Jilin Daxue Xuebao (Gongxueban)/Journal of Jilin University (Engineering and Technology Edition)
ISSN：1671-5497
E-Publication：Online Open Access
Vol: 40 Issue: 11-2021
DOI 10.17605/OSF.IO/9P4S6

DEVELOPMENT OF NANO FORMUATION AND ITS OPTIMIZATION EVALUATION OF EFINACONAZOLE LOADED TRANSFERSOMAL GEL FOR TREATMENT OF NAIL INFECTION

ANJANA DEVI[1], HIMANSHU CHAURASIA*[2], RANJIT SINGH[3]

[1]Research Scholar, Department of Pharmaceutical Sciences, Adarsh Vijendra Institute of Pharmaceutical Sciences, Shobhit University, Gangoh, Saharanpur, Uttar Pradesh – 247341, India.
Email: anjanasapna@gmail.com
[2]Associate Professor, Department of Pharmaceutical Sciences, Adarsh Vijendra Institute of Pharmaceutical Sciences, Shobhit University, Gangoh, Saharanpur, Uttar Pradesh – 247341, India.
Email: himanshuj02@gmail.com | himanshuj1980@gmail.com
[3]Vice Chancellor, Shobhit University, Saharanpur, Uttar Pradesh – 247341, India.
Email: ranjitsps@gmail.com
*Corresponding Author Email: himanshuj02@gmail.com | himanshuj1980@gmail.com

ABSTRACT

The aim of the study is to formulate Efinaconazole-loaded transfersomes using different proportions of Phospholipon® 90 G and Tween® 80 (95-75: 5-25 percent weight-to-weight) in order to determine the optimal formulation for treatment of nail infection. Each of the transferred vesicles were evaluated for the following characteristics features such as size, shape, polydispersity index, zeta potential, entrapment efficiency, *in vitro* skin permeation, and in vivo skin irritation studies. The results revealed that the formulated transfersomes were negatively charged, had a spherical uni-lamellar structure ranging from 132.97 nm to 553.06 nm in size and had an entrapment efficiency ranging from 40.37 to 84.61 percent. The transdermal flux index of the gel was found to be 0.024±0.002 mg/cm^2/h across the rat skin. It is indicated that the gel formulation was effective in transporting the drugs. Furthermore, *in vivo* skin irritation studies revealed that the transfersomes prepared were completely free of skin irritation potential (erythema and edema). The present study will aid in understanding the prepared transfersomes with Tween® 80 could be used for improving the transdermal delivery of Efinaconazole in a given formulation. In conclusion, it can be stated that the transdermal transfersomes formulation may have the ability to be a potential drug carrier. The Efinaconazole it is possible to prepare the Efinaconazole treated rats showed good activity and has got proven studies by which it can be scaled up with ease by using the above mentioned factors.

Key words: Transfersomes, Phospholipon® 90 G, Efinaconazole, Uni-lamellar structure, Transdermal flux.

Introduction:

According to the recent studies onychomycosis was not considered to be as dangerous infection [1]. Approximately 5% of the world's population is affected by this condition, which affects toe-nails much more severely than finger-nails [2]. It is estimated that onychomycosis effects between 0.5 to 5 percent of the population in India, with a higher prevalence in warm and humid climates [3]. Brittleness of the nails, distortion of the nail structure and discoloration are physical manifestations of

Jilin Daxue Xuebao (Gongxueban)/Journal of Jilin University (Engineering and Technology Edition)
ISSN：1671-5497
E-Publication：Online Open Access
Vol: 40 Issue: 11-2021
DOI 10.17605/OSF.IO/9P4S6

the disease [4]. There are few treatment options for onychomycosis, which is primarily due to the deep-seated infection and impermeable nature of the nail bed. The di-sulfide linkage and hydrogen bonds in the keratin network are extremely stable and strong and are responsible for the impermeability of the nail. The nail plate is one of the most difficult biological barriers to overcome, to its hard keratin fibers and globular proteins that hold them together [5]. Treatment with oral antifungal agents for onychomycosis is typically prolonged and associated with a number of side effects, particularly hepatotoxicity and drug interactions. Because of their limited availability in the site of action, the prescribed dose is increased, and the frequency of administration is also increased as a result of its availability [6]. This not only increases the severity of the associated side effects, but also increases the treatment cost [7]. While topical therapy does not have these side effects and is more convenient for patients and its effectiveness is limited due to the rigid keratin structure of the nails, which make it difficult to penetrate deeply into the nail bed [8].

Traditional liposomes are transformed into transfersomes (TF), also known as ultra-deformable nano-liposomes, by the addition of surfactants (such as sodium cholate) [9] to the phospholipid bilayers. The high deformability and skin penetration of transfersomes [10] are the most important characteristics associated with them, aside from the advantages of their good affinity for skin efficacy and safety. The drugs move across the skin from the epidermis to the dermis, and then into lymphatic vessels and blood vessels, where they exert a therapeutic effect [11].

Materials and methods

Materials

Different phospholipids phospholipon 90 G and Lipoid S100 were obtained from Lipoid® GmbH Germany. Tween 80 was purchased from Thomas Bakers (Mumbai, India). Ethanol was purchased from med Chem Pharma, India. Efinaconazole was also obtained as a gift sample from Sigma Aldrich. The solvents like water used in the HPLC and other required chemical are of local grade. The analytical method development were conducted using are HPLC solvents and other reagents of analytical grade.

Preparation of Efinaconazole Transfersomes

Transfersomes (TFS) were prepared by conventional rotary evaporation method. Precisely, Soya lecithin mixed with Edge activator (EA) was taken in a clean, dry, round bottom flask and the lipid mixture was dissolved in organic solvent. The organic solvent was removed by rotary evaporator above the lipid transition temperature. Final traces of solvent were removed under vacuum overnight. The deposited lipid film was hydrated with PBS (pH 7.4) containing Drug Efinaconazole to furnish the desired concentration in the final preparation by rotation for 1 hr at specified temperature [12]. The resulting vesicles were swollen for 2 hr at room temperature to get large multi lamellar vesicles (LMLVs). The thick suspension thus obtained was broken by sonication for 30 min at 40C at a frequency of 53 kHz to achieve desired vesicle size (200-300 nm)

ISSN：1671-5497
E-Publication：Online Open Access
Vol: 40 Issue: 11-2021
DOI 10.17605/OSF.IO/9P4S6

Screening of solvents

Solvent screening was required for homogeneous dispersion of soya lecithin and EA and plays an important role in thin film formation. To get uniform dispersion and clear mixture, different solvents and solvents mixtures were tried as listed in Table [13].

Screening of Edge activator

First step of preparation of transfersomes was deposition of thin lipid film at the base of round bottom flask (RBF). This step was requiring a skill and definite amount of lipid and surfactant ratio with other experimental conditions. If thin film was not properly deposited, it could affect the structure, size and shape of vesicles. Primary screening was done to select edge activator for best thin film formation at the base of round bottom flask. During this stage other parameters were considered constant. Four different EAs were selected and their different ratios with soya lecithin were tried (Table 1). Thin film was observed visually for evaluation [14].

Table 1: Screening of edge activators

SPC:EA	PC	EA	Solvent	Expt. Condition
1:0.25	Soya Lecithin	Tween 20	Methanol: Chloroform (1:2)	Temp 50°C
1:0.5		Tween 80		RPM 50
1.1		Span 20		
1:2		Span 80		
1:3				

Identification of the significant factors by Box-Behnken design

A three-factor, 3-level full factorial design was used for the optimization of the transfersomal formulations where the three factors were evaluated each at three different levels (low, medium and high) and experimental trails were performed using all possible 17 combinations using the Design-Expert® software (Version 12.0.3.0, Stat-Ease Inc., Minneapolis, USA). The Independent variables chosen for transfersomes were the percentage of phospholipid (X1) and ethanol (X2) whereas the amount of phospholipid (X1), edge activator (X2) and temperature (X3) was taken as independent variables for transfersomes. Percent entrapment efficiency (Y1), Vesicle size (Y2) and cumulative drug release (Y3) were selected as the dependent variable for transfersomes. Amount of Efinaconazole was kept constant in all 17 batches for the preparation of transfersomes. The extensive literature search was carried out for the selection of formulation parameters, which may alter the properties of transfersomes. Various factors like percentage of phospholipid, amount of tween and temperature for transfersomes were recognized as critical formulation parameters that could affect the entrapment efficiency, vesicle size and %

Jilin Daxue Xuebao (Gongxueban)/Journal of Jilin University (Engineering and Technology Edition)
ISSN：1671-5497
E-Publication：Online Open Access
Vol: 40 Issue: 11-2021
DOI 10.17605/OSF.IO/9P4S6

cumulative drug release. The initial screening studies were conducted to decide the levels of the formulation variables that influence the characteristics of transfersomes [15].

Table 2: Dependent factors and coded values of Transfersomes

Factor	Name	Units	Type	Minimum	Maximum	Coded Low	Coded High	Mean	Std. Dev.
A	Soya	Mg	Numeric	50.00	100.00	-1 ↔ 50.00	+1 ↔ 100.00	75.00	17.68
B	Tween	Mg	Numeric	25.00	100.00	-1 ↔ 25.00	+1 ↔ 100.00	62.50	26.52
C	Temp	C	Numeric	50.00	70.00	-1 ↔ 50.00	+1 ↔ 70.00	60.00	7.07

Characterization of optimized Efinaconazole transfersomal formulation

Physical Evaluation:

Visual observations were made on the color, phase separation, and precipitation of all the formulations. It was decided to use formulations with good physical properties in order to evaluate their characteristics [16].

Compatibility Evaluation using Differential Scanning Calorimetry (DSC) and Fourier Transform Infra-Red Spectroscopy (FTIR)

Identification of the drug, investigation of drug interactions and their physicochemical compatibility with polymers, diluting agents, and lubricants were studied in all the formulation of transfersomes was accomplished by using DSC (TA-Q20 series, USA). Samples weighing less than 2 mg were placed in aluminum pans and heated at a scanning rate of 10 degrees Celsius per minute between -50 degrees Celsius to 300 degrees Celsius, with nitrogen as the inert gas (50 ml/min). The instrument was calibrated using the element Indium as a reference standard. Transfersomes formulations were tested at 1:1 concentrations of the drug and excipients in order to provide the greatest possible chance of interaction [17]. The transferosomes were mixed (1 percent w/w) with KBr, the drug-excipient interaction was also confirmed using FTIR (Schimadzu IR Affinity, Japan) in the scanning range 4000-650 cm^{-1}, using the KBr method, and the results were published.

Vesicle size determination:

The size and size distribution of the vesicles were determined by using the Dynamic Light Scattering (DLS) technique (Malvern Zetasizer, Nano-ZS, Malvern, United Kingdom) with DTS (nano) software®. The optimized vesicular suspension and gel

Jilin Daxue Xuebao (Gongxueban)/Journal of Jilin University (Engineering and Technology Edition)
ISSN：1671-5497
E-Publication：Online Open Access
Vol: 40 Issue: 11-2021
DOI 10.17605/OSF.IO/9P4S6

was diluted with distilled water and placed in a quartz cuvette, after that it was subjected to size analysis, with the measurements being carried out in triplicate [18].

Zeta potential:

The zeta potential was calculated in accordance with Helmholtz–Smoluchowsky from the electrophoretic mobility of the particles in the solution. For the measurement of the zeta potential, the zeta sizer with field strength of 20 V/cm was used on a large bore measure cell. In this experiment, the samples were diluted with 0.9 percent sodium chloride and adjusted to have a conductivity of 50 lS/cm [19].

Vesicles size, polydispersity index, and zeta potential

Prior to analysis, transfersomes were diluted with filtered phosphate buffered saline solution to remove any traces of protein. [20] The size of transfersomes, as well as their polydispersity index and zeta potential, were measured at 25± 1°C using a Zetasizer (Nano ZS, Malvern Instruments, United Kingdom) (Dragicevic-Curic and colleagues 2009).

Entrapment efficiency determination:

Ultracentrifuge (Optima TM Max-E, Beckman Coulter, CA) was used to centrifuge transfersomes for about 1 hour at 40,000 rpm and 4°C using transfersomes. 1 ml was transferred to eppendorf tubes (Alomrani et al. 2014; Meng et al. 2013). After centrifuging, the supernatant was carefully collected and appropriate dilution were done using mobile phase and the samples were analyzed using high-performance liquid chromatography (HPLC) (Ahad et al. 2015). Entrapment efficiency was calculated using the following equation [21].

Entrapment efficiency (%) = (Total amount of drug in the vesicles − Amount of drug detected only in the supernatant) / Total amount of drug in the vesicles ×100.

Scanning Electron Microscopy (SEM)

The surface morphology of optimized transfersomes was investigated using scanning electron microscopy (SEM). Double-sided carbon adhesive tape was used to mount 1-2 drops of vesicular dispersion on to glass, which was then pasted over a grid, after which it was sputter-coated with conductive gold-palladium. A round coverslip was carefully placed over the stub in order to ensure uniform conductivity. A silver paint lining was then painted around the edges of the coverslip, allowing the narrow space between stub and coverslip to be filled with silver paint. **Preparation of gel base carpool**

Carpool 934 (1 percent w/v) sample was weighed and dissolved into 80mL of double distilled water in a beaker to maintain the required concentration. After the solution was stirred continuously at 800 rpm for 1 hour, after which 10ml of propylene glycol was added to it. It was necessary to adjust the volume of the gel to 100 mL before using a bath sonicator for 10 minutes to remove air bubbles. The pH of the gel base was adjusted to 6.8 in the final step. In order to achieve the desired concentration of Efinaconazole in the gel base, a transfersomal preparation containing 2 percent w/v weight-averaged Efinaconazole was incorporated into the gel base.

Jilin Daxue Xuebao (Gongxueban)/Journal of Jilin University (Engineering and Technology Edition)
ISSN：1671-5497
E-Publication：Online Open Access
Vol: 40 Issue: 11-2021
DOI 10.17605/OSF.IO/9P4S6

Evaluation of Efinaconazole loaded transfersomal gel

Homogeneity

The homogeneity of the developed gels was checked by the visual inspection after the gels had settled in the container for a period of time. They were examined for the appearance and presence of any aggregates [22].

PH measurements

It was possible to determine the pH of selected optimized formulations with the help of a digital pH-meter. The electrode was dipped into the vesicles after it had been calibrated. After that, the pH of the selected formulation was measured, and the readings displayed were noted.

Drug content

One hundred milli grams of topical transfersomal gel were accurately weighed and placed in a beaker with twenty milli liters of methanol. This solution was thoroughly mixed and filtered through Whatman filter paper No. 1 before being used. Then, 1.0 ml of the filtrate solution was transferred to a volumetric flask of capacity 10 ml and the volume was increased to 10 ml by adding methanol to the flask. Using an ultraviolet visible spectrophotometer with a maximum wavelength of 262 nm [23], this solution was analyzed.

Measurement of viscosity

The viscosity of the topical transfersomes-based gel that had been prepared was measured using a Brookfield viscometer with spindle no. 63 and at an optimum speed of 10 rpm [24].

Extrudability

A standard method was used to determine the extrudability, which was expressed in terms of the weight in gram required to extrude a 0.5 cm ribbon of gel in 10 seconds from a collapsible tube [25]. A crimped end of a closed collapsible tube containing gel was firmly pressed against the crimped end of the tube. When the cap was removed, the gel was extruded until it was firmly pressed against the crimped. The gel extruded until the cap was removed, at which point the pressure was released. The greater the amount of material extruded, the better the extrudability.

Spread ability

Spread ability of the formulation is required in order to ensure that a sufficient dose is available for absorption through the skin in order to achieve a good therapeutic response. A device consisting of a lower slide that is fixed to a wooden block and an upper slide that is movable, with one end of the movable slide tied to a weight pan. It was necessary to measure spread ability using 2-5 gm of gel sandwiched between two slides and the weight was gradually increased by placing it on a weighing pan. The time required for the top plate to face the distance of 10 cm after adding 80 g of

ISSN：1671-5497
E-Publication：Online Open Access
Vol: 40 Issue: 11-2021
DOI 10.17605/OSF.IO/9P4S6

weight was recorded. Spread ability that is good indicated if it has got less time to spread. It was calculated using the formula shown below [26].

S=mXl/T

Where, S=Spread ability (gcm/sec), m = weight tied to the upper slide (20 grams),

l= length of glass slide (6cms), t = time taken is seconds.

In- vitro release study of Efinaconazole loaded transfersomal gel

The *in-vitro* release study was conducted out in a 100 mL of Phosphate buffer pH 6.8 at 100 rpm and 37±0.5^{o}C with the motor running at 100 rpm. Transfersomal gel formulation (molecular weight cut off 12000 g/mol) was placed in a treated dialysis bag and 3 mL samples were taken at regular intervals (0, 5, 10, 15, 30, 1, 2, 4, 6, 8, and 10 hours) and the same volume of buffer was replaced each time. The amount of drug present in the withdrawn 1 mL of sample was determined using a UV-spectrophotometer operating at a wave length of 262 nm [27]. For the purpose of analyzing the release behavior from a dosage form, the *in vitro* release data was subjected to kinetic models.

In vitro skin permeation study

The transdermal diffusion system was used to conduct the *in vitro* skin permeation studies across the rat skin surface (SFDC-6, Logan, USA). Diethyl ether was used to sacrifice the rats, and skin samples were cut and removed from their bodies. A hair clipper was used to remove the hair from the rat skin. It was necessary to carefully remove fat and connective tissues, and skin samples were examined for any cuts or holes (Sood et al. 2015). The thickness of the skin was measured using Vernier calipers, and it was found to be between 0.3 to 0.1 mm in thickness. To use the Franz diffusion cell, the rat skin samples were cut to the appropriate size and adhered to the cell. During the experiment, the donor chamber contained the transfersomes formulations, while the receptor chamber contained the vehicle (12 mL, ethanol: phosphate buffered saline 20:80 v/v), which was maintained at 37 °C and stirred at 100 rotations per minute by using a magnetic stirrer. Using a high-performance liquid chromatography (HPLC), samples (1 ml) were taken from the receptor chamber at predetermined time intervals and analyzed for *in vitro* release [28]. The volume of vehicle in the receptor chambers was replenished with new vehicle after the previous vehicle was exhausted.

In vitro Anti-fungal Activity

In vitro anti-fungal activity of different formulation was performed using cup plate technique. The agar medium was prepared by dissolving 3 g of sabouraud dextrose agar powder in 100 ml distilled water. Of Mueller-Hinton broth powder, 0.1 g was dissolved in 60 ml distilled water with 1.5 g of agar and 2 g of glucose powder for the preparation of Mueller-Hinton broth and was sterilized using autoclave at 121°C and 15 lb. pressure for 20 min. The plates were sterilized using hot air oven at 160°C for 1 h before use. Sixty milliliters of Mueller-Hinton broth was poured into the plate and allowed to solidify. The plate was then streak plated with Candida albicans

Jilin Daxue Xuebao (Gongxueban)/Journal of Jilin University (Engineering and Technology Edition)
ISSN : 1671-5497
E-Publication : Online Open Access
Vol: 40 Issue: 11-2021
DOI 10.17605/OSF.IO/9P4S6

suspension. Using the cork borer, 5 cups, each with a diameter of 6 mm, were bored in the medium on each plate. One hundred microliters of blank formulation (negative control), optimized formulation equivalent to 3 mg of the drug (test), marketed product equivalent to 3 mg of drug (positive control), conventional formulation equivalent to 3 mg of drug and one more negative control containing only the vehicle (ethanol and buffer) were placed in the respective cups. The entire process of operation was carried out in the aseptic condition. The plates were incubated at 25°C for 24 h after allowing the formulation to diffuse for 3 h in refrigerated conditions, and the in vitro antifungal activity of each formulation towards Candida albicans was checked [29].

Stability study as per ICH Q1A (R2)

According to the recommendations in the ICH Q1A (R2) (ICH topic Q1 (R2), 2009), a stability study of the Transfersomal formulation was carried out. Three batches of Efinaconazole transfersomal formulation were stored for three months at different temperatures ranging from 25± 2°C and 65 ±5 percent relative humidity to 40±2°C and 75±5 percent relative humidity. After 0, 30, 60, and 90 days, samples were withdrawn and analyzed for particle size distribution, precipitation, and phase separation, among other characteristics.

RESULTS & DISCUSSION

Experimental design for preparation and optimization of Efinaconazole Transfersomes

The conventional rotary evaporation method was employed to prepare Efinaconazole transfersomes. The method was selected because it provides high entrapment efficiency for lipophilic drugs like Efinaconazole into transfersomes. A three-factor, 3-level factorial design (BBD) using Design-Expert® software was applied to optimize the Efinaconazole-loaded transfersomal formulations. The independent variables chosen were percent soya lecithin (X1) Tweens (X2) and temperature (X3) was selected for Efinaconazole transfersomes. The independent variables and their levels were selected on the basis of the extensive literature search and preliminary trails. The dependent variables chosen are % EE (Y1) vesicle size (Y2) and %CDR (Y3) for transfersomes. A total of 17 formulations were prepared transfersomes according to the BBD and the observed responses for % EE, vesicle size and % CDR is shown in Table 2. The obtained responses were fitted into various mathematical models such as linear, two-factor interactions (2FI) and quadratic model using Design-Expert® software. The best fit model was decided on the basis on the high values of multiple correlation coefficient (R2), adjusted R2 and predicted R2 and low values of standard deviation (SD), the coefficient of variation (% CV) and Predicted residual sum of square (PRESS). To evaluate the effect of independent variables on each response, the obtained responses were subjected to multiple linear regression analysis to generate second-order polynomial equation including interaction and quadratic terms.

Jilin Daxue Xuebao (Gongxueban)/Journal of Jilin University (Engineering and Technology Edition)
ISSN：1671-5497
E-Publication：Online Open Access
Vol: 40 Issue: 11-2021
DOI 10.17605/OSF.IO/9P4S6

Optimization of Transfersomal formulation

The most suitable and effective Efinaconazole Transfersomal formulation systems were selected based on the particle size and entrapment efficiency [23]. A composition shown in Table 2 was chosen after a thorough investigation of the various comprehensive evaluations of feasibility search exhaustive grid search options. Particles with an average size ranging from 124±0.2 to 364±2.1nm were prepared using eight different transfersomal formulations. The average size of the particles ranged from 124±0.2 to 364±2.1nm (Table.4). Formulation (F5), a composition containing Lipoid S 100 (60 mg), Tween 80 (40 mg), ethanol (20 ml), and 10 mg of Efinaconazole, was found to be suitable for meeting all of the required optimal parameters for an formulation. The SEM was used to evaluate the size and morphology of the optimized formulation, which was then incorporated into a gel.

Screening of solvents

Preliminary screening was done to optimize minimum volume required to dissolve lipid and surfactant uniformly. Target for solubility was 50 mg/ml for lipid SPC. Methanol, chloroform, Isopropyl alcohol and Acetone were tried in different combinations (Table 5) to solubilize 50 mg lipid. From the trials, it was clear that combination of methanol-chloroform was required in a less quantity than all other solvents and solvent mixtures. 1 part of methanol and 2 part of chloroform were used for next step in the formulation of transfersomes.

Table 3: Screening solvents in different ratios

Trail	Solvents	Ratios	Quantity required to solubilize
1	Chloroform	-	15 mL
2	Acetone	-	12 mL
3	Methanol	-	10 mL
4	Methanol: Chloroform	1:1	7 mL
5	Methanol: Chloroform	1:2	5 mL
6	Methanol: Acetone	1:1	9 mL
7	Methanol: Acetone	1:2	8 mL
8	Acetone: Chloroform	1:1	11 mL
9	Acetone: Chloroform	1:2	9 mL

Jilin Daxue Xuebao (Gongxueban)/Journal of Jilin University (Engineering and Technology Edition)
ISSN：1671-5497
E-Publication：Online Open Access
Vol: 40 Issue: 11-2021
DOI 10.17605/OSF.IO/9P4S6

Table 4: Dependent and independent variables of Transfersomal formulations

Std	Run	X1	X2	X3	Y1	Y2	Y3	PDI	Zeta
15	1	75	62.5	60	65.23	298.46	67.12	0.389	-29.84
11	2	75	25	70	70.84	295.28	78.34	0.324	-20.64
4	3	100	100	60	56.18	443.09	58.38	0.453	-26.76
17	4	75	62.5	60	68.49	374.21	72.21	0.381	-26.91
6	5	100	62.5	50	59.08	359.07	62.03	0.421	-33.56
14	6	75	62.5	60	72.53	261.54	70.24	0.549	-23.54
1	7	50	25	60	40.37	553.06	49.09	0.546	-22.34
2	8	100	25	60	53.92	356.72	59.08	0.531	-25.31
7	9	50	62.5	70	67.05	387.34	73.04	0.591	-26.01
16	10	75	62.5	60	63.39	325.28	67.12	0.642	-22.86
9	11	75	25	50	58.16	312.53	62.31	0.594	-25.32
12	12	75	100	70	79.32	247.61	82.26	0.312	-26.87
13	13	75	62.5	60	70.42	224.17	75.25	0.483	-25.54
10	14	75	100	50	65.84	254.38	69.08	0.421	-22.23
8	15	100	62.5	70	84.61	132.97	88.25	0.268	-32.67
5	16	50	62.5	50	59.84	197.85	63.32	0.381	-23.21
3	17	50	100	60	49.08	369.54	57.51	0.354	-25.21

Fitting of data to the model

The observed responses for all 17 formulations were simultaneously fitted to various mathematical models using Design-Expert® software to find the best fit model. The comparative values of SD, R2, adjusted R2, predicted R2, CV, and PRESS for all models were shown in Table 5. The quadratic model was found to be the best fit model for both the responses i.e. %EE (Y1) vesicle size (Y2) and %CDR (Y3) as specified by high values of R2, adjusted R2 value and predicted R2 value and low values of SD, CV, and PRESS. The PRESS statistic shows how well the model fits the data. The PRESS for the selected model should be small compared to the other models.

Jilin Daxue Xuebao (Gongxueban)/Journal of Jilin University (Engineering and Technology Edition)
ISSN：1671-5497
E-Publication：Online Open Access
Vol: 40 Issue: 11-2021
DOI 10.17605/OSF.IO/9P4S6

Table 5: Analysis of variance in RSM and regression analysis predicted second-order polynomial model for entrapment efficiency.

Source	Y1 (% EE)			Y2 (Vesicle size)			Y3 ()		
Model	**R^2**	**Adjusted R^2**	**Predicted R^2**	**R^2**	**Adjusted R^2**	**Predicted R^2**	**R^2**	**Adjusted R^2**	**Predicted R^2**
Linear	0.3741	0.2297	-0.2355	0.0736	-0.1402	-0.8434	0.4168	0.2823	-0.1723
2FI	0.4246	0.0793	-1.6465	0.4690	0.1504	-1.3302	0.4726	0.1562	-1.5084
Quadratic	**0.9647**	**0.9193**	**0.8629**	**0.9133**	**0.8019**	**0.8517**	**0.9639**	**0.9174**	**0.8575**
p value	0.0003			0.0056			0.0003		

Effect of independent variable of % EE (Y1) of Efinaconazole loaded Transfersomes

The maximum entrapment efficiency was obtained for F 15 (84.61 ± 2.15%) while the minimum value was obtained for F7 (40.37± 0.26%). The effect of independent variables on the % EE can be elucidated by the following polynomial quadratic equation.

$$\%EE=+68.01+4.68A+3.39B+7.36C-1.61AB+4.58AC+0.2000\,BC-9.51A^2-8.61B^2+9.14C^2$$

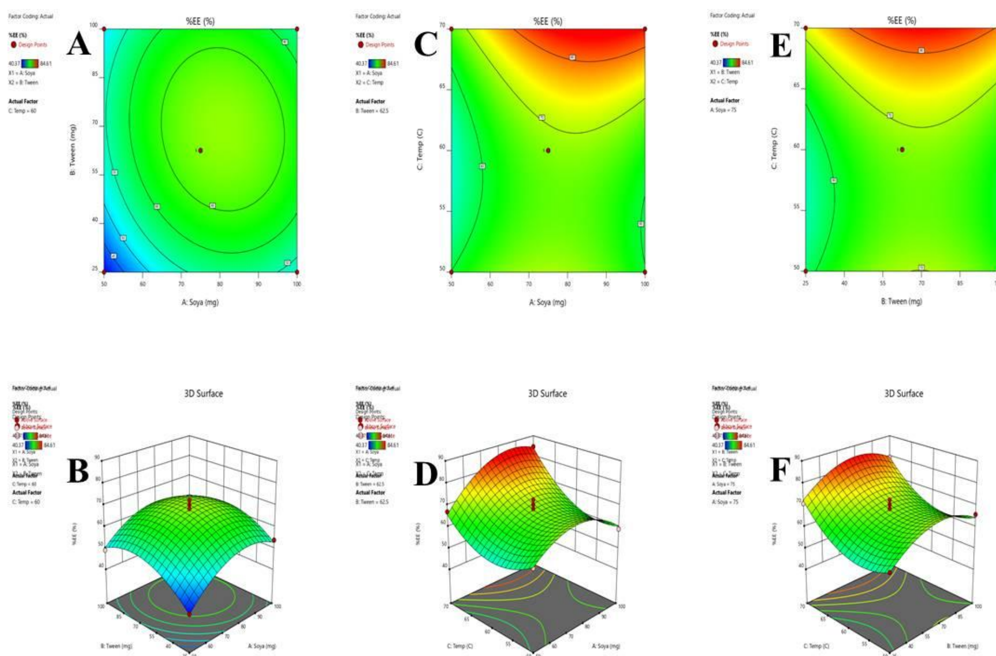

Figure 1: Counter and response surface plots of % EE in different independent variables effect

Jilin Daxue Xuebao (Gongxueban)/Journal of Jilin University (Engineering and Technology Edition)
ISSN：1671-5497
E-Publication：Online Open Access
Vol: 40 Issue: 11-2021
DOI 10.17605/OSF.IO/9P4S6

Effect of independent variables on vesicle size (Y2) of Efinaconazole loaded transfersomes

The vesicle sizes of the Efinaconazole loaded transfersomes formulations are shown in Table 4. The smallest vesicle size was observed for Efinaconazole loaded transfersomes formulation F15 (132.97 nm) while the maximum vesicle size was obtained as 553.06 nm for F7. The effect of independent variables on the vesicle size can be best described by the below quadratic equation.

$$Size=+296.73-26.99A-25.37B-7.58C+67.47AB-103.90AC+2.62BC+62.86A^2+71.01B^2-90.29C^2$$

Table 6: ANOVA test results and adequate precision for each response of Efinaconazole transfersomes according to quadratic model

Parameter	Source	DF	Sum of squares	Mean of squares	F Value	*p* Value
%EE	**Model**	1807.37	9	200.82	21.24	0.0003
	Residual	66.17	7	9.45		
	Lack of Fit	10.63	3	3.54	0.2552	0.8545
	Pure Error	55.54	4	13.89		
Vesicle size	**Model**	1.419E+05	9	15764.94	8.20	0.0056
	Residual	13463.56	7	1923.37		
	Lack of Fit	139.03	3	46.34	0.0139	0.9974
	Pure Error	13324.54	4	3331.13		
%CDR	**Model**	1495.48	9	166.16	20.75	0.0003
	Residual	56.05	7	8.01		
	Lack of Fit	9.25	3	3.08	0.2635	0.8490
	Pure Error	46.80	4	11.70		

Jilin Daxue Xuebao (Gongxueban)/Journal of Jilin University (Engineering and Technology Edition)
ISSN：1671-5497
E-Publication：Online Open Access
Vol: 40 Issue: 11-2021
DOI 10.17605/OSF.IO/9P4S6

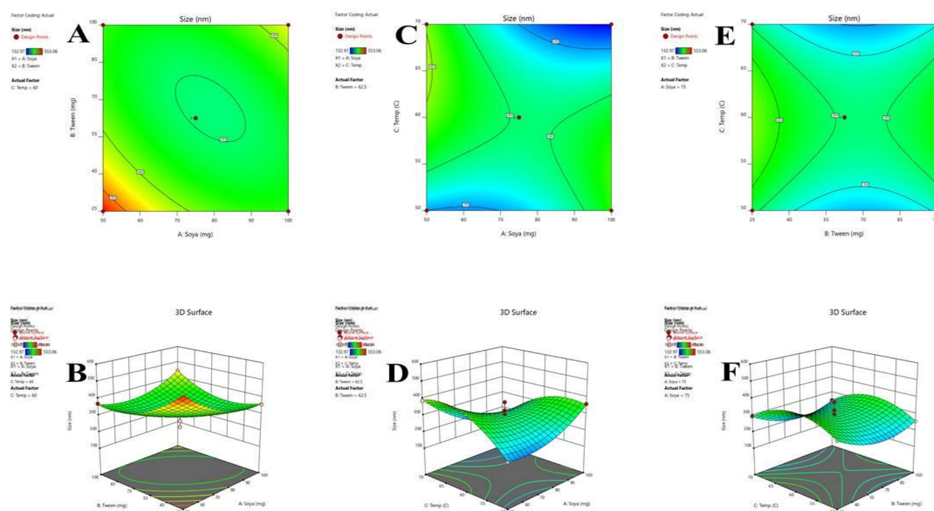

Figure 2: Counter and response surface plots of Vesicle size in different independent variables effect

Effect of independent variables on %CDR (Y3) of Efinaconazole loaded Transfersomes

The smallest % CDR was observed for F7 (49.09%) while the highest was obtained for F15 (88.25%). The effect of independent variables on the % CDR can be described by the following quadratic equation.

$$\%CDR = +70.20+3.12A+2.25B+8.13C-2.25AB+4.00AC-0.7500BC-7.85A^2-6.60B^2+9.15C^2$$

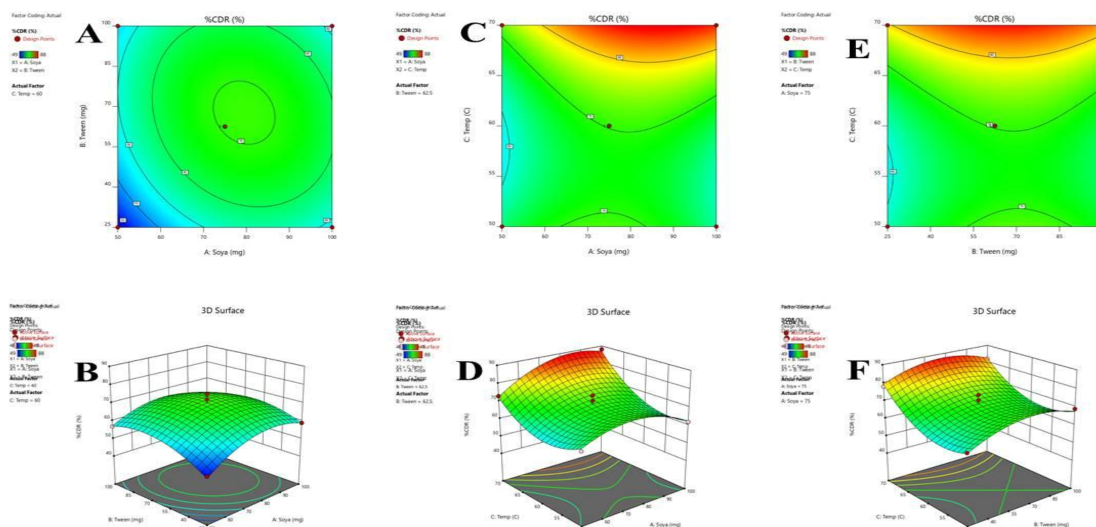

Figure 3: Counter and response surface plots of % CDR in different independent variables effect

Jilin Daxue Xuebao (Gongxueban)/Journal of Jilin University (Engineering and Technology Edition)
ISSN：1671-5497
E-Publication：Online Open Access
Vol: 40 Issue: 11-2021
DOI 10.17605/OSF.IO/9P4S6

Compatibility studies

FT-IR Spectroscopy

FT-IR spectroscopic studies shows interaction between the Drug and the excipients. The samples were dispersed in KBr pellet and scanned using Bruker FTIR Spectrophotometer between 4000-400 cm^{-1} with resolution of 4 cm^{-1}.

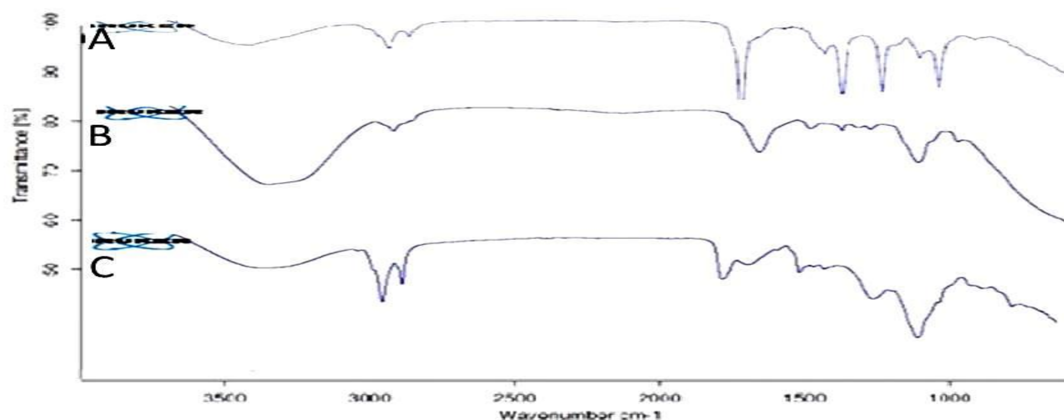

Figure 4: FTIR analysis of (A) Drug (B) Transfersomal suspension (C) Transfersomal gel

Differential Scanning Calorimetry:

Differential Scanning Calorimetry (DSC) experiments were carried out in order to determine whether or not there was any interaction between the drug and excipients, as well as whether or not there was any alteration in the drug properties. It measures the changes in enthalpy that occur during endothermic or exothermic reactions. The melting point and heat of fusion of indium (calibration standard with purity greater than 99.99 percent) were measured and calibrated with the instrument. For the analysis, approximately 5-15 mg of the drug sample was placed into standard aluminium pans. As a point of reference, an empty pan was used. The rate of temperature flow was kept at 10 degrees Celsius per minute, nitrogen was used as a purge gas, and liquid nitrogen was used to cool the system. This was accomplished through the use of a differential thermal analyzer.

Jilin Daxue Xuebao (Gongxueban)/Journal of Jilin University (Engineering and Technology Edition)
ISSN：1671-5497
E-Publication：Online Open Access
Vol: 40 Issue: 11-2021
DOI 10.17605/OSF.IO/9P4S6

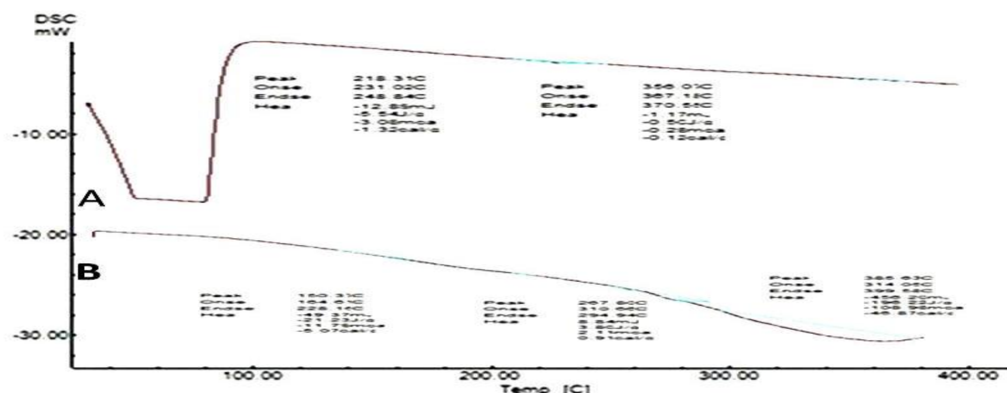

Figure 5: DSC Analysis of (A) Transfersomal suspension (B) Transfersomal gel

Characterization of optimized Efinaconazole transfersomal formulation

Vesicular Shape, Morphology and PDE

In SEM images, the transfersomes showed and an irregular shape, as well as a smooth and even surface on which the information need to be transferred (Fig.6). The particle size of the sample reveled that they are very close to the standard and this was determined by using the Malvern Zetasizer (126.9 ±5.45 nm).

Entrapment efficiency

The entrapment efficiency was determined by using centrifugation method in which the transfersomal vesicles formed in the precipitate was studied in terms of percent EE. The vesicles were separated in a centrifuge at 15000 rpm for 60 minutes to separate them from one another. The sediment and supernatant liquids were separated and the amount of drug present in the sediment was determined by analyzing the vesicles with ethanol. The vesicles were broken open in order to release the drug, based on dose dependent manner. The absorbance of the drug was measured at a wavelength of 262 nm. The following equation was used to calculate the entrapment efficiency as a result of this:

Jilin Daxue Xuebao (Gongxueban)/Journal of Jilin University (Engineering and Technology Edition)
ISSN：1671-5497
E-Publication：Online Open Access
Vol: 40 Issue: 11-2021
DOI 10.17605/OSF.IO/9P4S6

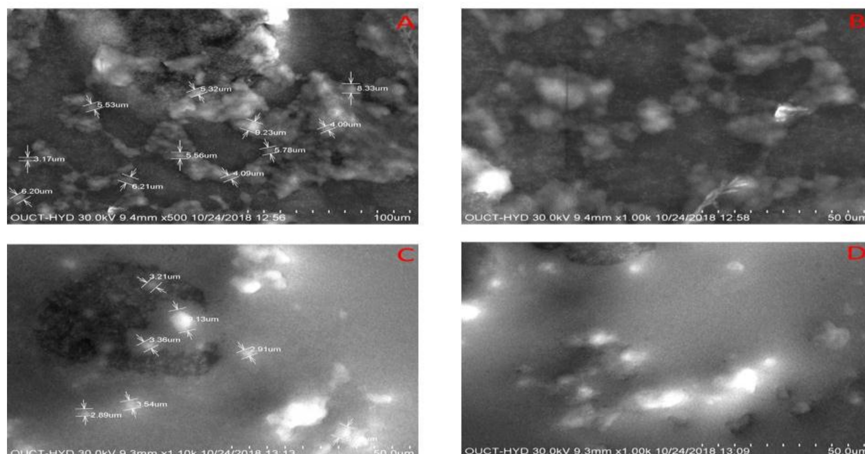

Figure 6: SEM Analysis of optimized Transfersomal Suspension and gel formulations

Evaluation of Efinaconazole gel formulation

The efficacy of Efinaconazole Transfersomal gel prepared with 1 percent Carpool 940 P was assessed for the critical parameters listed in Table 1. The G4 formulation exhibited excellent homogeneity, adequate skin retention due to its permissible viscosity, high spread ability and extrudability with low evaporation rate. Obtaining apparent viscosity values for gel as a function of increasing shear rate was accomplished and was illustrated in Figure 1. The G5 formulation rheogram showed characteristics of pseudo plastic flow, which indicated that their viscosity decreases with increasing shear rate [24]. The gel was found to be uniform in its appearance and also in terms of pourable viscosity.

Table No 7: Evaluation of Efinaconazole Transfersomal gel formulation

Homogenity	Mean Viscosity (Pa.s)±S.D. (n=3)	Mean Drug content (%)±S.D.(n=3)	Mean pH±S.D. (n=3)	Mean spread ability (gcm/sec)±S.D. (n=3)	Mean Extrudability (gm)±S.D. (n=3)
Good	5.8± 0.67	96.25 ± 1.13	7.4±0.12	9.68±0.342	155±2.51

Diffusion Study

The diffusion studies were carried out by selecting pH 7.4 phosphate buffer solutions as the diffusion medium. The egg membrane was mounted between the donor and receptor compartments of the Franz diffusion cell in order to create a diffusion cell gradient. Both compartments were separated by an egg membrane. The membrane

ISSN：1671-5497
E-Publication：Online Open Access
Vol: 40 Issue: 11-2021
DOI 10.17605/OSF.IO/9P4S6

separating the two compartments had a surface area of 1.7662 cm^2. It was necessary to accurately pipette the transfersomal suspension (200 mL volume) into the donor compartment, which was then covered with aluminum foil to prevent any evaporation. The temperature of the diffusion medium (7 mL) was maintained at 37±1 ^oC, so that the membrane only just touches the surface of the receptor medium. The diffusion medium was continuously stirred by a magnetic bar rotating at 100 rpm. Each 0.5 ml volume was withdrawn at the specified time intervals and replaced with equal volume of receptor medium (phosphate buffer pH 7.4) in order to maintain the sink condition in the cell culture system. Using an ultraviolet spectrophotometer a maximum wavelength of 262 nm, these samples were analysed.

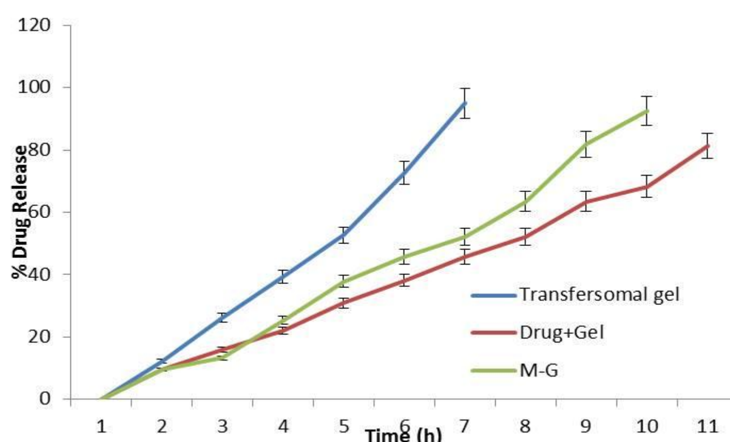

Figure 7: Comparison of Diffusion studies of Transfersomal gel and Marketed formulation

Ex-vivo permeation Studies

Transient flux values of the gels, i.e. transfersomal suspension, transfersomal gel, and drug suspension gel, were determined to be in the range of 0.016 ±003, 0.024±0.002, and 0.008±0.002 mg/cm^2/h respectively (Table.6.). Transfersomal gel had a significantly higher flux value than drug suspension gel and showed a significant difference ($p \leq 0.05$). This can be attributed to the presence of polymers, which play a significant role in the diffusion and permeation of pharmaceutical agents. Increased permeation of transfersomal gel could be caused by different of factors including the Nano sized ultra-transformable nature of the transfersomal vesicles and an increased in the interfacial area, which can influence the transportation of the medication [19].

Table: The *ex vivo* permeation profiles of Transfersomal gel and drug suspension gel were evaluated and compared. The mean and standard deviation were calculated from the slope of the linear portion of the graph. Kp was calculated by dividing Flux by the concentration of the drug present in the donor cell, as shown in the graph. A standard deviation has not been showed in the case of Kp, due to the small values and bulkiness of the table.

Jilin Daxue Xuebao (Gongxueban)/Journal of Jilin University (Engineering and Technology Edition)
ISSN：1671-5497
E-Publication：Online Open Access
Vol: 40 Issue: 11-2021
DOI 10.17605/OSF.IO/9P4S6

Table No 8: Ex-vivo Permeation studies of Transfersomes

Samples	Flux mg/cm^2/h	Kp
Transfersomal suspension	0.016±0.003	0.00380027
Transfersomal gel	0.024±0.002	0.00520036
Drug suspension gel	0.008±0.0002	0.00260013

In vitro Anti-fungal Activity

In vitro anti-fungal activity for the optimized transfersomes formulation was further assessed by cup plate method against Candida albicans. The zones of inhibition of different formulation are represented graphically. As shown in Fig. 5, the developed and optimized transfersomes formulation, transfersomal gel and marketed formulation showed resultant zones of inhibition than compared with blank formulation and control. Transfersomal gel shows the zones of inhibition diameter; it was found that the anti-fungal activity area of the optimized Efinaconazole-loaded transfersomes formulation was found to be greater (3.1 cm) than that of marketed formulation, transfersomal suspension, blank formulation and negative control. So the results demonstrated that the optimized transfersomal gel formulation was sensitive towards Candida albicans and has got anti-fungal activity against it. The transfersomal formulation acts as a carrier for the delivery of the drug at a particular site of action. The anti-fungal activity of the Efinaconazole-loaded transfersomes formulation is due to the high flexibility of the transfersomal suspension, facilitating its penetration through the fungal cell wall and inhibiting ergosterol synthesis resulting in fungal cell membrane lysis and cell death (7). The Efinaconazole loaded transfersomal formulation showed the slow and prolonged release of the drug and indicates the longer duration of anti-fungal activity as compared to transfersomal suspension and marketed formulation. The transfersomal gel formulation acts as a reservoir system, enabling slow, uniform and sustained release of the drug (8).

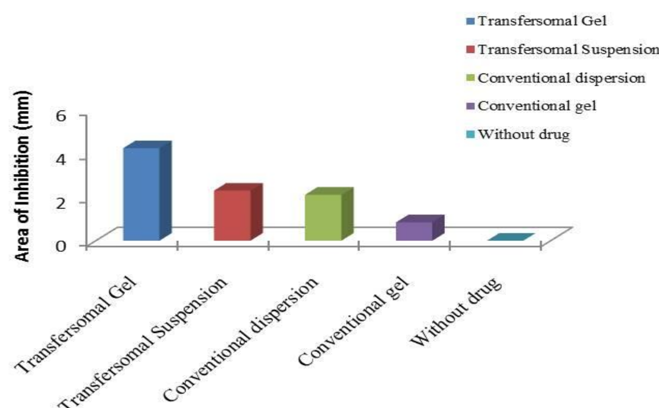

Figure No 8: invitro anti-fungal activity of different formulations

Jilin Daxue Xuebao (Gongxueban)/Journal of Jilin University (Engineering and Technology Edition)
ISSN：1671-5497
E-Publication：Online Open Access
Vol: 40 Issue: 11-2021
DOI 10.17605/OSF.IO/9P4S6

Stability study as per ICH Q1A (R2) and determination of shelf life.

Samples were kept for stability studies at predetermined time intervals and were analyzed for particle size distribution, precipitate formation, phase separation, PDI, percent EE and drug content. It was proved that transfersomes at the end of 90 days showed no precipitation or phase separation but had a particle size of 132.97, a PDI of 0.381, and a percent EE of 84.61, with no precipitation or phase separation (Table.12). Table 13 [21] depicts the results on slope calculations and degradation rate constants (K) [21]. The shelf life of formulation transfersomes was determined to be 452 days, or 1.252 years, when stored at 25 degrees Celsius.

Table No 9: Stability studies of Efinaconazole loaded transfersomes at 25 ^{0}C and 40 ^{0}C

Time (Days)	Temp (^{0}C)	% Drug Remaining	Log % Drug remaining	Slope	K
0	25	98.4	1.9929	0.000241	0.0002303
30		97.7	1.9898		
60		96.9	1.9863		
90		96.2	1.9831		
0	40	98.4	1.9929		
30		97.5	1.9890		
60		96.4	1.9840		
90		95.8	1.9813		

Conclusion:

The transdermal route of Drug Delivery is found to be interesting option due to its convenience and safety. Transferosomes are especially highly deformable vesicles and the flexibility of the transferosomes is achieved by mixing suitable surfactants in the proper ratio. Prepared transferosomes showed higher flux and accumulation of drug in the skin compared with ethanolic preparations of Efinaconazole. Moreover, incorporation of vesicles in aqueous gel also enhanced the permeation and deposition of drug. Likewise, transfersomal gel showed higher permeation and deposition compared with transfersomal suspension. Therefore, transferosomes seem to be a promising carrier for skin penetration or deposition of Efinaconazole and can work as a transporter and localizer. The deposition of transferosomes into the skin creates a reservoir and targeting to deeper skin layers is beneficial for various skin diseases. Further investigation in this area will allow better control over drug release *in vivo* and the long-term safety data and lead to more effective therapy.

Jilin Daxue Xuebao (Gongxueban)/Journal of Jilin University (Engineering and Technology Edition)
ISSN：1671-5497
E-Publication：Online Open Access
Vol: 40 Issue: 11-2021
DOI 10.17605/OSF.IO/9P4S6

Efinaconazole loaded with transfersomes prepared by reverse phase evaporation technique. Total 8 formulations were prepared in different ratios and evaluated drug entrapment efficiency and drug release studies. Among 8 formulations of transfersomes, F8 formulation was maximum drug entrapment efficiency compared with other formulations.

Transfersomes F8 formulation was optimized due to its high permeability and good release of drug. The F8 formulation showed drug entrapment efficiency of 97.4 % and drug release of 96.6% sustained upto 32 hrs. Drug release followed zero order kinetics with Fickian diffusion mechanism.

References:

1) Thapa RK, Choi JY, Go TG, et al. Development of ciclopirox nail lacquer with enhanced permeation and retention. Arch Pharm Res 2016; 39(7): 953-9.
2) McAuley WJ, Jones SA, Traynor MJ, Guesné S, Murdan S, Brown MB. An investigation of how fungal infection influences drug penetration through onychomycosis patients nails plates. Eur J Pharm Biopharm 2016; 102: 178-84.
3) Adekhandi S, Pal S, Sharma N, Juyal D. Incidence and epidemiology of onychomycosis in patients visiting a tertiary care hospital in India. Cutis 2015; 95: E20-E5.
4) Ghannoum M, Isham N. Fungal nail infections [onychomycosis]: a never-ending story? PLoS Pathog 2014; 10(6): e1004105.
5) Kushwaha A, Murthy RN, Murthy SN, Elkeeb R, Hui X, Maibach HI. Emerging therapies for the treatment of ungual onychomycosis. Drug Dev Ind Pharm 2015; 41(10): 1-7.
6) Bseiso EA, Nasr M, Sammour OA, Abd El Gawad NA. Novel nail penetration enhancer containing vesicles “nPEVs” for treatment of onychomycosis. Drug Deliv 2015; 23: 1-7.
7) Rocha KAD, Krawczyk-Santos AP, Andrade LM, et al. Voriconazole-loaded nanostructured lipid carriers [NLC] for drug delivery in deeper regions of the nail plate. Int J Pharm 2017; 531(1): 292-8.
8) Shah VH, Jobanputra A. Enhanced ungual permeation of terbinafine HCL delivered through liposome loaded nail lacquer formulation optimized by QbD approach. AAPS Pharm Sci Tech 2018; 19(1): 213-24.
9) Flores FC, Rosso RS, Cruz L, Beck RCR, Silva CB. An innovative polysaccharide nano based nail formulation for improvement of onychomycosis treatment. Eur J Pharm Sci 2017; 100: 56-63.
10) Turner R, Weaver S, Caserta F, Brown MB. The inadequacies of the current treatment plans give rise to the pressing need for a topical ungual treatment, allowing site specific delivery and minimizing systemic exposure. AAPS Pharm SciTech 2016; 20(1): 71-80.
11) Cevc, G., 1996. Transtersome, liposomes and other lipid suspensions on the skin: permeation enhancement, vesicle penetration, and transdermal drug delivery. Crit. Rev. Ther. Drug Carrier Syst. 13, 257–388.
12) Cevc, G., 2004. Lipid vesicles and other colloids as drug carriers on the skin. Adv. Drug Deliv. Rev. 56, 675–711.
13) Cevc, G., Gebauer, D., Stieber, J., Schatzlein, A., Blume, G., 1998. Ultraflexible vesicles, transfersomes, have an extremely low pore penetration resistance and

Jilin Daxue Xuebao (Gongxueban)/Journal of Jilin University (Engineering and Technology Edition)
ISSN：1671-5497
E-Publication：Online Open Access
Vol: 40 Issue: 11-2021
DOI 10.17605/OSF.IO/9P4S6

transport therapeutic amounts of insulin across the intact mammalian skin. Biochim. Biophys. Acta 1368, 201–215.
14) Cevc, G., Vierl, U., Mazgareanu, S., 2008. Functional characterization of novel analgesic product based on self-regulating drug carriers. Int. J. Pharm. 360, 18–28.
15) Chen, T.K., Lin, H.Q., Li, Y., 2009. The progress of new carrier transdermal delivery of flexible nano-liposomes. Prog. Pharm. Sci. 12, 732–734.
16) Ning, Y.M., Guo, Y.Z., 2004. Liposome for transdermal absorption of drugs progress. J. Fore Med. Pharm. 31, 302–307.
17) Wang, H., Guo, D.H., Guo, S.L., 2006. New non-invasive transdermals delver of drugs: transfersomes. Chin. J. New Drugs 15, 341–344.
18) J.Shaji, M. Lal, Preparation, Optimization and Evaluation of transferosomal formulation for enhanced transdermal delivery of a Cox-2 inhibitor, Int. J. Pharm. Pharm. Sci. 6 (2014) 467-477
19) M.K. Anwer, S. Jamil, M.J. Ansari, R. Al-Shdefat, B.E. Ali, M.A. Ganaie, M.S. Abdel-Kader, F. Shakeel, Water soluble binary and ternary complexes of diosmin with β-cyclodextrin: Spectroscopic characterization, release studies and anti-oxidant activity, J. Mol. Liquids. 199 (2014) 35-41.
20) M.K. Anwer, S. Jamil, E.O. Ibnouf, F. Shakeel, Enhanced antibacterial effects of clove essential oil by nanoemulsion, J. Oleo. Sci. 63 (2014) 347-354.
21) A.A. Abdellatif, H.M. Tawfeek, Transfersomal Nanoparticles for Enhanced Transdermal Delivery of Clindamycin, AAPS. PharmSciTech. 17 (2016) 1067-1074.
22) M. R. Sunilkumar, J. Adlin Jino Nesalin and T. Tamiz Mani. Development and evaluation of niosomes containing Ketoconazole. World J Pham & Pharm. Sci. 5 (2016) 1318-1327.
23) S.S. Deshkar, A.T. Patil.Development of mucoadhesive gel of fluconazole for vaginal candidiasis, Indo. Am. J. Pharm. Res. (2015) 3599-1610.
24) Mir-Palomo S, Nácher A, Díez-Sales O, Busó M, Caddeo C, Manca M et al. Inhibition of skin inflammation by baicalin ultradeformable vesicles. Int JPharm 2016; 511: 23-29.
25) Aljaeid B, Hosny K. Miconazole-loaded solid lipid nanoparticles: formulation and evaluation of a novel formula with high bioavailability and antifungal activity. Int J Nanomed 2016; 11: 441-447.
26) Thomas AP, Dubey R, Jain P. Formulation and Evaluation of Ethosomal Gel of Tazarotene for Topical Delivery. Asian J Pharm 2019; 13 (1):38-45.
27) Nimker V, Jamal H, Gosh P, Jain S, Beotra A. Liposomes; drug delivery system or possible doping agent. J Drug Deliv Ther 2017; 7: 25-9.
28) Zhaoa YZ, Zhanga Y, Xiaoa J, Zhaob YP, Tianc JL, Xud YY, et al. Selection of high efficient transdermal lipid vesicle for curcumin skin delivery. Int J Pharm 2013; 454:1-15.
29) Ai X, Zhong L, Niu H, He Z. 2014. Thin-film hydration preparation method and stability test of DOX-loaded disulfide-linked polyethylene glycol 5000-lysine-ditocopherol succinate nanomicelles. Asian J Pharm Sci. 9:244-250.