface appearance and shape. The transferosomes were dispersed indistilled water and 10 µltr of diluted dispersion was placed on the carbon-coated grid.

Incorporation into hydrophilic gels

Optimized transfersomal formulations showing highest EE, optimum PDI and ZP which was further incorporated into Carboxyvinyl polymer carbomer (Carbopol934P) gel formulations. Carbopol 934P (1.5% w/w) was soaked in a minimum amount of water for an hour followed by addition of 10 ml of transfersomal dispersion containing Fusdic acid (20 mg). It was then stirred continuously at 700 rpm in a closed vessel whose temperature was maintained at 30°C until homogeneous transfersomal gel was formed.

pH measurement: pH measurements of the formulations were done using digital pH meter (RI-152-R).

Spreading diameter: The spread ability of gel formulation was determined by measuring the spreading diameter of 1 g of gel between 2 horizontal plates (20 cm × 20 cm) after 1 min. The standard weight applied on the upper plate was 125 gm.

Drug content of the formed gels: 500 mg of the gel was taken and dissolved in 50 ml of pH 5.5 Phosphate Buffer (PBS). The solution was then passed through the filter paper and 50 µl of the filtrate was withdrawn. The filtrate was diluted by adding 3.5 ml of distilled water and the drug content was measured spectrophotometrically for FA at 228 nm against corresponding gel concentration.

Rheological studies: The rheological properties of formed gels were evaluated using Brookfield cone and plate viscometer (model LV DV-III+ Rheometer). 0.5 ml of sample was placed in plate of viscometer and analyzed for its viscosity, shear stress, rate of shear at various speeds and also tested for its thixotropic phenomena at 25°C using CP 52 spindle and Rheocalc software of the instrument.

Extrudability test: After applying the weight in gram necessary to extrude a gel ribbon of at least 0.5 cm in length in 10 seconds, the amount of gel (g/cm^2) that was extruded from the lacquered aluminium collapsible tube was calculated. The formula provided can be used to calculate the extrudability.

$$\textbf{Extrudability} = \frac{\text{Weight applied to extrude gel from the tube (g)}}{\text{Area in } Cm^2}$$

Flux of Gel formulation: Amount of Mupirocin from transferosomal gel was permeated through goat skin and was plotted against the function of time. The slope and intercept of the linear portion of plot were derived by regression. The flux (J, $lg/cm^2/h$) was calculated as the slope is divided by the skin surface area.

Skin irritation test: Skin irritation study was conducted on 3 healthy rat groups; each group included 6 rats of either sex. The animals were kept on standard animal feed and had free water access. Before one day the study hair was shaved from the back of the rats and 5 cm^2 of the area was marked on both sides, one side served as control while the other side was being tested. Prepared transferosomal gel was applied for 7 days and the site was observed for any sensitivity and reaction if any, graded as 0, 1, 2, 3 for no reaction, slight patchy erythema, slight but confluent or moderate but patchy erythema, and severe erythema with or without edema, respectively.

Diffusion studies: Transferosomal gel permeation experiments were conducted using the Franz diffusion cell through an excised rat abdominal skin. one gm

transferosomal gel has been placed in intimate contact with the skin excised. The donor compartment was charged with an adequate sample amount to keep the drug quantity constant and the receptor compartment was filled with 20 ml of pH 5.5 phosphate buffer saline and stirred at room temperature with a magnetic stirrer at 300 rpm. The samples were withdrawn at different intervals of time, filtered, adequately diluted and then analyzed at 228 nm using a UV spectrophotometer and replaced with the same fresh buffer volume.

CLSM studies: Mechanisms and depth of skin permeation of the FF7 loaded vesicle was investigated using CLSM. The FF7 loaded vesicles was prepared and labeled bilayer by Rhodamine-DHPE (100: 1 M ratio). The labelled vesicles were applied on the hairless goat skin for 12 h (2 h, 4 h, 6 h, 8 h, 10 h, 12 h). After removing the excess amount of vesicle formulation, the skin was washed with distilled water and then dried with cotton swab. The skin was sectioned into the pieces of 1 cm^2 size and evaluated for depth of fluorescent probe penetration. The full skin thickness was optically scanned at the different increments through the z-axis of the CLSM. (CLSM, Radiance 2100, Bio-Rad Laboratories, Inc., Hercules, CA, U.S.A). Before starting the studies, goat skin was soaked in drug solution along with rhodamine dye. Studies were performed using Franz diffusion cell for every 2 h up to 12 h. To study using CLSM, the skin was preserved in formalin solution.

Results and Discussion

λmax of FA in 6.4 pH phosphate buffer was found to be 228 nm as shown in Figure 1. From the results it was revealed that the values matches with the values reported in the literature so given sample complies with the standard.

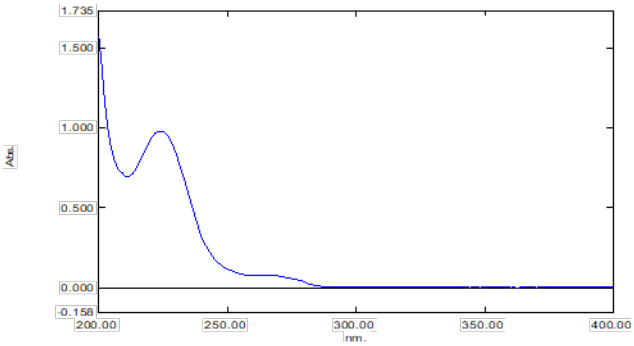

Figure 1: Wavelength of fusidic acid at 228nm

From the Table 7, Figure 2 standard plot of Fusdic acid was obtained by taking the ranges of concentration from 0-25 µg/ml. It was seen that R2 value is 0.9982.

Table 7: Calibration curve of fusdic acid

S.no.	Concentration (µg/ml)	Absorbance (nm)
1	0	0
2	2	0.205 ± 0.001
3	4	0.410 ± 0.005
4	6	0.615 ± 0.01
5	8	0.820 ± 0.01
6	10	0.990 ± 0.02

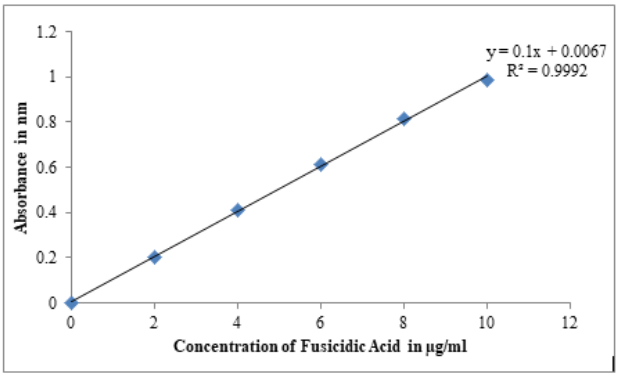

Figure 2: Standard plot of fusdic acid

The FTIR results active drug (Fusidic acid), excipients (Tween 80 and Dimyristolyphosphatidylcholine). The characteristic absorption peaks of fusidic acid were found at 35359.78 cm^{-1}, 2951.2 cm^{-1}, (carboxylic acid O-H stretching), 1685.08 cm^{-1} (carboxylic acid C=O stretching), 1441.8 cm^{-1}, and 1374 cm^{-1} (aromatic C=C) peaks at 1251.0 cm^{-1}, which confirm the aromatic structure of fusidic acid. On the other hand, fusidic acid, when incorporated in transferosomes, exhibited significant physical interaction as most of the fusidic acid peaks were diffused in the FTIR spectra of transferosomal formulations FF7. This might be due to the effect of high temperature during transferosomes development, which resulted in the molecular dispersion of drug within the microenvironment of transferosomes (Figures 3 and 4).

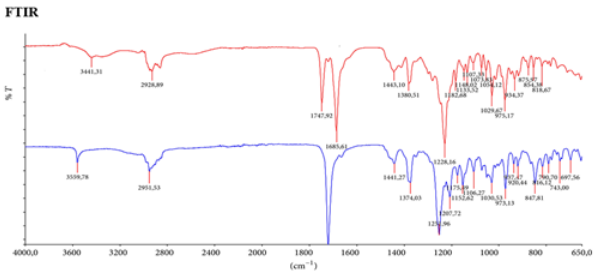

Figure 3: FTIR of fusdic acid and optimized formulation

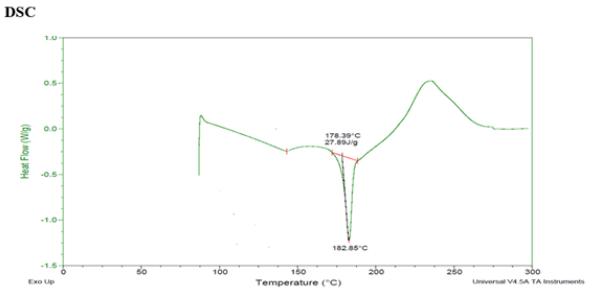

Figure 4: DSC of pure fusdic acid

From the DSC results obtained as shown in Figure 5, the melting point of pure drug was found to be 182°c and optimized formulation of Fusdic acid transferosomes also consist of same temperature, which means that, it has no deviation. Hence, it can be concluded that the formulation

can be proceeded for further studies.

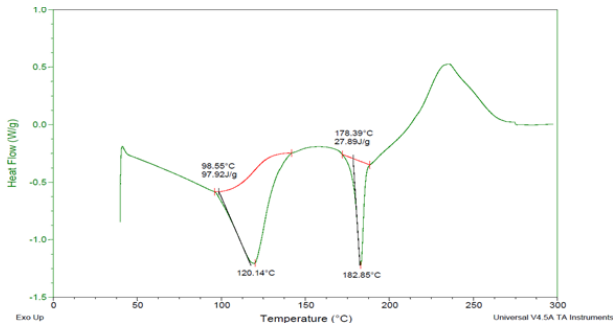

Figure 5: DSC of fusdic acid optimized formulation

The X-ray diffraction studies were carried out to identify the presence or absence of crystalline state of Fusdic acid in Transferosomes. XRD patterns with distinctive crystalline peaks of FA are shown in Figure 6. As seen in figure the XRD spectrum of FA displayed sharp and intense peaks of crystallinity, which suggested a highly crystalline nature. The XRD spectra of the Transferosomes of FA showed a reduction of peak intensity (Figure 7), as compared to the pure drug, which indicated decreased crystallinity or changes into an amorphous phase of the drug.

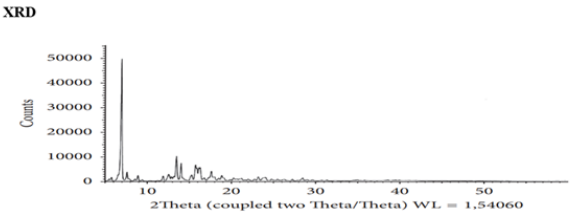

Figure 6: XRD of pure fusdic acid

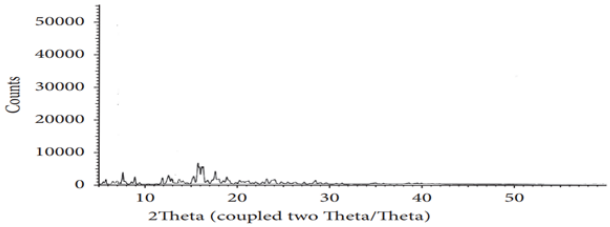

Figure 7: XRD of optimized fusdic acid formulation

Optimization of transfersomes

The process of optimization of Fusdic acid Transferosomes was mentioned in the section 2.3. The results obtained from the studies clearly explain that the ratio of Lipid: Edge activator (%) was 85:15 using Dimyristolyphosphatidylcholine (DMPC): Tween 80. Considering this ratio %EE, Vesicle size in nm, PDI and ZP (mV) were performed. The results are shown in the Table 8.

Table 8: Entrapment efficiency, vesicle size, polydispersity index and zeta potential of fusdic acid transferosomal preparations

Form code	Entrapment efficiency (%)	Vesicle size (nm)	Polydispersibility Index (PDI)	Zeta Potential (mV)
FF1	57.5 ± 0.20	302 ± 12	0.44 ± 0.052	-39
FF2	62 ± 0.32	295 ± 14	0.63 ± 0.038	-39.2
FF3	79.4 ± 0.71	288 ± 13	0.56 ± 0.024	-30.2
FF4	70.5 ± 0.64	270 ± 8	0.39 ± 0.033	-35.6
FF5	42.5 ± 0.33	345 ± 15	0.41 ± 0.035	-32.2
FF6	78 ± 0.48	330 ± 19	0.29 ± 0.037	-33.2
FF7	90.01 ± 0.51	277 ± 12	0.10 ± 0.021	-37.7
FF8	77.03 ± 0.36	275 ± 10	0.47 ± 0.027	-28.2
FF9	59.01 ± 0.49	300 ± 11	0.37 ± 0.031	-21.1
FF10	70.01 ± 0.25	290 ± 13	0.26 ± 0.033	-19.3
FF11	76.70 ± 0.36	285 ± 14	0.39 ± 0.049	-22.9
FF12	68.14 ± 0.44	260 ± 11	0.46 ± 0.025	-31
It is considered, FF7 as best since it has high (%EE), Larger vesicle size, high PDI and ZP				

In vitro drug release studies

From the results obtained, the transferosomes that were prepared for Fusdic acid, it was observed that highest entrapment efficiency, smaller particle size was found in FF7 (Tables 9-12). The edge activator that was used was Tween 80, when compared to cetomagragol and span 80 it has shown better release. These transferosomes were incorporated into gels and further studies were performed. The kinetic studies also showed that the drug release was in a controlled manner (Figures 8-14).

Table 9: Drug release profiles of fusdic acid formulations FF1-FF4

Time (hrs)	Cumulative % drug released ± S.D* (n=6)			
	FF1	FF2	FF3	FF4
1	10.55 ± 0.74	9.44 ± 0.33	8.19 ± 0.15	7.44 ± 0.19
2	13.23 ± 0.21	12.33 ± 0.19	17.33 ± 0.64	16.56 ± 0.45
4	19.11 ± 0.65	17.39 ± 0.37	20.33 ± 0.89	22.19 ± 0.49

6	22.44 ± 0.26	24.36 ± 0.41	21.74 ± 0.53	24.32 ± 0.54
8	24.39 ± 0.94	25.19 ± 0.29	25.62 ± 0.47	26.16 ± 0.16
10	25.21 ± 0.89	26.19 ± 0.20	27.07 ± 0.81	28.57 ± 0.33
12	27.15 ± 0.57	27.22 ± 0.31	29.20 ± 0.55	31.33 ± 0.28
14	28.37 ± 0.23	28.22 ± 0.67	33.52 ± 0.43	34.17 ± 0.25
16	30.29 ± 0.49	31.47 ± 0.22	37.81 ± 0.79	38.33 ± 0.85
18	31.19 ± 0.44	32.40 ± 0.61	39.41 ± 0.52	43.44 ± 0.14
20	34.47 ± 0.15	33.44 ± 0.72	40.17 ± 0.59	44.03 ± 0.60
22	35.12 ± 0.19	34.20 ± 0.15	41.34 ± 0.52	45.19 ± 0.23
24	36.44 ± 0.57	39.47 ± 0.23	42.44 ± 0.16	46.23 ± 0.66

Table 10: Drug release profiles of fusdic acid formulations FF5-FF8

Time (hrs)	Cumulative % drug released ± S.D* (n=6)			
	FF5	FF6	FF7	FF8
1	9.55 ± 0.61	8.41 ± 0.44	9.44 ± 0.35	10.19 ± 0.33
2	14.23 ± 0.41	18.93 ± 0.22	20.51 ± 0.25	25.33 ± 0.69
4	18.20 ± 0.50	22.39 ± 0.67	36.19 ± 0.42	33.33 ± 0.89
6	20.15 ± 0.31	23.19 ± 0.52	42.32 ± 0.54	41.74 ± 0.53
8	22.23 ± 0.57	25.61 ± 0.29	48.16 ± 0.33	45.39 ± 0.23
10	25.19 ± 0.40	27.22 ± 0.48	52.57 ± 0.85	51.62 ± 0.47
12	26.11 ± 0.44	29.47 ± 0.42	54.33 ± .0.15	54.07 ± 0.19
14	29.57 ± 0.19	28.17 ± 0.53	57.17 ± 0.11	56.20 ± 0.54
16	31.21 ± 0.44	30.31 ± 0.58	60.33 ± 0.19	58.50 ± 0.57
18	33.19 ± 0.25	32.17 ± 0.41	65.51 ± 0.40	60.33 ± 0.29
20	36.33 ± 0.21	35.44 ± 0.39	68.22 ± 0.15	62.52 ± 0.43
22	37.11 ± 0.36	37.20 ± 0.69	71.28 ± 0.11	64.81 ± 0.52
24	38.44 ± 0.14	40.07 ± 0.33	74.35 ± 0.15	68.19 ± 0.31

Table 11: Drug release profiles of fusdic acid formulations FF9-FF12

Time (hrs)	Cumulative % drug released ± S.D* (n=6)			
	FF9	FF10	FF11	FF12
1	9.11 ± 0.39	16.23 ± 0.67	8.88 ± 0.37	7.44 ± 0.19
2	15.19 ± 0.47	25.30 ± 0.22	17.19 ± 0.52	15.83 ± 0.21
4	24.17 ± 0.59	30.12 ± 0.69	25.19 ± 0.19	28.44 ± 0.93
6	30.28 ± 0.65	34.68 ± 0.22	29.63 ± 0.45	35.44 ± 0.57
8	34.22 ± 0.61	37.21 ± 0.59	33.22 ± 0.26	44.15 ± 0.19
10	36.33 ± 0.47	38.14 ± 0.27	37.41 ± 0.32	46.15 ± 0.35
12	39.51 ± 0.20	40.37 ± 0.66	40.65 ± 0.50	48.33 ± .0.74
14	40.61 ± 0.39	42.19 ± 0.71	44.33 ± 0.67	52.27 ± 0.45
16	43.51 ± 0.66	44.15 ± 0.10	49.63 ± 0.85	54.23 ± 0.63
18	45.61 ± 0.45	46.43 ± 0.37	52.19 ± 0.49	56.37 ± 0.33
20	47.14 ± 0.20	48.19 ± 0.34	54.29 ± 0.19	57.14 ± 0.45
22	49.33 ± 0.14	49.31 ± 0.67	57.63 ± 0.16	59.33 ± 0.79
24	51.14 ± 0.57	53.43 ± 0.18	59.57 ± 0.41	63.51 ± 0.49

Table 12: Correlation Coefficient (r2) values of formulations FF1–FF12 as per various kinetic models

Formulation	Correlation Coefficient (r^2) values	24	24	24	24
	Zero order	First order	Higuchi's	Peppas's	n value
FF1	0.974	0.8704	0.939	0.964	0.87
FF2	0.9552	0.9411	0.949	0.979	0.68
FF3	0.996	0.6306	0.9905	0.981	0.81

FF4	0.934	0.7097	0.9405	0.986	0.74
FF5	0.9938	0.7997	0.9585	0.9945	0.82
FF6	0.9687	0.8926	0.9788	0.9957	0.76
FF7	0.9978	0.7936	0.9934	0.9977	0.76
FF8	0.995	0.7394	0.9555	0.9934	0.77
FF9	0.9925	0.6997	0.9115	0.983	0.82
FF10	0.9956	0.784	0.9551	0.9961	0.8
FF11	0.9924	0.789	0.963	0.997	0.877
FF12	0.9905	0.833	0.954	0.993	0.927
24	24	24	24	24	24

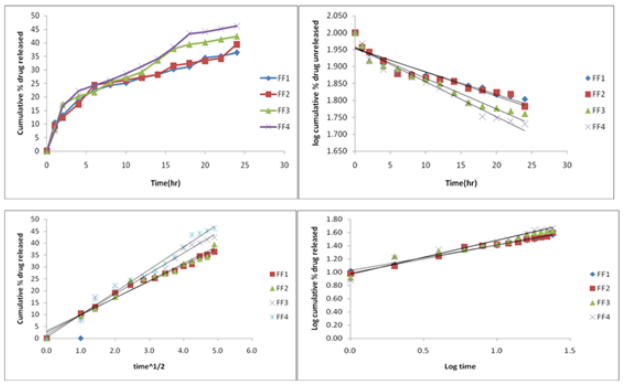

Figure 8: Drug release kinetic models of fusdic acid from FF1-FF4 showing zero order, first order, higuchi and peppas plots

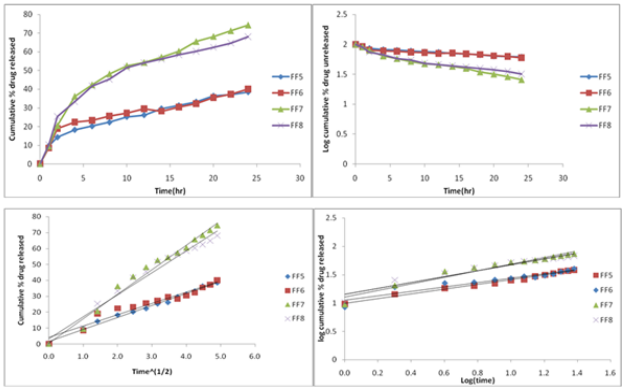

Figure 9: Drug release kinetic models of fusdic acid from FF5-FF8 showing zero order, first order, higuchi and peppas plots

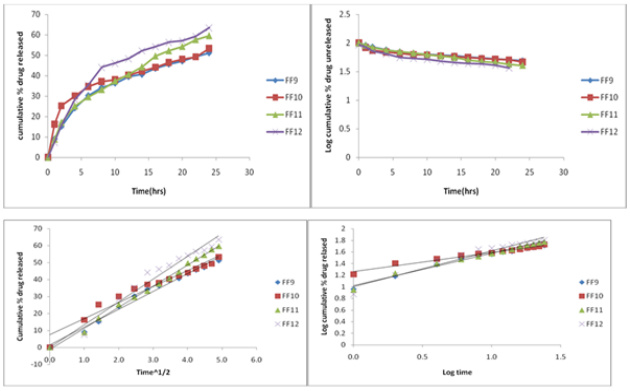

Figure 10: Drug release kinetic models of fusdic acid from FF9-FF12 showing zero order, first order, higuchi and peppas plots

Optimization of transferosomes

Phospholipid and surfactant have a greater impact on formulation of transferosomes, hence Dimyristoly phosphatidyl Choline and Tween 80 were used for formulation of FA transferosomes using thin film hydration technique and critical parameters like drug loading, entrapment efficiency and particle size as assessed. The optimization of formulated FA transferosomes were done employing CCD in which varying concentration of Dimyristoly phosphatidyl Choline and Tween 80 were done and they served as independent factors and on other side entrapment efficiency (%w/w) and Particle size (nm) were dependent factors. The table depicts that the different concentration of Dimyristoly phosphatidyl Choline (200 mg to 400 mg) and Tween 80 (100 mg to 200 mg) have an obvious influence on the critical dependent parameters of transferosomes.

Considering the obtained results, it was concluded that batch FF7(F4) was the optimized batch as it possesses the maximum entrapment of FA (87.5 ± 0.58) along with particle size of 170.1 nm ± 1.27 nm and therefore proved superior to other batches and selected for further studies. It is usually observed that transferosomes have ability to squeeze themselves and transport the entrapped drug across various biological membranes.

Concentration of Dimyristoly phosphatidyl Choline has vital role on drug loading and entrapment of drug and simultaneously higher concentration may lead to increase size of particle and flocculation of transferosomes may be seen. Moreover, enhancing the total lipid concentration it lowers the amount of Dimyristoly phosphatidyl Choline involved in formulation of FA transferosomes and batch 4 was having ideal concentration of phospholipid and this assisted in overcoming the above-mentioned flaws of formulation. The results direct that rise in amount of Phospholipid has a prominent role on the particle size and besides that initially hike in entrapment efficiency was seen and decreases subsequently. Higher Tween 80 content also contributes towards turbidity phenomenon and hence the batch F4 was transparent and clear.

The role of surfactant is important governing the surface attributes of transferosomes, optimal presence of sodium deoxycholate improves the flexibility and elasticity of FAs up to a larger extent and assists in easy pass through the pores of skin. The major factors which are responsible for interaction between the transferosomes and the outermost layer of the skin include size, hydrophobicity along with the

hydrogen bonds formation capability with other molecules. Henceforth as per this theory transferosomes having an average size of 500 nm can easily pass through skin and penetrate. The average size in this study ranged between 170.1 ± 1.27 to 370.44 ± 2.72, which were relatively lower than the upper limit and stated i.e., FA for management of Impetigo have the ability to penetrate through the skin. The presence of edge activators in transferosomes classifies them as the first generation of elastic vesicles. In FATs the Tween 80 helps in vesicular bilayer destabilization and improving elasticity of bilayer by plummeting the interfacial tension and results in lower particle size. The unique characteristic properties of Dimyristoly phosphatidyl Choline like hydrophilic lipophilic balance and chemical structure has vastly affected the entrapment efficiency, which can be clinched form results. FATs having surfactant of ideal HLB value, which leads to higher drug entrapment values. Tween 80 satisfies the parameter of critical packing and which causes the improved entrapment of highly lipophilic drug in the vesicle bilayer.

The results were better implicit with Design Expert® DX 13 by generating mathematical model equation, entrapment efficiency (% EE) (Y1) and particle size (nm) Y2 as response is shown in equations:

Y1 (% Entrapment Efficiency): 0.0484-0.2957+0.0073-1.66-1.39 ($p<0.0007$)–Equation 3

Y2 (Particle size nm): 0.7328-0.4289-0.0900-7.65-9.71 ($p< 0.0004$)-Equation 4.

The fitted polynomial equation (quadratic model) related to (Y1) entrapment efficiency (% w/w) and (Y2) Particle size (nm), were used to confirm the obtained result by taking in consideration the coefficient and the mathematical sign present their in. The positive and negative values favour the results and esteem contradicts the demonstrate fit respectively. The relationship coefficient (r2) of the quadratic show (0.9409) for reaction entrapment efficiency (% EE) (Y1) and (0.7880) for particle size (nm) Y2 was found to be critical. Since Dimyristoly phosphatidyl Choline and Tween 80 have their notable influence on entrapment efficiency (% EE) and particle size (nm), reactions can be examined evidently in their surface plot.

Equation 3, 4 signifies the computable impact on independent factors (Y1, Y2). The quadratic equation relating to the independent variables assisted in overcoming with the correlation coefficient (r2) values. The r2 values were 0.9409 and 0.7880 for Y1, Y2 respectively.

Validation of CCD results

The validation of the process method and the results generated was done and comparison of the predicted value and observed value of the independent factors (Y1, Y2) can be seen in Table 13. From the equation 3 and 4, percent relative error (PRE) of the independent factors was generated and was found to be 1.31 (Y1), 0.0072 (Y2) respectively. As the PRE value was <2 which indicates the higher precision and suitability of experimental design. As a conclusion batch 4 possess which is FF7 (F4) is in close agreement between the predicted value and observed value and was considered to be optimized batch.

Table 13: Comparison of the predicted value and observed value of the independent factors (Y1, Y2)

Response	Predicted mean	Predicted median	Observed	Std deviation	n
Entrapment Efficiency	99.014	99.014	99.1	1.2	1
Particle size	270.15	270.15	270.1	1.5	1

Evaluation of transferosomes

By using statistical optimization technique it was observed that FF7 (F4) is the optimized formulation consisting of particle size 270 nm which was also similar with FF7, similarly the entrapment efficiency was also 99%. Further zetapotential was calculated and found-37.7 mV (Figure 11).

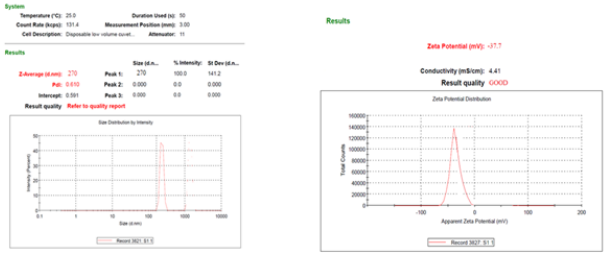

Figure 11: Zeta potential and particle size of fusdic acid transferosomes

Rheological studies: Rheograms of the prepared gel are represented in Figures 12-14. The examined gel sample exhibited a thixotropic pseudoplastic behavior, which was a preferable property in some pharmaceutical preparations such as gel. Self-alignment in the parallel directions of applied shear may be the cause of the pseudoplasticity results. The prepared gel samples were exposed to different rates of shear. From the obtained results, the rate of shear was plotted against shearing stress as shown. Thixotropic behavior was determined in the examined gel. By applying ascending shearing stress on the examined gel may be positioned as weak construction of parallel lines, leading to more and more ease of flow (up-curve). On contrary, by applying descending shearing stress on the gel samples, the construction begin to reform and gradual restoration of viscosity occurs (down-curve). All viscosity values on down-curve were less than on up curve at the same shearing stress.

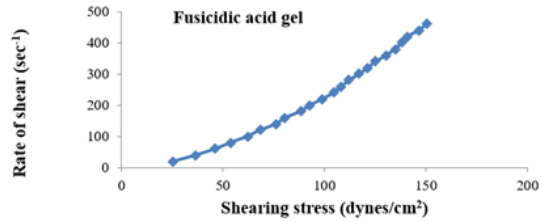

Figure 12: Rheogram plotted showing flow property of gel

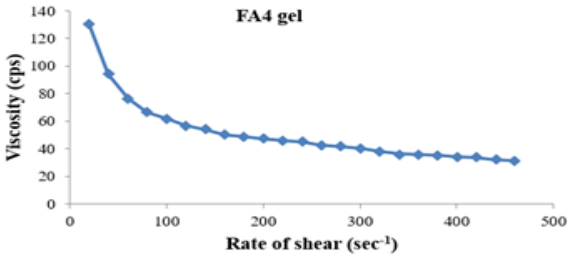

Figure 13: Rheological behavior of transferosomal gel formulation FF7(F4) at different shear rate (n=3)

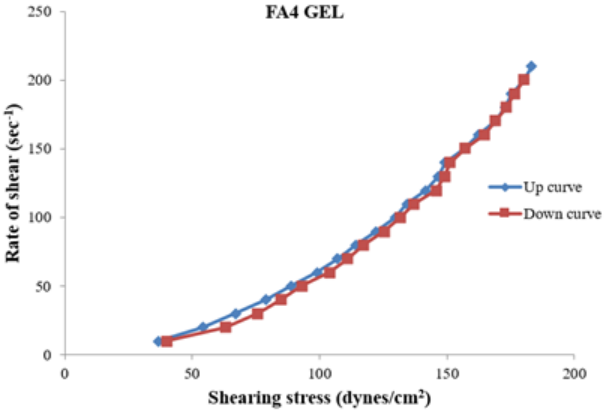

Figure 14: Thixotrophic behaviour for FF7(F4) gel

Table 14 shows the evaluation properties of transferosomal gel of fusdic acid.

Table 14: Evaluation properties of transferosomal gel of fusdic acid

Formulation	Colour	Homogenity	Texture	Viscosity (cps)	pH	Spreading diameter (mm)	Drug content (%)	Extrudability	Skin irritation test
FA4 gel	Dull white	Homogeneous	Smooth	133 ± 0.04	6.4	49	95.6	Excellent	No irritation

Flux: Fusdic acid flux for transferosomal gel formulations was found to be 50.732 µg/cm²/hr, 53.0 µg/cm²/hr, 56.6 µg/cm²/hr and 64.2 µg/cm²/hr for FF7(F1),FF7(F2),FF 7(F3),FF7(F4), respectively as shown in Figure 15. The better skin permeation of Fusdic acid from transferosomal gel was explained by the ability of transferosomal gel to penetrate through the relatively smaller pores of skin. The shape transformation of transferosomal gel was originated from the presence of "edge activator" in their lipid bilayers. The impact of particle size on the improvement in the permeation of a drug through the skin also has a significant role in the effectiveness of the formulation.

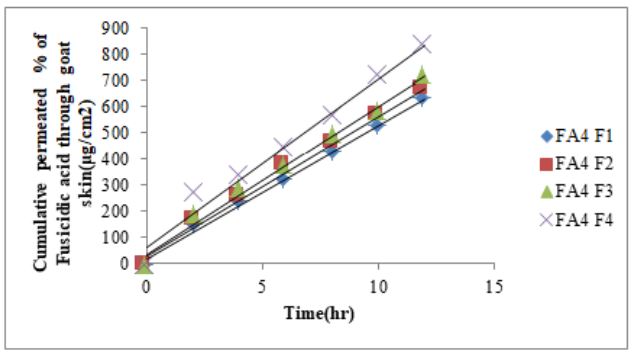

Figure 15: Comparison flux between the fusdic acid gel formulations from FF7 F1,FF7 F2,FF7 F3,FF7 F4.

Diffusion studies: Conducted for FF7 F1, FF7 F2, FF7 F3, FF7 F4 transferosomal gels for 12 hours using Franz diffusion cell on skin pH to evaluate release parameters. The amount of drug that permeated through the skin after 24 h from FA4 F4 was 94.5% and in case of FA4 F1 gel 69.8%. The permeation of transferosomal gel FA4 F4 was higher than that of FA4 F1, FA4 F2, FA4 F3 gels as shown in Figure 16. It can be due to the presence of Dimyristolyphosphatidylcholine which contributes towards deformation of transferosomes and lead to release drug. Transferosomes have the capacity to alter the anatomy of the skin by losing intercellular lipid barrier and hence increase its permeability.

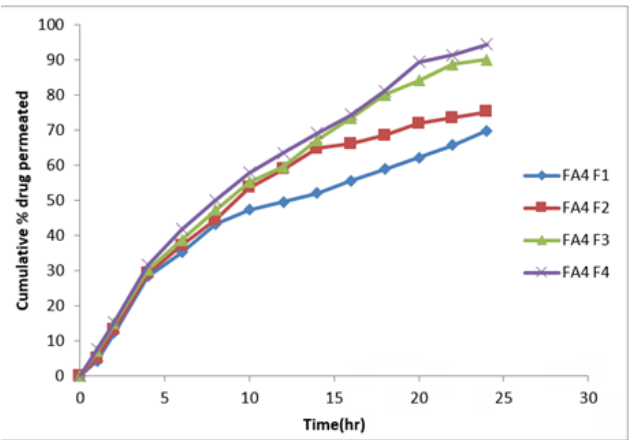

Figure 16: Amount of drug that permeated through the skin after 24 h from FF7 F4

CLSM studies: From the studies it was observed that the prepared gel has high permeability. The result indicated that the low elasticity value vesicles had also low permeability,

could not penetrate into the deep layer of the epidermis and only remained to the upper layer of the stratum corneum. On the other hand, the high elasticity value vesicles showed effective permeability up to viable epidermis as high fluorescence intensity in the skin between 15 µm–40 µm (viable epidermis layer) was observed. The obtained results indicated that high elastic value vesicle penetrated across the skin greater than low elasticity vesicles (Figure 17).

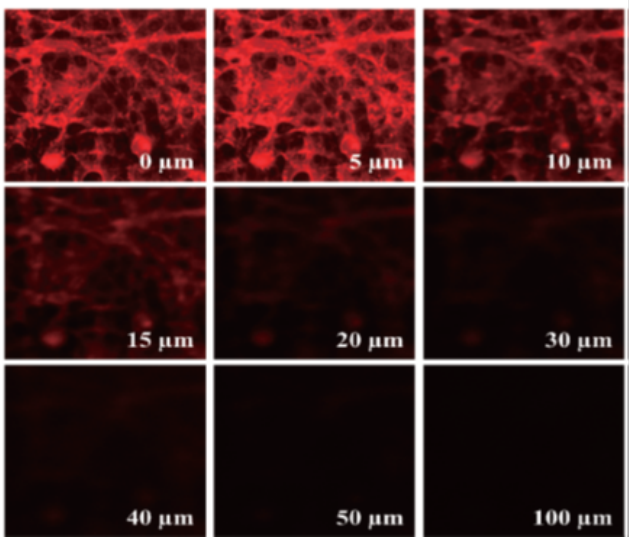

Figure 17: The hairless goat skin after 12 h skin permeation of transferosomes

Conclusion

The preparation and optimization of transferosomal gel containing Fusdic acid (FA) using Central Composite Design demonstrated promising results for the treatment of impetigo. Through systematic experimentation, the use of Dimyristolyphosphatidylcholine and Tween 80 was identified as the optimal lipid and edge activator combination, respectively, due to their superior solubility characteristics. The rotary thin film hydration method proved effective in preparing stable transferosomes, with key parameters such as lipid-to-surfactant ratio, stirring speed, entrapment efficiency, drug content, and zeta potential being meticulously optimized.

The Central Composite Design facilitated the identification of the best formulation, ensuring maximum efficacy and stability. Subsequent evaluations of the transferosomal gel, including extrudability, viscosity, and spreadability tests, confirmed its suitability for topical application. Confocal Laser Scanning Microscopy (CLSM) studies further validated the gel's capability to permeate skin layers effectively.

In conclusion, the optimized transferosomal gel of FA shows significant potential as a topical treatment for impetigo, offering enhanced drug release and skin permeability. This study underscores the importance of systematic optimization in developing effective dermatological therapies and paves the way for further clinical investigations.

Acknowledgement

None.

Conflict of Interest

Authors declare no conflict of interest.

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