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FORMULATION AND OPTIMIZATION OF KETOCONAZOLE LOADED ETHOSOMAL GEL FOR TOPICAL APPLICATION: IN VITRO & EX-VIVO CHARACTERIZATION

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ABSTRACT

The present study aimed to develop Ketoconazole -loaded ethosomal gel intended to be applied topically for treating skin infections. Ethosomes were prepared using the cold method. The formulation variables were optimize dusing[3] factorial designand Design Expert® software for analyzing the data statistically and graphically using response surfaceplots. Phospholipid (X1) and ethanol (X2) and propylene glycol (X3) were chosen as the independent variables, while the dependent variables comprised entrapment efficiency (Y1), vesicles size (Y2) and zeta potential (Y3). Ultra-centrifugation was used to assess the encapsulated medication after confirmingthe presence and size of vesicles. There was a greater increase in value (79.62%) in sonicated particles containing 30% w/w ethanol. The optimized ethosomes were subsequently incorporated into Carbopol® 940 gel and characterized for rheological behaviour, *in-vitro* release, *ex-vivo* skin permeation and deposition. Morphologically, the produced ethosome formulations were consistent when examined by SEM. All of

the vesicles met or exceeded the criteria for nanotechnology in terms of size (less than 200 nm), polydispersity index (PDI), and entrapment efficiency (of the intended medication). The percentage of Ketoconazole released after 24 hours was significantlydecreased (p 0.05) when the ethosomes were included into a variety of gel bases. By contrast, ethosomal gel showed considerably greater anthralin penetration than the other tested preparations ($p<0.05$). Compared to the ethosomal gel, the drug solution in receptor medium, and the drug hydroalcoholic solution, the total quantity of drug penetrated from the ethosomal gelwasaround2.5-,3.5-, and4.5-foldgreater ($p<0.05$). Stability studies displayed that after 2 months, all of the gels' physicochemical characteristics, including viscosity and color, remained unchanged, passing the tests.

KEYWORDS: Topicalapplication; ethosomes; Ketoconazole, ex-vivopermeation, stability studies.

1. INTRODUCTION

Modern medicine relies heavily on improvements in medication administration via the skin. Transdermal medication delivery has recently been at the forefront of promising new developments in the field, competing with oral administration as the gold standard.[1]

And over 20 years have passed since the advent of the transdermal medication delivery technology. Intheeightiesandnineties, this technology wasthesubjectofintenseattention and enthusiasm from the world's leading pharmaceutical corporations. Transdermal medication delivery systems were increasingly being acquired by bigger companies beginning in the mid-to late-1990s. The skin serves as a barrier between the medicine and the body's internal organsin a transdermal drug delivery method. It has shown promising results over the past year compared to oral drug deliverysystems because it avoids gastrointestinal interferences and first pass metabolism of the drug. However, the main drawback of TDDS is that it encounters the barrier properties of the Stratum Corneum, so only lipophilic drugs with a molecular weight of less than 500 Daltons can pass through it.[2]

Transdermal delivery would be a crucial route because it allows for the controlled administration of a medicine via the skin, which then has a systemic effect.

There are two primary justifications for the pursuit of more effective drug delivery systems for biopharmaceuticals:

- Theymakeupanever-increasing share of the latest therapies, and
- Typically, the yare administered through injection.

Novel pharmaceuticals have been successfully implemented thanks to advances in drugdelivery systems, but current medications have also been used to generate new medical treatments. Develop dover the course of two decades, transdermalpatcheshave shown to be a viable method for delivering a variety of compounds via the skin. And over 35 transdermal patch medicines covering 13 compounds have been licensed by the FDA during the last 22years to treat symptoms including nausea and vomiting caused by motion sickness, which hadits first approval in 1981.[3]

ROUTESOFPENETRATION

The dermis, which is made up of connective tissue, lies underneath the stratified avascular cellular epidermis on human skin. Below the dermis lies a layer of subcutaneous fat. The hair follicles and sebaceous glands of hairy skin are supported by the dermis's extensive blood supply, while the epidermis's pores allow the sweat glands (apocrine and eccrine) toreach the skin's surface. The stratum corneum, or horny layer, is the most critical part of this complex membrane in terms of drug absorption since it often offers the rate-limiting or slowest stage in the penetration process.[4]

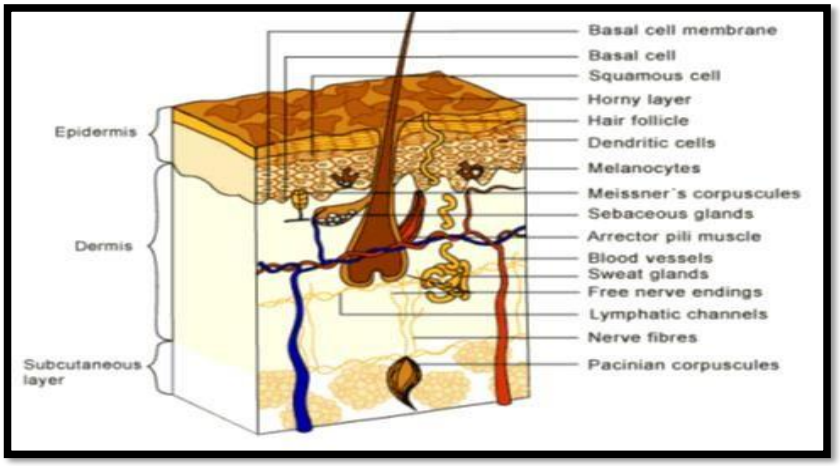


Figure 1: Structure of skin.

Molecules come into touch with dead cells, bacteria, sebum, and other substances at the skin, although these encounters have a negligible impact on the rate at which theyare absorbed. In order to reach healthy tissue, the penetration might use one of three routes:

i. Involvement of the sebaceousglands of hair follicles

ii. Through the sweatglands

MATERIALS ANDMETHODS

Table-1-Chemicals and Materials.

Chemicals	Manufacturedby
Ketoconazole	NATCOPHARMALABS
Propyleneglycol	Researchlabfinechem.Industries(Mumbai)
Alcohol	JiangsuHuaxiInternationalTradeCo.Ltd(CHINA)
Cholesterol	Viratlab(Mumbai).
Carbopol-934	Researchlabfinechem.Industries(Mumbai)
Triethanolamine	Researchlabfinechem.Industries(Mumbai)

EQUIPMENTS

Table 2: Instruments and company.

Instruments	Company
Electronic weighing balance	Scimadzucorporation(JAPAN)
U.V.spectrophotometer	Schimadzu1800(JAPAN)
Magneticstirrer	REMelektrotechniklimited.vasai(INDIA)
Refrigerator	Allwyn(INDIA).
Sonicator	SISCOScientificInstrumentssalesCorporation,Thana, Mumbai.
pHmeter	REMI
Scanning electron microscope	Scimadzucorporation(JAPAN).
FTIR	Scimadzucorporation(JAPAN).

Preformulation Studies

Rational dosage form development begins with a thorough preformulation analysis. Effective and stable dosage forms need examination of the physicochemical characteristics of both the drug molecule and any excipients used.[5]

Identification Tests

1) **Solubility of Drug:** An essential factor in the design of ethosomes is the drug's solubility. For the purpose of selecting an appropriate solvent system, the solubility of Ketoconazole was measured in a wide variety of solvents, including but not limited to distilled water, methanol, ethanol, chloroform, and certain buffer solutions. Five milligrams of ketoconazole were dissolved in ten milliliters of the aforementioned solvents and sonicated for an additional ten minutes. The solubilityof the solutions was visually evaluated and compared to industrynorms.[6]
2) **Melting Point Determination:** Pure Ketoconazole's melting point was measured using a capillary technique. Capillary was loaded with a measured quantity of Ketoconazole

powder, and the melting point was recorded digitally using a digital melting point device.

3) **Determination of Absorption Maxima**: The UV-visible spectrophotometer was used toscan a Ketoconazole solution in ethanol at 10 g/mL across the range of 200 to 400 nm. The measured max was compared to the theoretical maximum value.
4) **Calibration Curve of TH in PBS (pH 7.4):** For the preparation of the standard stock solution, 10 mg of Ketoconazole was added to a 100 mL volumetric flask and the weights were checked. The stock concentration was brought up to 100 g/mL by diluting the sample to the appropriate amount with PBS (pH 7.4).[7]

Preparation of Sample: All of the diluted samples were made from the stock solution (100 g/mL). Toachieveserialdilutions of 5,10,15,20, and 25g/mL, aUV-Visible spectrophotometer was used to record the absorbance at 223 nm when 0.5, 1.0, 1.5, 2.0, 2.5,3.0,3.5, and 4.0mL of the stock solution were transferredtoa10mL volumetric flask.

Table 3: Ingredients in test batches.

Formulation (F)	Lecithin (%)	Propylene Glycol (%)	Ethanol (%)	Cholesterol (mg)	Drug (mg)	Water
F1	2	10	20	0.05	100	Q.s
F2	3	10	20	0.05	100	Q.s
F3	4	10	20	0.05	100	Q.s
F4	3	10	30	0.05	100	Q.s
F5	3	10	40	0.05	100	Q.s
F6	3	10	50	0.05	100	Q.s
F7	-	10	30	0.05	100	Q.s

Formulation of Ketoconazole-Loaded Ethosomal Gel

For in vitro, ex vivo skin permeation, and in vivo screening, the improved ethosomes were earmarked for incorporation into the gel basis. Carbopol's hydrophilicity, cross-linking capabilities, and water insolubility make it an ideal drug delivery vehicle.[8] The Carbopol934 was dissolved in the water and let to sit for a whole night. A magnetic stirrer was then used to ensure an even distribution of the ingredients. The Carbopol dispersion was evenly infused with a 1% w/v solution of ketoconazole ethosome. As a preservative, benzyl alcohol was used. The pH was then adjusted to the desired range of 6.5 - 7.4.[9,10] by adding triethanolamine drop by drop while swirling gently.

Table 4: Composition of different gel formulation.

Gel formulation	Ketoconazole suspension(ml)	Carbopol (%)	Tri ethanol amine (ml)	Water
G1	100	1	0.5	Q.s
G2	100	1.5	0.5	Q.s
G3	100	2	0.5	Q.s
*G4	100	1.5	0.5	Q.s

*G-4freedruggel

RESULTS AND DISCUSSION

Analytical study Scanning of drug

An ultraviolet spectrophotometer was used to scan pure ketoconazole in methanol between 200 and 400 nanometers. Light absorbed by ketoconazole has a characteristic spectrum, with a maximum absorbance at 234 nm and a wavelength range of 220 to 360 nm. It has been determined that Ketoconazole is present because of the presence of a wide shoulder at about257 nm. As a result of finding that ketoconazole had the largest peak at 257 nm, it was chosen for further testing.

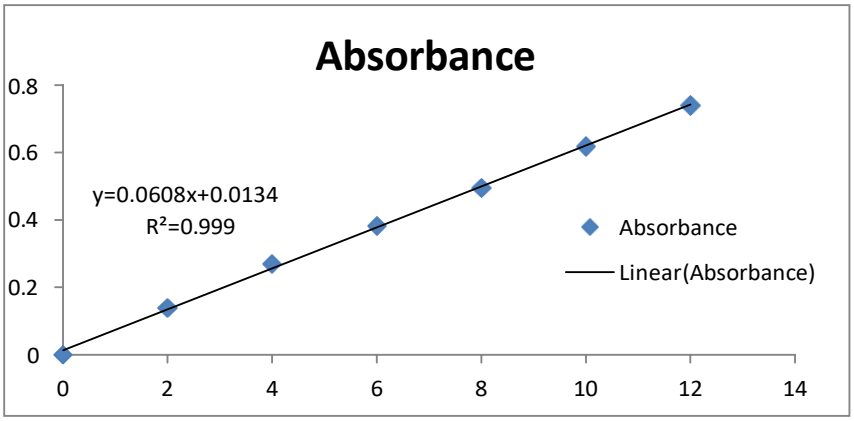


Figure 1: Standard graph of Ketoconazole.

Preformulation Study

Identification of Pure Drug:

(1) Solubility of Drug

Ketoconazole is lipophilic, as shown by its low water solubility. Drug was insoluble in waterbut soluble in a wide variety of organic solvents (pH 7.4.).[11,12]

(2) Melting Point Determination

It has been determined that 152 degrees Celsius is the melting point of Ketoconazole. Ketoconazole's melting point is often reported to fall between 148 and 152 degrees Celsius, a

range consistent with its high level of purity.[13]

(3) Determination of Absorption Maxima

An ultraviolet (UV) spectrophotometer was usedto examine a Ketoconazole solution with a concentration of 10 g/mL in the range of 200 to 400 nm. Absorbance maximum (max) was observed at 257 nm in the recorded spectra, as shown in Fig.9.1.[14]

Table 5: Ketoconazole formulation batch composition and characterization.

Run	X1	X2	X3	R1	R2	R3
1	5	30	10	65.51	750	78.56
2	2	20	8.5	71.72	112	90.23
3	3.5	30	8.5	73.94	520	68.73
4	3.5	20	10	88.89	98	96.49
5	3.5	40	7	50.82	235	69.41
6	3.5	40	10	55.69	490	60.28
7	3.5	30	8.5	75.93	450	68.72
8	3.5	30	8.5	73.84	521	70.62
9	5	40	8.5	46.19	759	70.27
10	2	40	8.5	45.76	1275	68.81
11	2	30	7	59.79	300	70.34
12	3.5	20	7	82.38	115	65.24
13	3.5	30	8.5	74.49	535	68.34
14	2	30	10	51.61	220	66.13
15	5	20	8.5	72.38	1025	88.69
16	3.5	30	8.5	73.49	495	69.76
17	5	30	7	50.25	285	48.98

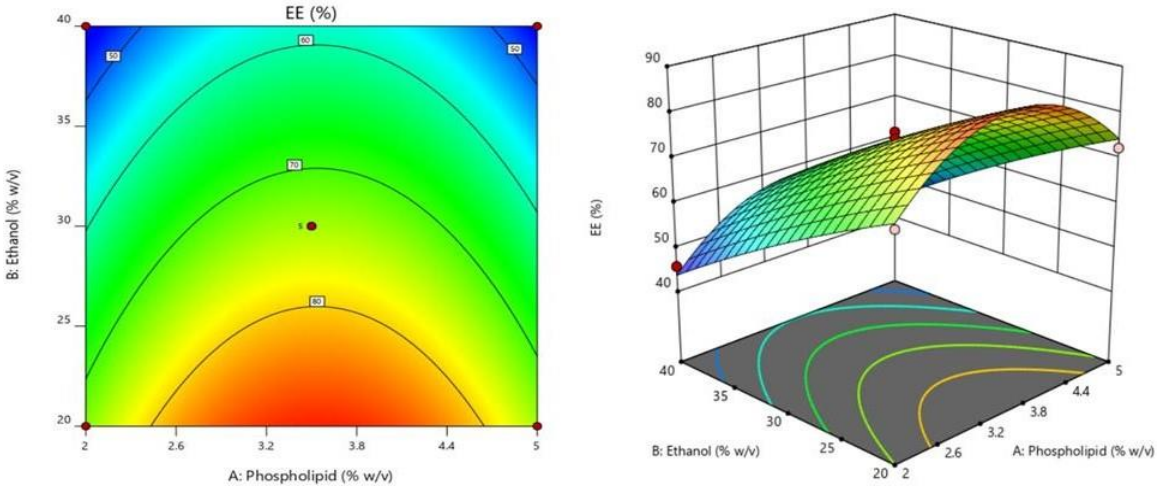


Fig. 2: 3D Counter and RS Counter Effect of Independent Variables on Total Electrical Energy Consumption as a Percentage (%) Plot.

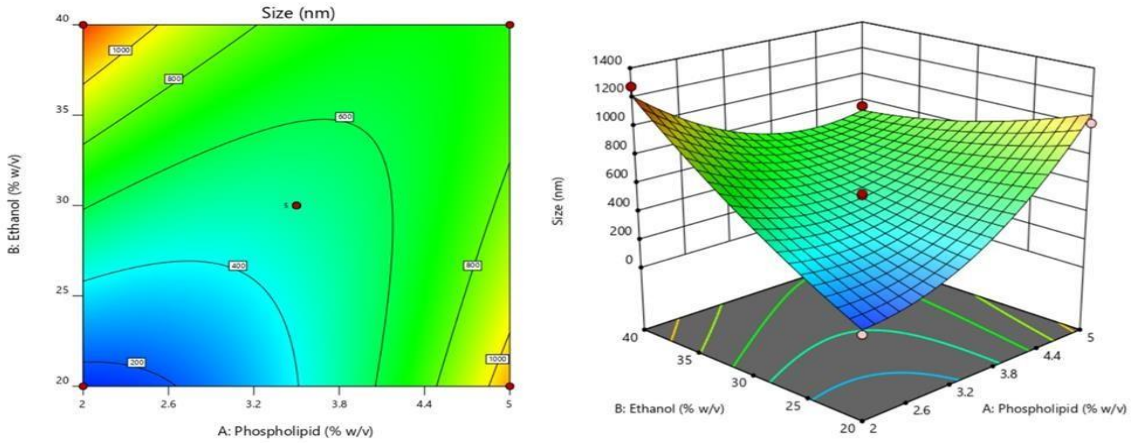


Fig. 3: 3D counter and a response board Influence of Independent Variables on: Size, Location, and Time (3-D Plot).

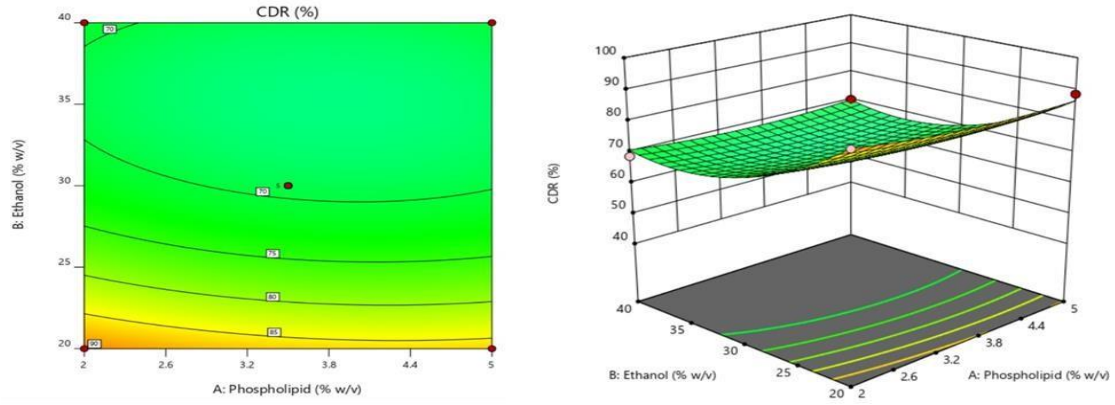


Fig. 4: 3D Counter and RS Counter Diagrammatic Display of the Influence of Independent Factors on: CDR.

Table 6: Size distribution of gel formulation #2F2(3% Lecithin, 20% ethanol).

SIZERANGE					
Eye piece micrometer	**Inμm**	**Average size (d) μm**	**No of vesicles (n)**	**% No of vesicles (n/150 *100)**	**nxd**
0-1	0.00-3.33	1.665	60	40.000	99.9
1-2	3.33-6.66	4.995	45	30.000	224.775
2-3	6.66-9.99	8.325	30	20.000	249.75
3-4	9.99-13.32	11.655	10	6.667	116.55
4-5	13.32-16.65	14.985	5	3.333	74.925
			Σn=150		**Σnd=765.9**

Averagediameter(davg)=Σnd=5.106μm

Table 7: Sized is tribution of gel formulation #3F3 (4% Lecithin, 20% ethanol).

SIZERANGE					
Eye piece micrometer	**Inμm**	**Average size (d) μm**	**No of vesicles (n)**	**% No of vesicles (n/150 *100)**	**nxd**
0-1	0.00-3.33	1.665	58	38.667	96.57
1-2	3.33-6.66	4.995	40	26.667	199.8
2-3	6.66-9.99	8.325	27	18.000	224.775
3-4	9.99-13.32	11.655	22	14.667	256.41
4-5	13.32-16.65	14.985	3	2.000	44.955
			Σn=150		**Σnd = 822.51**

Table 8: Sized is tribution of gel formulation#4F4(3% Lecithin, 30% ethanol).

SIZERANGE					
Eye piece micrometer	**Inμm**	**Average size(d)μm**	**No of vesicles (n)**	**% No of vesicles (n/150 *100)**	**nxd**
0-1	0.00-3.33	1.665	59	39.333	98.235
1-2	3.33-6.66	4.995	48	32.000	239.76
2-3	6.66-9.99	8.325	26	17.333	216.45
3-4	9.99-13.32	11.655	15	10.000	174.825
4-5	13.32-16.65	14.985	2	1.333	29.97
			Σn=150		**Σnd= 765.9**

Averagediameter(davg)= ***Σnd****=5.062μm*

Table 9: Size distribution of gel formulation#5F5(3% Lecithin, 40% ethanol).

SIZERANGE					
Eye piece micrometer	**Inμm**	**Average size(d)μm**	**No of Vesicles (n)**	**% No of vesicles (n/150 *100)**	**nxd**
0-1	0.00-3.33	1.665	64	42.667	106.56
1-2	3.33-6.66	4.995	52	34.667	259.74
2-3	6.66-9.99	8.325	23	15.333	191.475
3-4	9.99-13.32	11.655	11	7.333	128.205
4-5	13.32-16.65	14.985	2	1.333	29.97
			Σn=150		**Σnd= 715.95**

Averagediameter(davg)=$^{\Sigma nd}$=4.71μmble-

The size and form findings agree with the empiricalevidence.

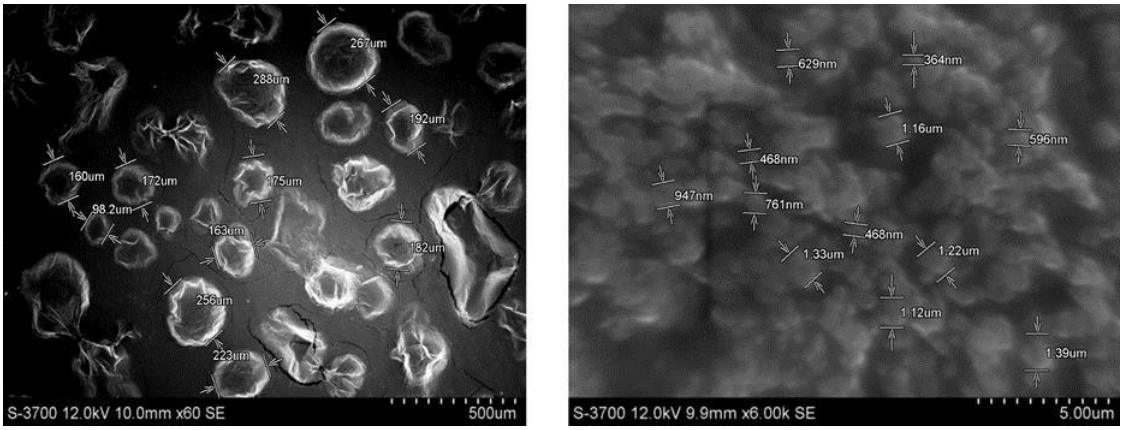


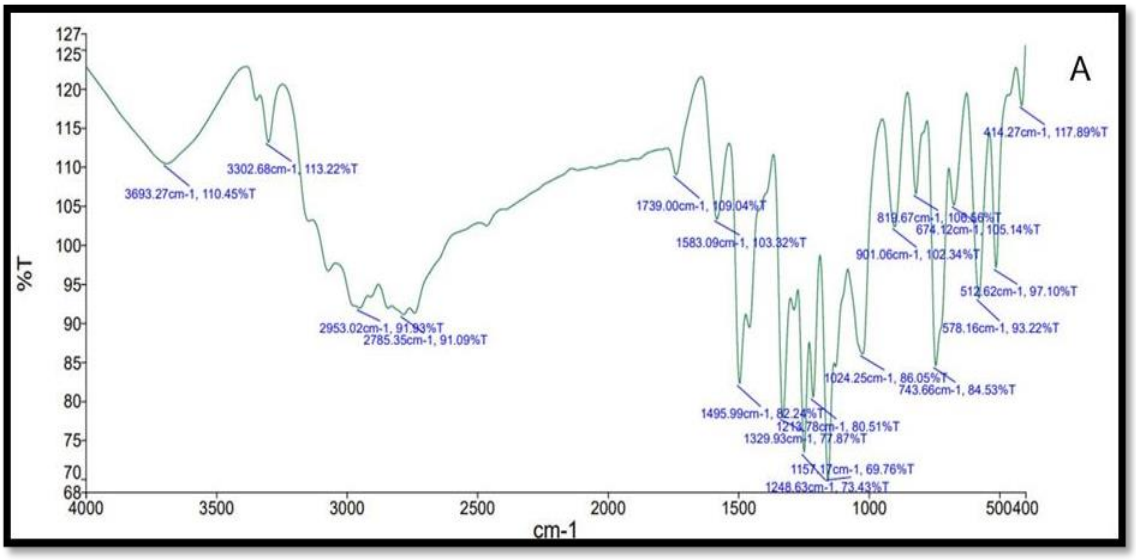
Figure 5: Scanning electron microscopy of ethosomal suspension and gel formulation.

Table 10: Drug entrapment efficiency of Ketoconazole Gel.

Formulationcode	Entrapmentefficiency(%)			MEAN
F1	72.19	71.75	71.82	71.92
F2	66.91	67.12	68.53	67.52
F3	60.05	60.00	60.01	60.02
F4	**79.91**	**79.62**	**79.33**	**79.62**
F5	58.01	55.96	54.96	56.31
F6	39.39	42.32	42.76	41.49

FTIR study

Pure drug IRspectra includedistinctpeaksat 1,643cm1, 813cm 1owing toC= O,- Cl, and aromatic groups, respectively. Similar maxima at 1,640, 796, and 1,288/cm were seen for the aforementioned categories in Formulation F4. That means the drug's structure has been preserved in the formulations.



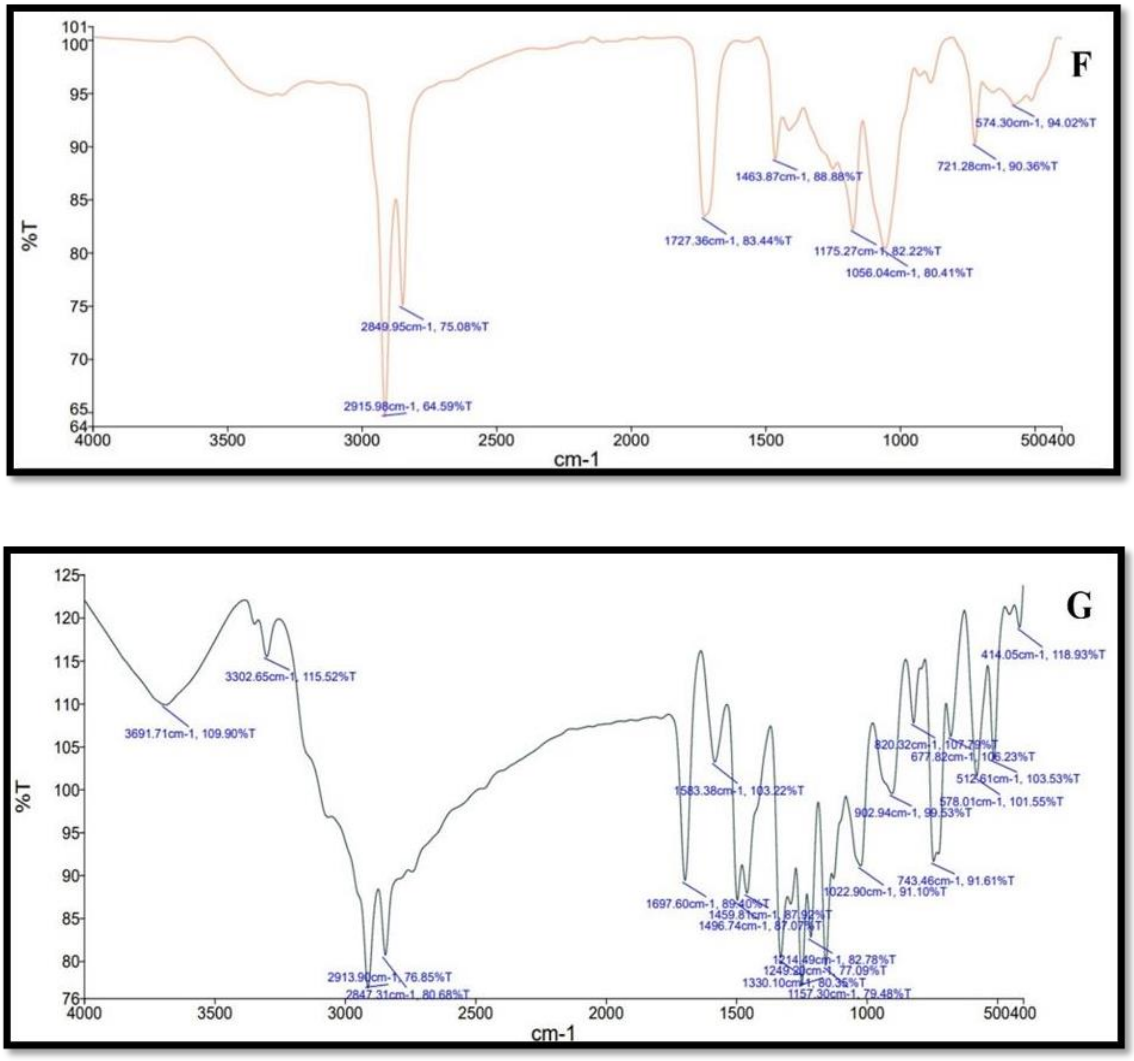


Figure 6: A. Ethosomal suspension, F. Ethosomal gel, and G. Ethosomal gel with optimization FTIR spectral investigations.

Drug-excipientcompatibility studies by DSC

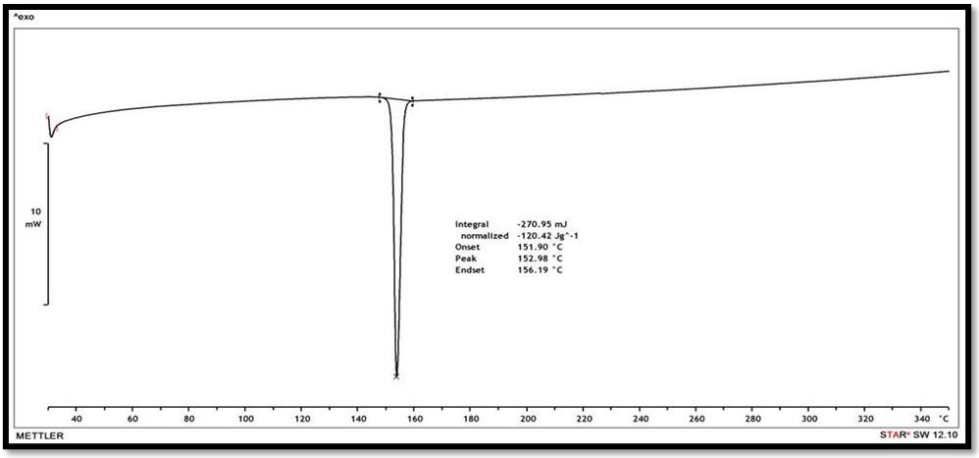


Figure 7: DSC thermogram of Puredrug(Ketoconazole).

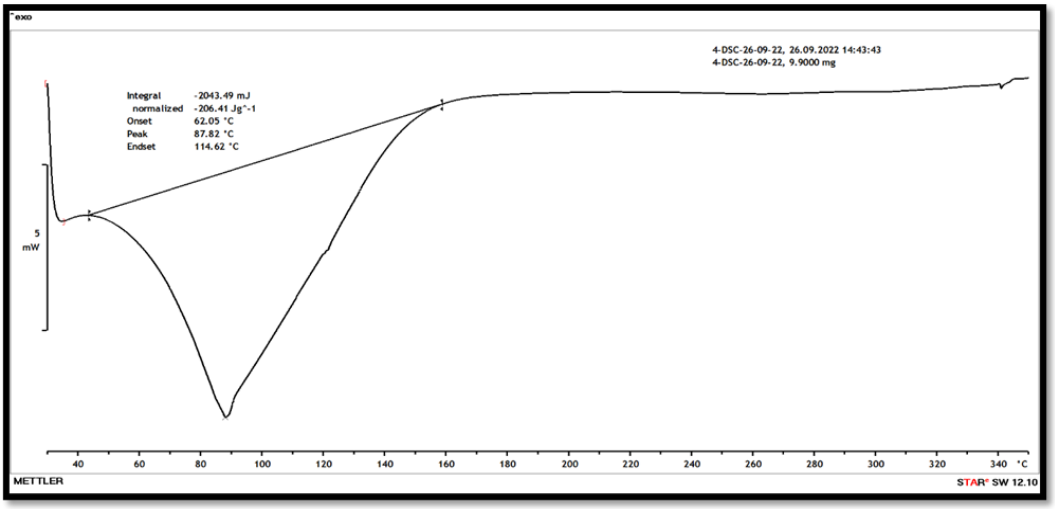


Figure 8: Differential Scanning Calorimetry Thermogram OF Ethmosomal Suspension Formulation.

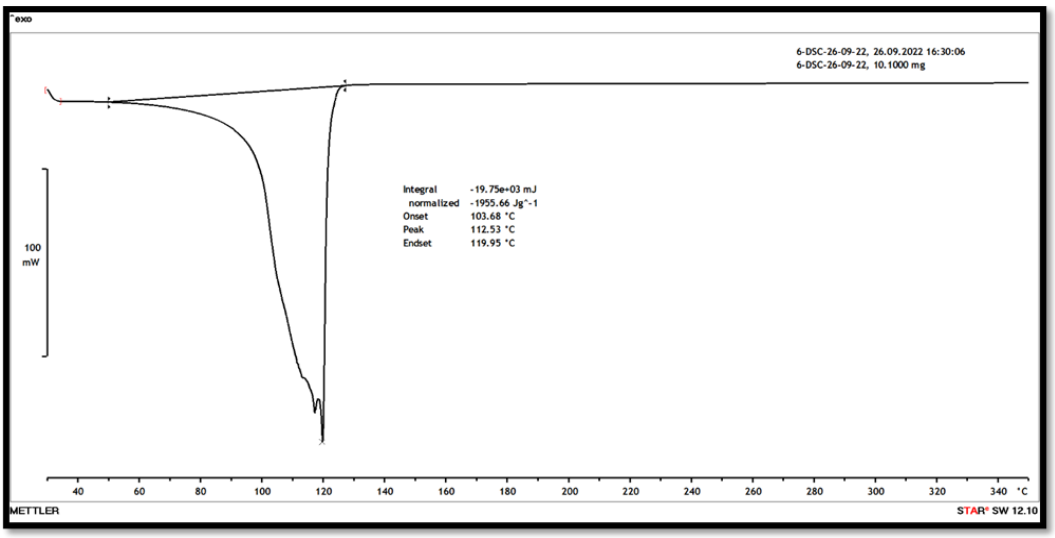


Figure 9: DSC thermogram showing the development of ethosom algel.

SUMMARY AND CONCLUSION

When compared to more traditional methods of dosing and administering topical and systemic medications, TDDS stands out as a promising new option. Transdermal medication delivery device design is complicated by the skin's inherent transport barrier. Different innovative methods have been evaluated to boost this rate temporarily and locally for this aim. There have been period ictechnological development saimedatmitigating such difficulties. The vesicular system is one strategy that has been met with debate. Ethosomal systems are used for transdermal distribution of a broad variety of medications, and are made up of phospholipids, ethanol, water, and a penetration enhancer (e.g. NSAIDS, antifungal, anti- rheumatoid, cosmetics, veterinary, etc).[15]

The Transdermal approach may have various benefits over the more common methods. These benefits include improved physiological and pharmacological response, stability of blood levels, reduced risk of adverse effects, increased utility of medications with a short half- life, and, most importantly, greater patient convenience. However, a poor penetration rate is a key obstacle to effective medication delivery.[16]

Ultra-centrifugation was used to assess the encapsulated medication after confirmingthe presence and size of vesicles. There was a greater increase in value (79.62%) in sonicated particles containing 30% w/w ethanol. Dialysis membrane was used to perform in vitro release. Drugreleasevalueswereas follows:F1=76.89%,F2=82.31%,F3=73.62,F4=86.42%, F5 = 72 %, F6 = 63 %, and F7 = 66 % when consumed with 20%, 40%, 50%, and 60% ethanol, respectively. All the formulations were determined to have a first order drug release. The results suggest that Ketoconazole gel is a viable topical medication delivery strategy for the management of hypertension.[17]

Morphologically, the produced ethosome formulations were consistent when examined by SEM. All of the vesicles met or exceeded the criteria for nanotechnology in terms of size (less than 200 nm), polydispersity index (PDI), and entrapment efficiency (of the intended medication). These factors were associated with the drug's abilityto crossthe skin's lipid barrier (SC). This is because the ethanol in ethosomes makes the medication more soluble, and the smaller vesicle size makes it easier for the drug to diffuse over the skin. However, traditional gel formulations are only able to reachthe surface of the skin, where they function as a reservoir.[18]

By observing the formulations with a microscope at several magnifications, we were able to demonstrate the existence of vesicular structure, includingthe lipid bilayer and spherical vesicles. Microscopy and the program "particle size analysis" were used to calculate the sonicatedvesicle size. The size of individual vesicleswasmeasuredtobeanythingfromzero to 5.483 m. Sonication was shownto be effectivein reducingvesiclesizebyas muchas afactor of three.

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