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Research Article

QbD Based Optimization Of Luliconazole Loaded Transferosomal Gel For Topical Fungal Infection

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ABSTRACT

Transferosomes overcome the skin penetration difficulty by squeezing themselves along the intracellular sealing lipids of stratum corneum. Advantages of Transferosomes are wide range of solubility, better penetration, biocompatible and biodegradable. The transferosomes were formulated by the conventional rotary evaporation sonication method. It contains phospholipids; surfactant and the drug were formulated. Evaluation parameters of Transferosomes are as Vesicle size distribution and zeta potential, Vesicle morphology, No. of vesicles per cubic mm, Entrapment efficiency, Drug content, Turbidity measurement, Degree of deformability or permeability measurement, Penetration ability, Surface charge and charge density, In-vitro drug release, in-vitro Skin permeation Studies, Physical stability. They can deform and pass through narrow constriction (from 5 to 10 times less than their own diameter) without significant loss. QbD aims to design quality into the product rather than relying solely on end-product testing. Luliconazole (LUL), an antifungal drug containing imidazole moiety with ketone dithioacetate, is a broad-spectrum agent. It disrupts the conversion of lanosterol to ergosterol, thus hinders the cell wall formation of fungi. transferosomal gel is selected to enhance the permeability of luliconazole that increases bioavailability, reduce the GI side effects, eliminate first pass metabolism, reduce large doses and increase the therapeutic efficacy

INTRODUCTION

INTRODUCTION TO TRANSFEROSOMES [1-3]

Transferosomes is a carrying body for targeted transdermal drug delivery system. This are special types of liposomes, consisting of

phosphatidylcholine and an edge activator. This system also takes advantage of phospholipids vesicles as transdermal drug carrier. It penetrates the stratum corneum by either intracellular route or the transcellular route by the generation of

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“osmotic gradient”. Advantages of Transferosomes are wide range of solubilities, better penetration, biocompatible and biodegradable etc. Advantages of Transferosomes are oxidative degradation, expensive, etc. [1] The transferosomes were formulated by the conventional rotary evaporation sonication method. It contains phospholipids, surfactant and the drug were formulated. Evaluation parameters of transferosome are as Vesicle size distribution and zeta potential, Vesicle morphology, No. of vesicles per cubic mm, Entrapment efficiency, Drug content, Turbidity measurement, Degree of deformability or permeability measurement, Penetration ability, Occlusion effect, Surface charge and charge density, In-vitro drug release, in-vitro Skin permeation Studies, Physical stability. Transferosomes can be applied in controlled release, transportation of large molecules weight compounds, target delivery to peripheral subcutaneous tissues, transdermal immunization etc. [2] Transferosome is a trademark registered by the German company IDEA AG, which refers to its proprietary drug delivery technology. The name means “carrying body” and is derived from the Latin word 'transferre', meaning 'to carry across' and the Greek word 'soma', meaning 'a body'. A Transferosome carrier is an artificial vesicle designed to exhibit the characteristics of a cell vesicle or a cell engaged in exocytosis, and thus suitable for controlled and potentially, targeted drug delivery. Transferosomes are complex vesicles that have extremely flexible & self-regulating membranes, which make the vesicle very deformable. Transferosome vesicle can cross microporous barriers efficiently, even if the porous are much smaller than the vesicles size. [3]

INTRODUCTION TO FUNGAL INFECTIONS [4-9]

The emergence of fungi causing human infection is a growing serious public health problem. Fungi of medical importance may be characterized as

primary or opportunistic. Primary which causes infection in healthy population who are not exposed to endemic fungi, whereas opportunistic infect immune suppressed individuals. State of art medical devices and services increases the depending population on healthcare which leads to increase in number of susceptible hosts. [4] The emergence and re-emergence of fungal infections such as candidiasis, mucormycosis (zygomycosis), aspergillosis, Cryptococcus's, and pneumocystis are quite common. However, Candidemia is one of the leading causes of bloodstream infections with mortality rate more than 30% whereas Aspergilla's can affect more than 45% of susceptible host. Zygomycosis is common among diabetics all around the globe including India. Mortality associated with invasive fungal infections among patients hospitalized in ICUs has reached to 67%. [5] A majority of children, at one time or other suffers some or the other form of fungal infection. For instance, if a child develops a rash on the buttocks or white patches in the mouth, it is as a result of fungal or yeast infection. Fungal infections which were quite rare at the beginning of this century are now increasingly growing at a rapid rate. [6] This is probably due to the result of the increase in number of immunocompromised children. Normally children have strong natural immunity to fungi. Only a couple of fungi out of thousands are pathogenic. Fungi are very good at taking advantage of some abnormality in the human host and, thus, virtually every fungal infection is opportunistic. [7] Fungi are eukaryotic, nonmotile, and usually aerobic. They can exist as parasites or free living organisms and need organic sources of nourishment. They have a dense rigid cell wall made of glucan and chitin (found in crab shells). Their cell membrane contains sterols (ergosterol), making them similar enough to human cell membranes to have negative implications for the membrane destroying properties of antifungal drugs.



Fungi come in many forms but only three are of our interest as they may cause disease in human being: [8, 9]

1. Yeasts –

round/oval, unicellular, and reproduce via budding

2. Molds –

long, floppy, fluffy colonies that microscopically can be seen as long tubular structures called hyphae and reproduce by forming spore-forming structures at the end of hyphae called conidia.

3. Dimorphs –

most medically important, can change from yeast to mold and back, and grow in environment as molds and in humans as yeast. Fungi can produce toxins but this is not relevant to human infections. Fungi can produce human disease because of their sheer size (50– 100 times larger than bacteria) and by eliciting an immune response as a result of themselves or their by-products.

TYPES OF FUNGAL INFECTIONS [6, 7]

Fungal infections in children are broadly classified into three types:

- I. Superficial/cutaneous – present on skin, hair, nails
- II. Subcutaneous – infection in tissues under the skin
- III. Systemic – they are of two types: “true pathogens” (term is becoming obsolete) – which have the ability to cause disease in healthy hosts and opportunists – which cause disease exclusively in immunocompromised individuals.

Drug profile[8, 9]

Luliconazole: Luliconazole is a topical antifungal agent that acts by unknown mechanisms but is postulated to involve altering the synthesis of fungi cell membranes. It was approved by the FDA (USA) in November 2013 and is marketed under the brand name Luzu. Luliconazole is also approved in Japan.

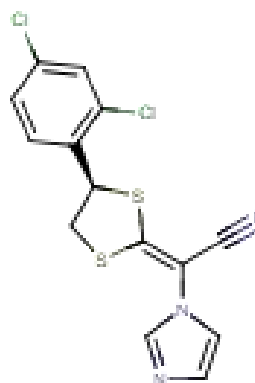


Figure no.1 - Structure of luliconazole

Chemical formula of luliconazole

C₁₄H₉Cl₂N₃S₂ .IUPAC name of luliconazole 2-[(2E,4R)-4-(2,4-dichlorophenyl)-1,3-dithiolan-2-ylidene]-2-(1H-imidazol-1-yl)acetone nitrile.

MECHANISM OF ACTION-

The exact mechanism of action for luliconazole anti-fungal activity is still not known, but luliconazole is thought to inhibit the enzyme lanosterol demethylase. Lanosterol demethylase is needed for the synthesis of ergosterol, which is a major component of the fungus cell membranes.

MATERIALS AND METHODS

Materials

Luliconazole was obtained as gift sample from Kivi Pvt. Ltd. Tween 80, Span 80, Lecithin Phosphatidylcholine, Ethanol, Methanol, 7.4 Phosphate buffer were procured from chemdyes corporation.

RESULT DISCUSSION:

Preparation of Calibration Curve for Luliconazole:

A luliconazole stock solution was made by dissolving 100 mg of the medication in a tiny amount of methanol, sonicating it for a few minutes, and diluting it with 100 ml of phosphate buffer (pH 7.4). The stock solution was serially diluted to get solutions in the range of 2- 10µg/ml, and the solution's maximum concentration was determined. At 296 nm, the absorbance of several diluted solutions was measured in a UV-visible spectrophotometer. A calibration curve was

created by plotting drug concentrations ($\mu\text{g/ml}$) against absorbance (nm) in displayed in figure 1.

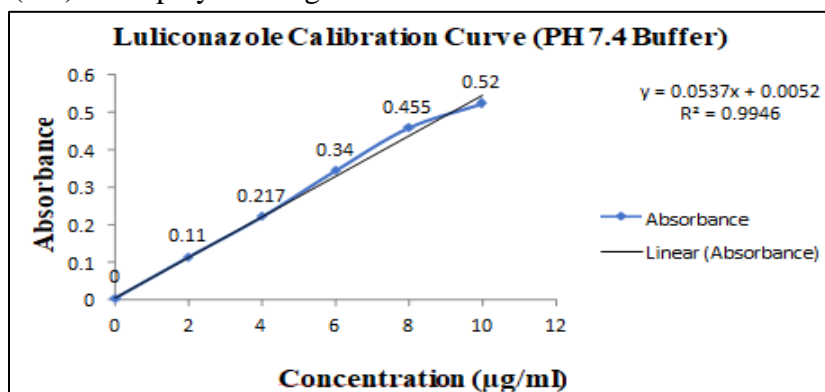


Figure 1: Calibration Curve of Luliconazole

Identification of Drug- Luliconazole by FT-IR Spectroscopy:

The identification of Luliconazole was carried out using the FTIR study. For this, the FTIR spectra of the pure drug were recorded using FTIR 8400 S

Shimadzu spectrophotometer. The powder of dried samples of the drug was prepared with the potassium bromide pellet method. The scans were performed at a resolution of 4000-400 cm^{-1} displayed in figure 2.

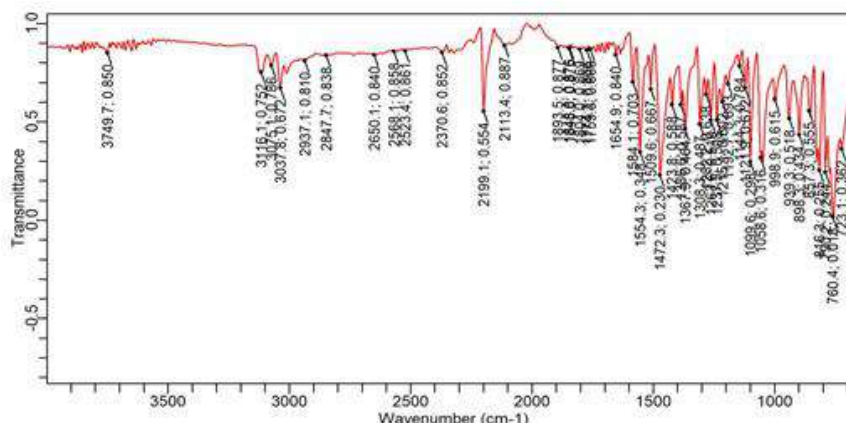


Figure 2: Identification of drug (Luliconazole) using FT-IR

Method of Preparation of Luliconazole loaded transfersomes[14, 15]

Lecithin (phospholipids) and mixed with edge activator Span 80 were dissolved in methanol: chloroform: ethanol (2:1:2). Then the solution was put in a round bottom flask. These were then dissolved by shaking. A thin film was then formed by keeping it in the rotator vacuum evaporator at 40 $^{\circ}\text{C}$. (evaporate organic solvents). Final traces of

solvent were removed under vacuum. The deposited lipid film is hydrated with the phosphate buffer (7.4) containing luliconazole 1% w/w by rotation at 60 rpm for 1 hour at room temperature. The resulting vesicles are swollen for 2 hours at 25 $^{\circ}\text{C}$ to get multilamellar lipid vesicles (MLV). The thick suspension was broken by sonication for 30 min at 20 $^{\circ}\text{C}$ -25 $^{\circ}\text{C}$ to achieve desired vesicle size.

Factorial Design Approach: [10-13]

Table 1: 32 Factorial Design Approach

Independent variables of formulations			
Independent variables	LOW (-1)	Medium (0)	High (+1)

Volume of Phospholipid (ml)	1.5	1.8	2.1
Amt of Surfactant	14	16	18
Dependent variables			
Y1=%Entrapment efficiency			
Y2= % Drug Release			

Formulation and Characterization of Luliconazole Transferosome by using QbD Approach

Table 2: Formulation and Characterization of Luliconazole Transferosome by using QbD Approach

Batch No	CQAs (X-inputs; Independent variables)		QTTPs (Y-Outputs; Independent variables)		
	Vol. of PL (ml) (X1)	Amt. of surfactant (mg) (X2)	Particle Size (nm) (Y1)	% E. E (Y2)	Drug Release 8h (%) (Y3)
Batch 1	1.5	14	210.89±0.85	82.36±0.67	70.54±0.78
Batch 2	1.8	14	220.50±0.21	83.49±0.78	69.26±0.64
Batch 3	2.1	14	230.56±1.24	88.35±0.96	81.54±0.89
Batch 4	1.5	16	225.65±0.98	76.64±0.45	77.35±0.16
Batch 5	1.8	16	241.32±0.65	80.26±0.78	61.64±0.34
Batch 6	2.1	16	245.65±0.67	74.37±0.69	75.68±0.46
Batch 7	1.5	18	235.49±0.19	84.59±0.34	82.46±0.67
Batch 8	1.8	18	255.68±0.49	78.35±0.56	62.67±0.22
Batch 9	2.1	18	265.67±0.34	68.23±0.64	57.35±0.12

Statistical Analysis

Effect on Particle size

Effect on Particle size (Y1) had been displayed in table 3 and figure 3, 4.

$$PS = +239.88 + 11.64 * A + 15.82 * B + 2.63 * AB - 3.52 * A^2 - 1.08 * B^2$$

Table 3: ANOVA response 1 of particle size

ANOVA for Response Surface Quadratic model						
Analysis of variance table [Partial sum of squares - Type III]						
	Sum of		Mean	F	P-value	
Source	Squares	df	Square	Value	Prob > F	significant
Model	2368.49	5	473.70	64.07	0.0030	
A-vol. of phospholipid	813.17	1	813.17	109.98	0.0019	
B-amt of surfactant	1500.69	1	1500.69	202.97	0.0007	
AB	27.62	1	27.62	3.73	0.1488	
A ²	24.71	1	24.71	3.34	0.1650	
B ²	2.31	1	2.31	0.31	0.6151	
Residual	22.18	3	7.39			

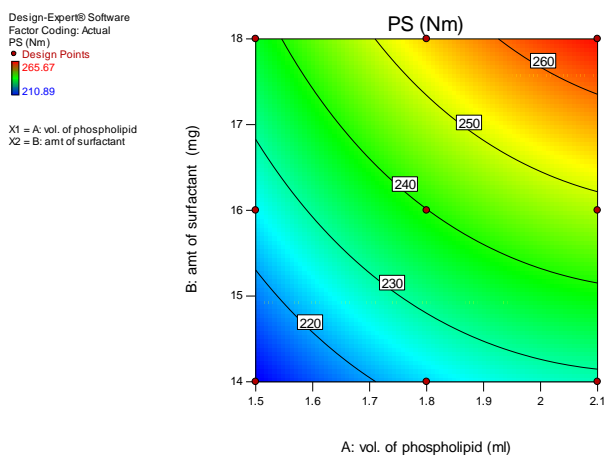


Figure 3: Contour plot 1

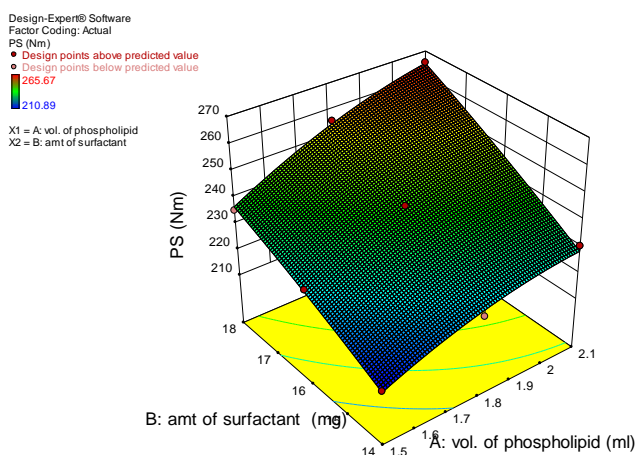


Figure 4: 3D Plot 1

Effect on %EE

$$\%EE = +78.14 - 2.09 * A - 3.84* B - 5.59* AB$$

Effect on % E.E (Y2) had been displayed in table 4 and figure 5, 6.

$$- 1.63* A^2 + 3.84* B^2$$

Table 4: ANOVA response 2 of %E.E.

ANOVA for Response Surface Quadratic model						
Analysis of variance table [Partial sum of squares – Type III]						
Source	Sum of Squares	df	Mean Square	F Value	p-value Prob > F	
Model	274.24	5	54.85	9.16	0.0489	significant
A-vol. of phospholipid	26.21	1	26.21	4.38	0.1275	
B-amt of surfactant	88.40	1	88.40	14.77	0.0311	
AB	124.88	1	124.88	20.86	0.0197	
A ²	5.29	1	5.29	0.88	0.4165	
B ²	29.47	1	29.47	4.92	0.1132	
Residual	17.96	3	5.99			
Cor Total	292.20	8				

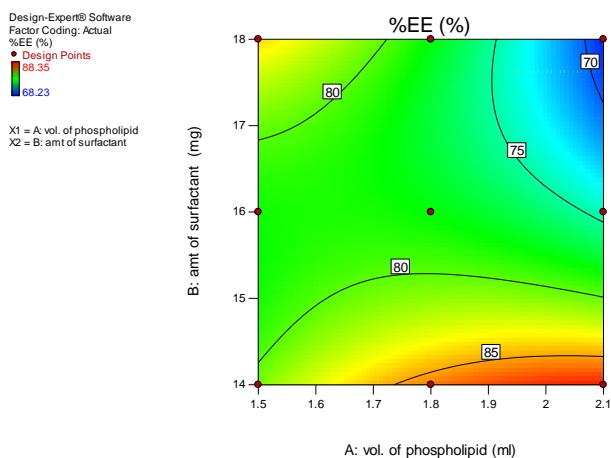


Figure 5: Contour plot 2

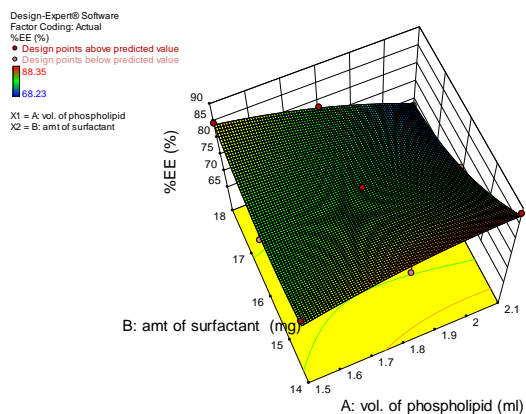


Figure 6: 3D Plot 2

Effect on Drug Release

Effect on drug release (Y3) had been displayed in table 5 and figure 7, 8.

$$\% \text{Drug release} = +65.14 - 2.63 * A - 3.14 * B -$$

$$9.03 * AB + 9.63 * A^2 - 0.92 * B^2$$

Table 5: ANOVA response 3 of %Drug release

ANOVA for Response Surface Quadratic model						
Analysis of variance table [Partial sum of squares - Type III]						
	Sum of		Mean	F	p-value	
Source	Squares	df	Square	Value	Prob > F	
Model	613.93	5	122.79	9.89	0.0441	significant
A-vol. of phospholipid	41.50	1	41.50	3.34	0.1649	
B-amt of surfactant	59.28	1	59.28	4.78	0.1168	
AB	325.98	1	325.98	26.26	0.0144	
A ²	185.47	1	185.47	14.94	0.0306	
B ²	1.69	1	1.69	0.14	0.7365	
Residual	37.25	3	12.42			
Cor Total	651.18	8				

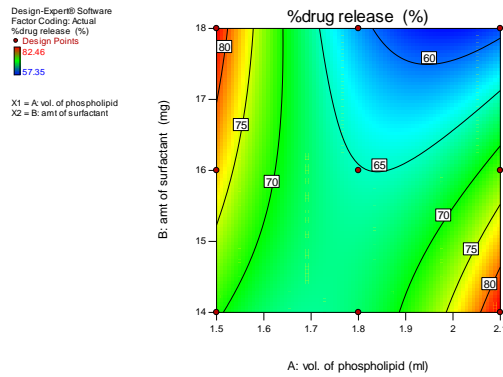


Figure 7: Contour plot 3

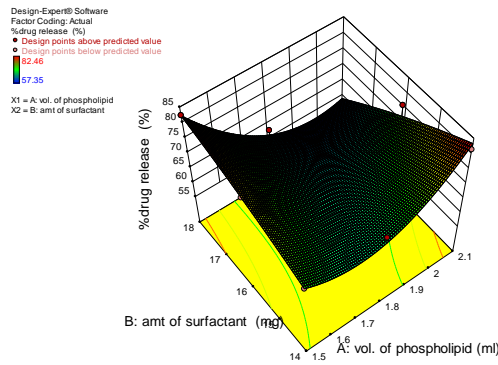


Figure 8: 3D Plot 3

Optimization of final batch through Check point Analysis

Final batch of luliconazole transferosome was obtained after undergoing check point analysis in the design

expert 10.0.1. Result was displayed in figure 9 and table 6.

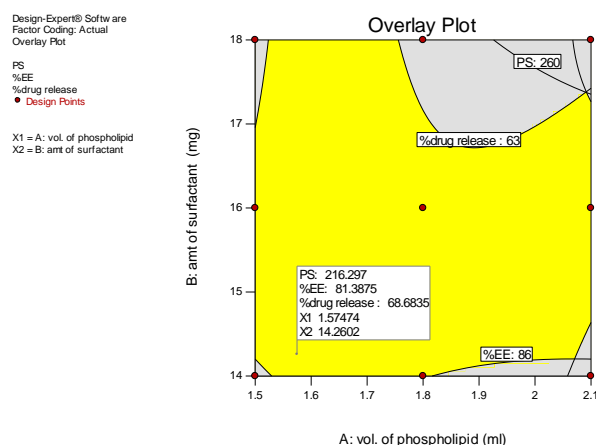


Figure 9: Overlay plot 1

Table 6: Optimised batch (based on overlay plot result)

Batch	Phospholipids conc. (X1)	Surfactant conc. (X2)	%E.E	%Drug release (8 h)
LULT 10 (Predicted Response)	1.57	14.26	81.38%	68.68%
LULT 10 (Actual Response)	1.6	14.3	82.56%	71.06%

% Entrapment efficiency

Prepared Transferosome formulation showed good entrapment efficiency of 82.56%. This behavior may be due to the presence of a higher concentration of ethanol which increases LUL solubility in Transferosome.

Drug Content:

The luliconazole Transferosomes dispersion was carefully collected and blended with a vortexing mixer until entirely dissolved at room temperature in an intermediate solvent containing a chloroform: methanol (40:60) mixture. Transferosomes dispersion samples were further diluted with identical amounts of diluent after mixing. UV was used in conjunction with UV analysis at a wavelength of max 295.6 nm to determine the drug content of luliconazole. [16] Drug content (%) of optimized batch was found to be 90.28 ± 0.75 . This indicates good amount of drug in Transferosome.

Preparation of gel base:

Take 80ml of Distilled water form total amount of distilled. Add required quantity of Propyl Paraben in preheated ($80 \pm 2^\circ$ C). After complete dissolution of it add Methyl paraben in above solution and dissolve till clear solution formed. (Solution 1). Cool down the solution 1 at room temperature. Then add required quantity of alginic acid (1% w/v) slowly by sprinkle with continuous strried at 500-600 RPM (15 min). (Solution 2). Solution 2 should be clear no lump should be remained. Add 5 gm Propylene glycol strried for 10 min. Add Triethanolamine (0.25 ml) with continuous stirring. Make up volume by DM water (qs 100 gm). Final pH of the gel base was adjusted to 6.5-7.5 Transferosomal preparation corresponding to Luliconazole was incorporated into gel base. the gel base to get the desired concentration of drug. [16,17]

Table 7: Formulation Design of Topical Gel Trial Batches

Ingredient	LTG1	LTG2	LTG2
Alginic acid (%w/v)	1	1.5	2
Propylene glycol (gm.)	5	5	5
Methyl paraben(gm.)	0.1	0.1	0.1
Propyl paraben (gm.)	0.05	0.05	0.05
Triethanolamine(mL)	0.25	0.25	0.25
Water(mL)	100	100	100

Characterization of Luliconazole-loaded Transfersomal gel: [15-17]

1. Measurement of viscosity:

Viscosity measurements of prepared topical Transfersomes based gel were measured by Brookfield viscometer using spindle no. 63 with the optimum speed of 10 rpm.

2. pH measurements

The pH of selected optimized formulations was established with the help of digital pH meter. The pH meter was calibrated with the help of buffer solution of pH 4, pH 7 and pH 9.2. After calibration, the electrode was dipped into the vesicles. Then, pH of selected formulation was measured and readings shown on display were noted.

3. Drug content

Accurately weighed 100 mg of topical Transfersomal gel was taken in beaker and added 20 ml of methanol. This solution was mixed thoroughly and filtered by means of Whatman filter paper No. 1. Then, 1.0 ml of filtered solution was engaged in 10 ml capacity of volumetric flask; moreover, volume was ready up to 10 ml by means of methanol. This solution was analyzed using UV spectrophotometer at λ_{max} 296 nm.

4. Homogeneity and grittiness

By pressing the produced gel between the thumb and index finger, the consistency was determined. A small amount of gel will be applied to the back of the hand to check for homogeneity and grittiness

5. Spreadability

Spreadability of formulation is necessary to provide sufficient dose available to absorb from skin to get good therapeutic response. An apparatus in which a slide fixed on wooden block and upper slide has movable and one end of movable slide tied with weight pan. To determine Spreadability, 2-5 gm of gel placed between two slides and gradually weight was increased by adding it on the weight pan and time required with the top plate to face the distance of 10 cm on adding 80 g of weight was noted. Good Spreadability shows lesser time to spread.

$$S = ML/T$$

Where, S is the Spreadability,

M is the weight tied to the upper side,

L is the length of the glass slide and

T is the time taken to separate the slide from each other

Preparation and characterization of Luliconazole Transferosomes loaded topical gel:**Table 8: Result of evaluation of gel**

Batch code	Color	Odor	pH (Mean \pm S.D.) (n = 3)	Viscosity Spindleno:62 (Mean \pm S.D.) (n = 3)	Spreadability (gm.cm/sec) (Mean \pm S.D.) (n = 3)
LTG 1	Colorless	Odorless	6.72 \pm 0.08	10014 \pm 447.49	12.84 \pm 0.47
LTG 2	Colorless	Odorless	6.75 \pm 0.05	13842 \pm 800.96	10.95 \pm 0.60
LTG 3	Colorless	Odorless	6.55 \pm 0.06	16975 \pm 474.97	9.04 \pm 0.32

In-Vitro Diffusion study

The in vitro diffusion study about is conveyed by utilizing Franz diffusion cell. Egg membrane is taken as semi penetrable membrane for diffusion. The Franz diffusion cell has receptor compartment with an effective volume roughly 60 ml and compelling surface area of permeation 3.14sq.cm. The egg membrane is placed between the donor and the receptor compartment. A 2cm² size patch taken and weighed then set on one face of membrane confronting donor compartment. The receptor medium is phosphate buffer pH 7.4. The receptor compartment is encompassed through

water casing to keep up the temperature at 32 \pm 0.5°C. Warmth is furnished utilizing a thermostatic hot plate with a magnetic stirrer. The receptor liquid is mixed by Teflon covered magnetic bead which is put in the diffusion cell. Amid each testing interim, samples are pulled back and replaced by equivalent volumes of fresh receptor liquid on each sampling. The samples withdrawn are analyzed spectrophotometrically at wavelength of drug 296 nm. [15] Drug release of marketed formulation with transferosomal gel had been carried out and displayed in table 9.

Table 9: Drug release data of marketed gel and Transferosomes gel:

Time (hr)	Marketed LULICONAZOLE Gel (Brand name: Lulimac gel) (Company: Macleods)	Optimized LLZ Transferosomal Gel
0	0	0
1	26.92	24.94
2	38.16	30.06
3	49.60	39.34
4	58.57	46.34
5	64.79	49.30
6	75.14	55.32
7	83.42	61.53
8	91.55	67.74
9	-	75.44
10	-	83.72
11	-	88.16
12	-	92.90

Stability studies of LLZ Transferosomal gel:**Stability Study**

The Physical Stability of Transferosomal gel were studied by leakage of the drug from Transferosome vesicles in the innate prepared form that is dispersion stored at room temperature

as well as under refrigeration. The optimized formulation was taken in the dispersion form and stored in sealed vessels at room temperature for a period of 30 days. The samples were withdrawn at regular interval of time, i.e., 0, 15 and 30 days and then evaluated for percentage drug retained.

Table 10: Stability Analysis

PARAMETER (N=3)	Room Temperature		
	Time (Days)		
	1 day	15 days	30 days
Clarity	Opaque	Opaque	Opaque
pH	6.72±0.01	6.72±0.01	6.71±0.01
Viscosity	10014±117.57	10009±117.57	10016±117.57
Spreadability	12.84±0.60	12.78±0.60	12.24±0.60
%CDR	92.90±0.51	92.49±0.51	92.15±0.51

Optimized Transferosomes gel was subjected to stability study and evaluated for all evaluation tests. At fixed time interval pH, viscosity, Spreadability and cumulative drug release determination of these formulations shows no significant changes when compared to the initial formulations as displayed in table 10.

CONCLUSION

It is concluded from the study that transferosomal gel formulated using, lecithin, Alginate acid, chloroform and methanol along with the pure drug Luliconazole can be used to improve the site specificity, increase the transdermal flux and prolong the release of the drug. Luliconazole could be entrapped into Transferosomes for penetration into skin pores much narrower than the vesicle diameter. The transferosomal gel formulation showed better results having maximum drug content, cumulative percent drug release and Entrapment efficacy. Transferosomes can alternatively be used as carriers for other transdermal drug delivery system as they possess simple scale up and can also act as a penetration enhancer by itself. Finally, it is confirmed that transferosomal gel formulation of Luliconazole is therapeutically effective for the treatment of local

skin infections and can be developed to improve the antifungal activity of the drug.

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