FORMULATION AND EVALUATION OF LULICONAZOLE LOADED ETHOSOMAL GEL FOR THE TREATMENT OF FUNGAL INFECTIONS

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Abstract: The present study was to formulate and evaluate ethosomal gel containing Luliconazole as a topical administration for fungal infection. The study was investigated by encapsulating the drug in various formulations composed different ratios of phospholipids, ethanol and Propylene glycol by hot method. The formulations were evaluated for Vesicle size, vesicle shape, spreadability, pH, %EE and in vitro diffusion study. The %EE of Luliconazole was 78.15%, the pH of the formulation was 5.3, and the spreadability was found to be 19.89 gm cm/sec. In vitro diffusion studies were compared with the marketed formulations. The release profile was faster for F5 formulation due to the increased concentration of phospholipids. The kinetic analysis was done and it showed Higuchi order kinetics. The stability studies were carried out for 45 days at 40° ± 2°C / 75% ± 5% RH it doesn't show any significant changes. So, the present study was evident that ethosomes are promising for the faster release of Luliconazole and has good stability. The work suggests that Luliconazole containing ethosomes can be potentially used as a topical administration.

Key words: Ethosomes, Topical delivery, fungal infection

1. INTRODUCTION

INTRODUCTION

Skin acts as a crucial protective barrier, shielding the body from external environmental factors while preventing water loss from underlying tissues. It is designed to be flexible enough to withstand movement without permanent distortion and thin enough to allow sensory perception. In addition to these primary functions, the skin also plays a significant role in temperature regulation, waste excretion through sweating, and immune defense. The outermost layer of the skin, the stratum corneum, is composed of dead, keratinized cells embedded in lipid bilayers and serves as the main barrier to drug penetration. When topical formulations are applied, the active drug must penetrate this layer to reach the viable tissue beneath, but the slow diffusion through the stratum corneum, which behaves like a hydrophobic membrane, can limit this process.[1]

Ethosomes, which are vesicular systems made up of phospholipids and high concentrations of ethanol, offer a promising approach to enhancing drug delivery through the skin. These systems are particularly flexible and capable of deep skin penetration, making them more effective for transdermal applications than traditional carriers like liposomes. Ethosomes come in various forms, including classical ethosomes, binary ethosomes, and transethosomes, each with specific modifications to improve drug delivery. While ethosomes have advantages such as being non-toxic, safe for pharmaceutical and cosmetic use, and suitable for immediate marketing, they also have some drawbacks, including the potential for allergic reactions, flammability, and economic concerns due to poor yield and product loss during manufacturing.[2]

Materials used

Luliconazole, Carbopol 934 P, Methylparaben, Propyline glycol , triethanolamine, Ethanol ,Best Care formulations ,Pondicherry .Soyaphosphatydl choline (SPC), Vinzi Chemical Industries ,Mumbai.

Equipment Used in the Study

Digital Weighing Balance - AX200, Shimadzu Corporation, Japan. pH Meter - Cyber Scan, Eutech Instruments, Singapore. UV-Visible Spectrophotometer - UV-1700 Pharmaspec, Shimadzu. High-Speed Homogenizer - RO-127A, Cole Parmer. , FTIR Spectrophotometer - 4100, JASCO. Zeta Sizer - MAL 1021384, Malvern Instruments Ltd. Scanning Electron Microscopy - JSM 6390, Jeol. Differential Scanning Calorimetry - DSC 60, Shimadzu. Glassware - Borosilicate, Mumbai, India

METHODOLOGY

FORMULATION AND DEVELOPMENT OF ETHOSOMES CONTAINING LULICONAZOLE: [3]

Phospholipid, ethanol, propylene glycol and distilled water were used to prepare ethosomes containing Luliconazole. Here, the phospholipid acts as vesicle forming agent, ethanol as penetration enhancer, for providing softness to the vesicles and also for reducing the size of the vesicles. Propylene glycol act as skin penetration enhancer and also provides viscosity to the ethosomal formulation. Distilled water was used as vehicle.In this method, a colloidal solution of phospholipid is obtained by dispersing it in water with the aid of heating at 40°C. Ethanol and propylene glycol are mixed in a separate vessel and heated to 40°C. Once the two mixtures reach 40°C, the organic phase is added to the aqueous one. The drug will be dissolved in water or ethanol based on its hydrophilic/ hydrophobic nature. Probe sonication or Homogenization can be used to reduce the vesicle size of ethosomal formulation to the desired extent.

Formulation chart of ethosomal suspension

FORMULATION	DRUG (mg)	SPC % w/v	ETHANOL (% v/v)	PROPYLENE GLYCOL (% v/v)
F1	300	2	10	10
F2	300	2	20	10
F3	300	2	30	10
F4	300	4	10	10
F5	300	4	20	10
F6	300	4	30	10
F7	300	6	10	10
F8	300	6	20	10
F9	300	6	30	10

PREPARATION OF ETHOSOMAL GEL[4]

1g of carbopol 934 was dissolved in 30 ml of water. Take 1 ml of ethosomal suspension and added to the solution of carbopol. Then separately 0.5g of propyl paraben and methyl paraben dissolved in 10 ml of water. Both the solution was mixed and triethanolamine was added until pH was adjusted.

EVALUATION OF ETHOSOMES OF LULICONAZOLE [5]

Vesicle size

The vesicle size of the best formulation was determined by using Dynamic light scattering technique.

Vesicle shape

The vesicle shape of best formulation was determined by using scanning electron microscopy.

Zeta Potential

The zeta potential of the ethosomal formulation was determined inorder to find out the permeability characteristics of the formulation. This was done using zetasizer.

Entrapment efficiency

Centrifugation method was used to determine the entrapment efficiency of Luliconazole ethosomal vesicles. The vesicles were separated in a high-speed centrifuge at 5,000rpm for 60 min. The sediment and supernatant liquids were collected individually. Amount of un entrapped drug was determined spectro photometrically at 297nm. From this, the entrapment efficiency was determined by the following equation.

Entrapment efficiency % = A2-A1/A2×100

Where, A1 = Amount of Luliconazole in sediment.

A2= Total amount of Luliconazole added.

EVALUATION OF ETHOSOMAL GEL [6]

Determination of pH

The pH measurements of the best formulation were carried out using a pH meter by dipping the glass electrode completely into the semisolid formulation

Spreadability Coefficient studies

Slip' and 'Drag characteristics of gels are the basis for measurement of Spreadability.4 gm gel under study was placed on a ground glass slide. The gel was then sandwiched between another glass slide having the dimension of fixed ground slide and provided with the hook. Excess of the gel was scrapped off from tine crises. The slides were placed one above the other and count the time taken for 2" Slide to slip out from other slide.

Spreadability coefficient-ML/T

M=Mass, L-Length, T-Time

Drug Content:

The drug content of the formulation was done by diluting 1 gm of the gel in ethanol and the absorbance was determined by UV-visible spectrophotometer at 297nm.

Viscosity:

The viscosity of gels was determined by using a Brookfield viscometer. The reading of each formulation was taken. Unit of viscosity is centipoise (cps)

In vitro diffusion study:[7]

In-vitro drug release study was carried out using open ended cylinder model. The ends of the cylinder were covered with egg shell membrane and the cylinder was loaded with 10 ml of Ethosomal formulation. This was then placed in 500 ml phosphate buffer (pH 7.4). The beaker was stirred continuously using magnetic bead stirrer at rate of 50 rpm. Samples were withdrawn at 1, 2, 3, 4, 5,6,7,8 hrs interval and analyzed using UV spectrophotometer at 297 nm.

Antimicrobial activity [8]

In the disc diffusion method, the drug potency is based on the measurement of the diameter of zone of inhibition surrounding cylindrical discs. The extract was placed at the surface of the solid nutrient media previously inoculated with a culture of a suitable microbe. Inhibition produced by the test compound is compared with that produced by the known concentration of reference standard.

FUNGI STRAINS

Anti-fungal activity

In this study, two fungi strains (Aspergillus *Niger, Candida albicans*) were selected for the disc diffusion method.

Standard drug used

Luliconazole at the concentration of 10µg/disc for fungal study.

Preparation of inoculum [9]

The inoculums for the experiment were prepared in fresh Sabouraud's broth from preserved slant culture. The inoculums were standardized by adjusting the turbidity of the culture to that of McFarland standards. The turbidity of the culture may be adjusted by the addition of sterile saline or broth (if excessive or by further incubation to get required turbidity.

Preparation of sterile swabs

Cotton wool swab on wooden applicator or plastics were prepared and sterilized by autoclaving or dry heat (only for wooden swabs) by packing the swabs in culture tubes, papers, or tins etc.

Sterilization of forceps

Sterilize forceps by dipping them in alcohol and burning off the alcohol

Drug release kinetics- model fitting of the dissolution data [10]

Whenever a new solid dosage form is developed, it should be made sure that the drug dissolution occurs inappropriate manner. The kinetic model for drug dissolution from solid dosage forms is described by the manner in which the dissolved amount of drug (O) is a function of the test time, t or Q = f (t). Some analytical definitions of the Q (t) function are commonly used such as zero order, first order, Higuchi, Korsenmeyer-Peppas models. Other release parameters, such as dissolution time (tx %), dissolution efficacy (ED), difference factor (fi), similarity factor (fs) can be used to characterize drug dissolution / release profile

STABILITY STUDIES:[11]

All regulatory bodies allow only real time data for any drug or pharmaceutical for the purpose of determining the shelf life and only accelerated stability studies serve as a tool for formulation, screening and stability issues related to shipping or storage at room temperature. The accelerated stability studies were conducted in accordance with the ICH guidelines. The capability of vesicles to hold the drug was assessed by keeping the ethosomal suspension at different temperature. The vesicular suspension was kept at 40 ± 2°C/ 75% ± 5% and at room temperature for 45 days. Optimized ethosome formulation was selected for stability studies of vesicles.

RESULTS & DISCUSSION

Compatibility studies (FT-IR interpretation):

IR spectrum analysis was carried out to assess the functional groups present in the sample and to explore any potential chemical interactions. There is no major shift or change in peaks, thus the compounds are compatible with each other

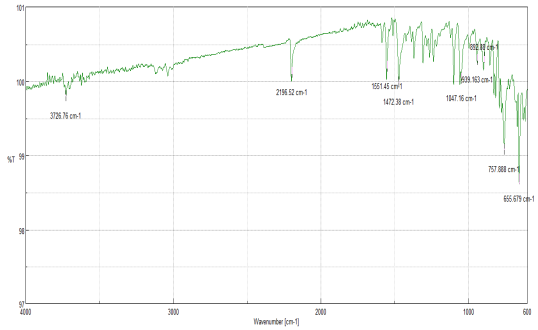


Fig 9: IR spectrum of Luliconazole

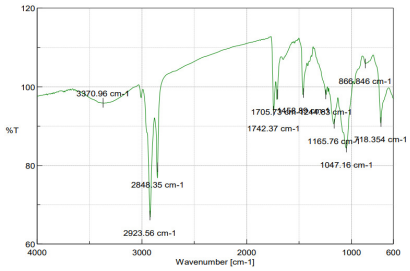
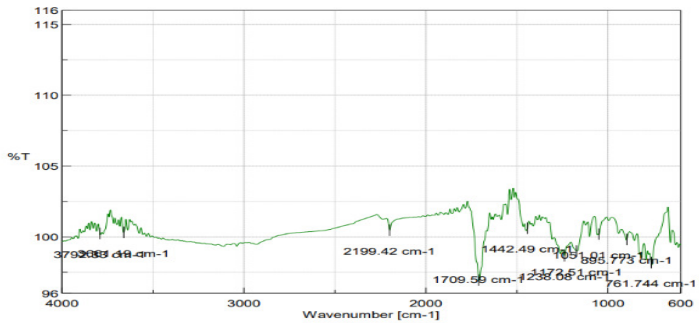


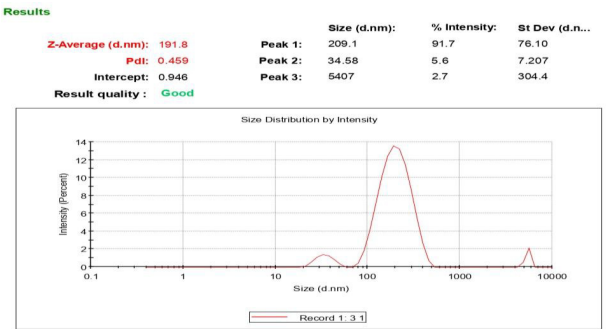
Fig 10: IR Spectrum of Lipids



IR spectrum of Luliconazole + Carbopol 934

Vesicle size:

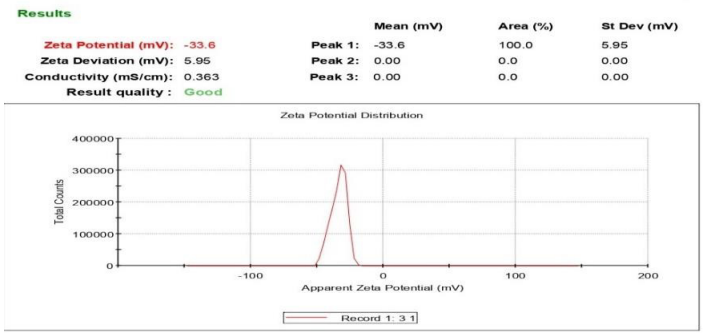
The vesicle size was analysed by Dynamic light scattering technique. It was found that formulation F5 was found to have least particle size i.e. 191.8 d.nm. Increasing in concentration of lipids results in increase of particle size. As stated by Lamsal et al.[12], the vesicle size of ethosomes of his work ranges from 134 to 266 nm. On increasing the concentration of phospholipid from 0.5 to 1% w/w, the size of vesicle was increased and on increasing the concentration of ethanol from 10 to 30% w/v, the size of vesicle decreases**.**



Dynamic light scattering of Ethosomes containing Luliconazole

Zeta potential:

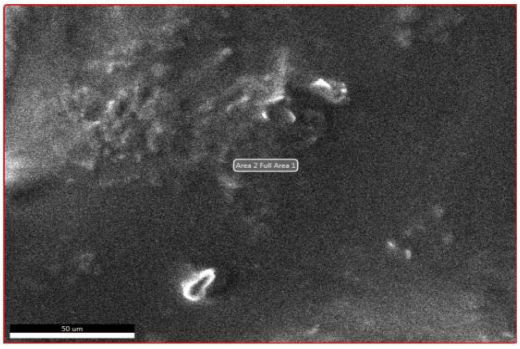
The zeta potential values of ethosomal formulations were highly negative and generally above −20 mV due to the presence of ethanol in the system which may provide the negative charge on vesicles surface in the range −20.9 to −35.6 mV. Out of this, Formulation F5 has zeta value of –33.6 mV.



Zeta potential graph of Ethosomes

Vesicle shape by SEM Technique:

The nature of formulated ethosomal vesicles F5 were confirmed by scanning electron microscopy. It was found to be spherical in size with rough surface. As stated by Lamsal *et al.,* the vesicle size was observed in scanning electron microscopy and his work proved that the ethosomes were spherical with smooth surface.[13]



Scanning electron microscope image of ethosomes containing Luliconazole

Entrapment Efficiency:

Entrapment efficiency of Ethosomes containing Luliconazole was found in the range of 53.92 to 84.29 %. The highest entrapment efficiency was observed in F5 formulation with **84.29%.** Thus, it was selected as best formulation. The entrapment efficiency percentage was found to increase with increasing the ethanol concentration from 6ml to 9ml but, was found to decrease when ethanol concentration was increased to 12ml. These may be due to more fluidity effect by ethanol which causes the drug to leak out form the lipid vesicle. Furthermore, increase in lipid concentration

increased the entrapment efficiency. High encapsulation efficacy of the developed formulations was due to the lipophilic nature of the drug. Ethanol used in ethosomes provided high fluidity to vesicular membranes and results in better mixing and retention of drug.

EVALUATION OF ETHOSOMAL GEL:
Ethosomal gel was prepared using Carbopol 934 and then characterized for pH, spreadability, drug content & *in vitro* diffusion study.
Table: Evaluation parameters of Ethosomes gel

FORMULATION CODE	pH	Spreadability (g cm/ sec)	Drug content (%)	Viscosity (cps)
F1	5.8	19.25	90.74	11658 ± 2.8
F2	5.6	19.54	92.31	11476 ± 2.1
F3	5.4	19.35	80.58	11656 ± 1.9
F4	5.2	19.63	88.47	11663 ± 2.6
F5	5.3	19.89	96.02	11780 ± 1.5
F6	5.9	19.21	79.65	11494 ± 2.9
F7	5.1	19.86	87.13	11635 ± 2.5
F8	5.3	19.78	93.77	11671 ± 2.3
F9	5.5	19.64	75.46	11646 ± 1.8

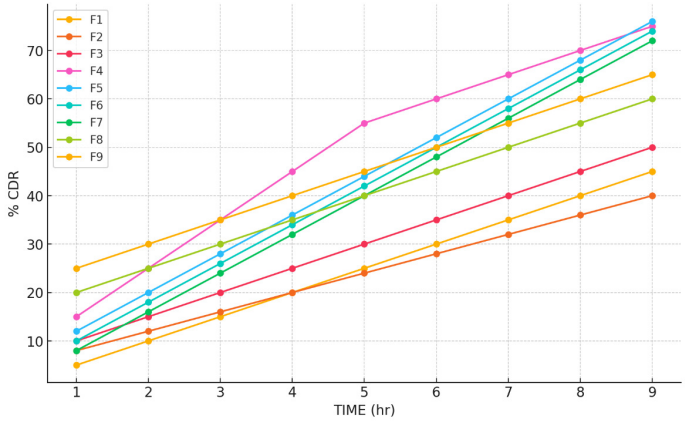
pH studies:
The pH of the best formulation F5 was found to be **5.3** which was found to be in skin pH. Thus, the prepared gel is compatible with the skin. As stated by Maha M.A Nasra *et al.,* the pH of the gel of his formulation was found to be 4 which maintains the stability of the drug and the form of Carbopol 934.[14]

Spreadability coefficient studies:
The spreadability coefficient of the best formulation F5 was found to be **19.89 g cm/ sec**. The value of spreadability indicates the gel was easily spreadable by small amount of shear.

Drug content:
The drug content of all the formulations was found to be in the range of 75.46 % to 96.02 % in which the best formulation F5 contained **96.02%** of the drug

Viscosity:
The viscosity of the optimized formulation F5 was found to be **11780±1.5** cps. As stated by Maha M.A Nasra *et al.,* viscosity is to be determined at low temperature not at room temperature due to the thickness of the gel.[15]

***In-vitro* diffusion studies:**
Drug diffusion of ethosomes containing Luliconazole was carried out through eggshell membrane. The cumulative percent drug release of ethosomal gel after 8 hrs was found to be in the range of 26.25% to 79.56%. The in vitro release data revealed that F5 formulation showed highest drug release with **79.56%** and it indicated the in vitro drug release depend upon ethanol and lipid concentration.There was increase in the amount of drug release with the gradual increase in concentration of ethanol from 10 to 30 % v/v. This may be due to the effect of ethanol, which acts as penetration enhancer.



Release kinetics:

In- vitro release data obtained for the formulations was subjected to kinetic analysis. The cumulative percentage drug release data obtained were fitted to zero order, first order, korsemeyer-peppas, Higuchi, Hixson crow well. The figures are mentioned below. From the regression values it was concluded that, the formulation follows higuchi order of diffusion. According to the literature review of Rekha Bhagya[16] the release kinetics of atorvastatin drug optimized formulation followed higuchi's order of diffusion and also stated that if the release follows higuchi order it was found to be an encouraging observation

Comparative *in vitro* diffusion profile of optimized formulation of Ethosomal gel and marketed formulation

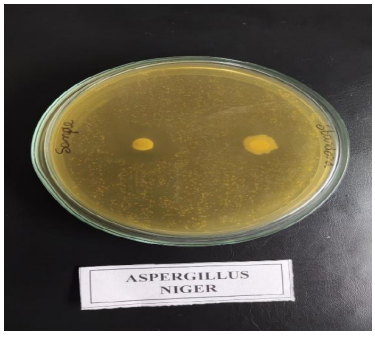
Anti-microbial activity

Disc diffusion method of *Aspergillusniger* and *Candidaalbicans*

S.NO	Micro organism	Standard(1mg/ml)	Sample(100 µ/ml)
1	***Aspergillus niger***	20 mm	17 mm
2	***Candida albicans***	22 mm	18 mm

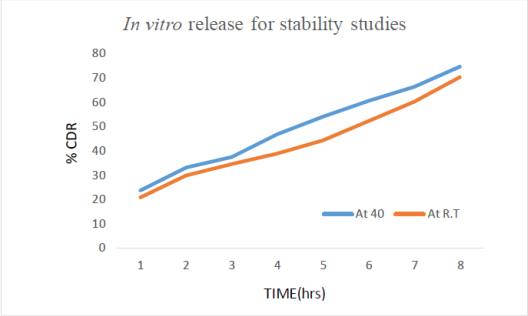
STABILITY STUDIES:

Stability studies for the most satisfactory formulation of Ethosomal gel F5 was carried out at accelerated temperature 40°C ± 2°C/75% ± 5% and at room temperature for 45 days. At the end of 45 days, samples were evaluated. Entrapment efficiency study showed that, there was no major change in the % of entrapped drug of F5 at 40°C ± 2°C/75% ± 5% and slight decrease at room temperature. There was no significant change in the *in vitro* drug diffusion study of F5 at 40°C ± 2°C/75% ± 5%. However, it showed decrease in in-vitro drug diffusion after stability at room temperature. This may be due to ethanol which avoids aggregation of vesicles. These results are in agreement with literature reports which was stated by Subheet Jain *et al.,* [17] indicating that ethosomes possess reasonably good stability in comparison to liposomes because of the presence of ethanol. Ethanol provides a net negative surface charge, which avoids aggregation of vesicles due to electrostatic repulsion.



Comparative diffusion profile after stability studies

S.NO	PARAMETERS	INITIAL	30th DAY	45th DAY
1	PHYSICAL APPEARANCE	Off – white colour	No change	No change
2	DRUG CONTENT	96.02	95.31	94.09
3	ENTRAPMENT EFFICIENCY	78.15	77.96	77.32
4	pH	5.3	5.3	5.3
5	SPREADABILITY	19.89	19.84	19.82



***In vitro* release for stability studies**

CONCLUSION

Luliconazole is used for the treatment of fungal infection like Ringworm of the body (tinea corporis); Ringworm of the foot between the toes (interdigital tinea pedis, athlete's foot) and Ringworm of the groin (tinea cruris, jock itch). In the present study, an attempt was made to formulate ethosomes of Luliconazole for efficient delivery of drug across the skin. FTIR spectra revealed that there was no interaction between the drug and the excipient. Various formulations were developed by using vesicle forming agent and penetration enhancers by hot method. Ethosomal suspension of Luliconazole were evaluated for the parameters such as vesicle size, vesicle shape, Zeta potential and entrapment efficiency. The size of the ethosomes is in nanometer range and shape confirms three dimensional natures of Ethosomes. Percentage entrapment efficiency and in-vitro diffusion increases with increase in phospholipid concentration and alcohol concentration and decrease on further increase of alcohol due to fluidity of vesicles. Ethosomal gel was prepared and evaluated for pH, spreadability, Viscosity, Drug content and *in-vitro* diffusion studies. All the values are in optimal range. Among all the developed formulations, F5 showed better entrapment efficiency and drug diffusion for a period of 8 hr and also had smaller vesicle size. Therefore, it was selected as the best formulation. The most satisfactory formulation-F5 had showed no significant change in entrapment efficiency percentage, in vitro diffusion pattern after stability studies at accelerated temperature 40°C ± 2°C/75% ± 5% RH for 30 days and 45 days. Thus, the objective of the present work of formulating and evaluating Ethosomes of Luliconazole has been achieved.

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