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FORMULATION OPTIMIZATION OF KETOCONAZOLE LOADED ETHOSOMAL GELFORTOPICAL APPLICATION: *IN VITRO* AND *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 optimized using 3^3 factorial design and Design Expert® software for analyzing the data statistically and graphically using response surface plots. 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 confirming the 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 200nm), polydispersity index (PDI), and entrapment efficiency (of the intended medication). The percentage of Ketoconazole released after 24 hours was significantly decreased (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 gel was around 2.5-, 3.5- and 4.5-fold greater ($p<0.05$). Stability studies displayed that after 2 months, all of the gels' physicochemical characteristics, including viscosity and color, remained unchanged, passing the tests.

KEYWORDS

Topical application, Ethosomes, Ketoconazole, *Ex-vivo* permeation and Stability studies.

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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].

It has shown promising results over the past year compared to oral drug delivery systems 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].

Theoretical advantages of transdermal route

Avoiding first-pass metabolism, having a predictable and gradually expanded duration of activity, reducing undesirable side effects, making use of short-half-life drugs, enhancing physiological and pharmacological reactions, eliminating fluctuations in drug levels and most importantly, ensuring patient compliance are all potential benefits of the transdermal route over more traditional administration methods. However, the limited rate of penetration into the outermost layer of skin isa key issue in transdermal medication administration[3].

Considering that the most common method of medicine administration is by mouth, it is common practice to draw parallels between the two delivery methods.

Since transdermal medication administration does not need gastrointestinal (GI) absorption, it has the potential to reduce or remove numerous of the potential confounding factors that are present when a medicine is taken orally. The pH of a molecule varies as it travels through the gastrointestinal system, from the very acidic stomach juices (as low as 1) to the more basic but still acidic intestine (up to 8). Gastric emptying, intestine motility as well as transit durations, human and bacterial enzyme activity, and the impact of food are all other factors that may be altered[4].

NOVEL APPROACHES OF TRANSDERMAL DRUG DELIVERY

ETHOSOMES

For quite some time, vesicles' role as messengers between cells and as carriers of small particles has been well recognized. To improve medication administration within their chambers, vesicle architectures are being studied by scientists so that they may be tagged for cell selectivity. As an added bonus, vesicles would make it possible to regulate the release of the medicine over a prolonged period of time, keeping it safe from elimination by the immune system or other mechanisms. The discovery of ethosomes, a kind of vesicle, was a big step forward in the study of vesicles[5-8].

To increase the flexibility of the lipids in the stratum corneum (SC), ethanol interacts with them in that polar hard group area. Ethanol's capacity to promote membrane permeability is due to its ability to intercalate into polar head group environments. The ethosome may connect with the stratum corneum barrier independently of ethanol's influence on the barrier's structure[9].

Types of ethosomes

According to their respective chemical make-ups, ethosomes are classified as either "classical" (Touitou E, 1996) or "transethosomes"[10,11].

REVIEW OF LITERATURE

Touitou *et al*, (2000) Ethosomal, a new carrier consisting of phospholipid, ethanol, and water, was characterized as having improved skin delivery. Diffusion cell tests proved that ethanol and phospholipid from Ethosomal may penetrate human skin. Electron microscopy revealed the presence of multilamelar vesicles in ethosomal systems containing 2% soybean Phosphotidyl choline, 30% ethanol and water. The lipid bilayer structure was verified by nuclear magnetic resonance (NMR) experiments. Both calorimetry and fluorescence tests indicated that vesicle bilayers are pliable and have a low Tm. By manipulating Ethosomal makeup, we were able to adjust the average vesicle size, as determined by dynamic light scattering. Ethosomes were shown to have a high trapping capacity for compounds of variable lyophilicity in experiments utilizing fluorescent probes and ultracentrifugation[12].

Dayan N, Touitou E, (2000) studied the transport of trihexyphenidyl HCl (THP) from Ethosomes as opposed to traditional liposomes and defined an unique Ethosomal carrier containing THP. Reduced

vesicle size (from 154mm to 90mm) was seen when THP concentration was raised from 0 to 3%. That was probably because of how active THP was on its surface. Ethosomes' entrapment effectiveness was higher and its capacity to transport anentrapment fluorescent probe to deeper layers of skin better than that of conventional liposomes. Through the skin of a naked mouse, the flow of THP from THP Ethosomes (0.21g/cm2/hr) was 87, 51 and 45 times greater than that from liposomes, phosphate buffer and hydro ethanolic solution, respectively[13].

Chetoni P *et al*, (2004) this study compared the pharmacokinetic profile of acyclovir (ACV) in the aqueous humor of rabbits following topical application of acyclovir (ACV) ointment including liposomal formulation to a commercial ACV ointment. In comparison to three reference formulations with the same ACV content, the ACV liposomal dispersion resulted in a considerably higher drug concentration profile in aqueous humor, with a plateau at 90 minutes. The AUC obtained by full strength 3% ointment was just 1.6 times bigger than that corresponding to liposomal vehicle, despite the significantly higher dosage (1.5 Vs 0.18ng)[14].

AIM AND OBJECTIVE OF THE STUDY

For topical administration, create Ketoconazole ethosomalgel.

The goal of this project is to conduct diffusion studies and other tests on the final Ketoconazole emulgel formulation.

To investigate the relevance of Carbopol concentration in gelformation.

PLAN OF WORK

A survey of relevant articles, books and online resources.

The procedure for determining Ketoconazole levels needs standardization.

Ketoconazole suspension was prepared by altering the soy lecithin content (2%, 3% and 4%), while maintaining the ethanol percentage (20%) constant.

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Ketoconazole suspension was prepared by altering the ethanol content from 30% to 40% to 50% while maintaining a constant lecithin concentration (optimized concentration).

Optimizing the ethanol content by contrasting the cumulative percent drug release of the afore mentioned Ketoconazole suspensions.

To create a Ketoconazole gel, we first tuned the amount of lecithin and ethanol in a Ketoconazole suspension.

DRUG PROFILE AND EXCIPIENT PROFILE

Drug name : Ketoconazole[15]

IUPAC name : 1-[4-(4-{[2-(2,4-dichlorophenyl)-2-(1H-imidazol-1-ylmethyl)-1, 3-dioxolan-4-yl] methoxy}phenyl)piperazin-1-yl]ethan-1-one

Synonyms : Ketoconazolum, Ketozole, Nizoral, Nizorala-D

Solubility : DMSO, ethanol, chloroform, water and methanol all havea 20mg/mL solubility threshold when warmed.

Description : Phenylpiperazines are a class of chemical compounds that include this substance. Phenylpiperazine compounds have a piperazine backbone that is chemically bonded to a phenyl group.

Melting point : 148-152°C

C**ASNO :** 65277-42-1

Molecular formula : C26H28Cl2N4O4

Molecular weight : 531.431

Dosage forms : cream, tablet, shampoo, aerosol

Dose : 20mg, 20.5mg, 200mg

Category : Antifungal Agents, 14-alpha Demethylase Inhibitors

Halflife : 2hours

RESULTS AND DISCUSSION

Analytical study

Calibration curve in water (makeup with PH6.8 phosphate buffer)

Absorbance was measured at 257nm from standard solutions of varying concentrations (Table No.13). Drug concentrations were plotted against absorbance to generate a calibration curve, per the protocol (Figure No.8). Beer's law was shown to be linear with a k2 value of 0.999.

Scanning electron microscope (SEM)

Images takenata high magnification (8-11 in the figures) revealed asizer education.

The size and form findings agree with the empirical evidence.

Analysis of vesicle size revealed that ethanol concentration influences vesicle size. Smaller vesicles were found at higher ethanol concentrations, with a maximum size of 5.483m found in 20% ethanol and a minimum size of 3.907m found in 50% ethanol. The findings of this study agree with those of previous studies.

ENTRAPMENT EFFICIENCY

After establishing that bilayer vesicles were present in the gel system, ultra-centrifugation was used to examine the vesicles' potential for drug entrapment. Ultra-centrifugation was employed to separate the drug-containing gel vesicles from the free drug and un-entrapped drug.

Ultra centrifugation showed that the gel formulation with 30% ethanol (EF4) had the highest entrapment efficiency of gel vesicles, at 79.62%, almost twice that of the gel.

FTIR study

Pure drug IR spectra included is tinct peaksat1, 643cm1, 813cm 1owing to C= 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.

Table No.1: Oils mostly used in emulsion preparation in Ethosomalgel

S.No	Ingredient Quantity	Dosage Form
1	Light liquid paraffin 7.5%	Ethosomalgel
2	Isopropyl myristate 7-7.5%	Emulsion
3	Isopropyl palmitate 7-7.5%	Emulsion
4	Propylene glycol 3-5%	Ethosomalgel

MATERIALS EQUIPMENTS AND METHODS

Chemicals

Table No.2: Chemicals and materials

S.No	Chemicals	Manufactured by
1	Ketoconazole	Natco Pharma Labs
2	Propylene glycol	Research lab fine Chem. Industries (Mumbai)
3	Alcohol	Jiangsu Huaxi International Trade Co. Ltd (CHINA)
4	Cholesterol	Virat lab (Mumbai).
5	Carbopol-934	Research lab fine chem. Industries (Mumbai)
6	Triethanolamine	Research lab fine chem. Industries (Mumbai)

EQUIPMENTS

Table No.3: Instruments and company

S.No	Instruments	Company
1	Electronic weighing balance	Scimadzu corporation (JAPAN)
2	U.V. spectrophotometer	Schimadzu 1800 (JAPAN)
3	Magnetic stirrer	REM elektrotechnik limited. Vasai (INDIA)
4	Refrigerator	Allwyn (INDIA).
5	Sonicator	SISCO Scientific Instruments sales Corporation, Thana, Mumbai.
6	pH meter	REMI
7	Scanning electron microscope	Scimadzu corporation (JAPAN).
8	FTIR	Scimadzu corporation (JAPAN).

Table No.4: Ingredient sin 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

Table No.5: 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-4 free drug gel

Table No.6: Determination of λmax of Ketoconazole in methanol--λmax=278nm

S.No	Concentration (µg/ml)	Absorbance
1	0	0
2	2	0.14
3	4	0.269
4	6	0.383
5	8	0.496
6	10	0.618
7	12	0.740

Table No.7: 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

S.No	Size range					
	Eye piece micrometer	In µm	Average size (d) µm	No of vesicles (n)	% No of vesicles (n/150 *100)	nxd
1	0-1	0.00-3.33	1.665	65	43.33	108.225
2	1-2	3.33-6.66	4.995	62	41.33	309.69
3	2-3	6.66-9.99	8.325	11	7.33	91.575
4	3-4	9.99-13.32	11.655	7	4.667	81.585
5	4-5	13.32-16.65	14.985	5	3.33	74.925
6	-	-	-	Σn=150	-	Σnd=666

Average diameter (d avg) = $\frac{\Sigma nd}{\Sigma n}$ = 4.44µm

Table No.8: Size distribution of gel formulation #2F2 (3% Lecithin, 20% ethanol)

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

Average diameter (davg) = $\frac{\Sigma nd}{\Sigma n}$=5.106µm

Table No.9: Size distribution of gel formulation #3F3 (4% Lecithin, 20% ethanol)

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

Average diameter (davg) = $\frac{\Sigma nd}{\Sigma n}$ = 5.483µm

Table No.10: Size distribution of gel formulation #4F4 (3% Lecithin, 30% ethanol)

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

Average diameter (davg) = $\frac{\Sigma nd}{\Sigma n}$ = 5.062µm

Table No.11: Size distribution of gel formulation #5F5 (3% Lecithin, 40% ethanol)

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

Average diameter (davg) = $\frac{\Sigma nd}{\Sigma n}$ = 4.71µm

Table No.12: Size distribution of gel formulation #6F6 (3% Lecithin, 50% ethanol)

S.No	Size range					
	Eye piece micrometer	In µm	Average size(d)µm	No of vesicles (n)	% No of vesicles (n/150 *100)	nxd
1	0-1	0.00-3.33	1.665	70	46.667	116.55
2	1-2	3.33-6.66	4.995	67	44.667	334.665
3	2-3	6.66-9.99	8.325	7	4.667	58.275
4	3-4	9.99-13.32	11.655	4	2.667	46.62
5	4-5	13.32-16.65	14.985	2	1.333	29.97
6	-	-	-	Σn=150	-	Σnd = 586.08

Average diameter (davg) = $\frac{\Sigma nd}{\Sigma n}$ = 3.907µm

Table No.13: Drug entrapment efficiency of Ketoconazole Gel

Formulation code	Entrapment efficiency (%)			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

EVALUATION OF GEL

Organoleptic characters of gel

Table No.14: Organoleptic characters of Ketoconazole gel

S.No	Organoleptic Characteristics	Color: golden yellow Greasiness: Non greasy Grittiness: Free from grittiness Ease of application: Easily/smoothly applied Skin irritation: No skin irritation
1	Wash ability	Easily washable without leaving any residue on the surface of the skin.
2	Spread ability	6.25cm/sec

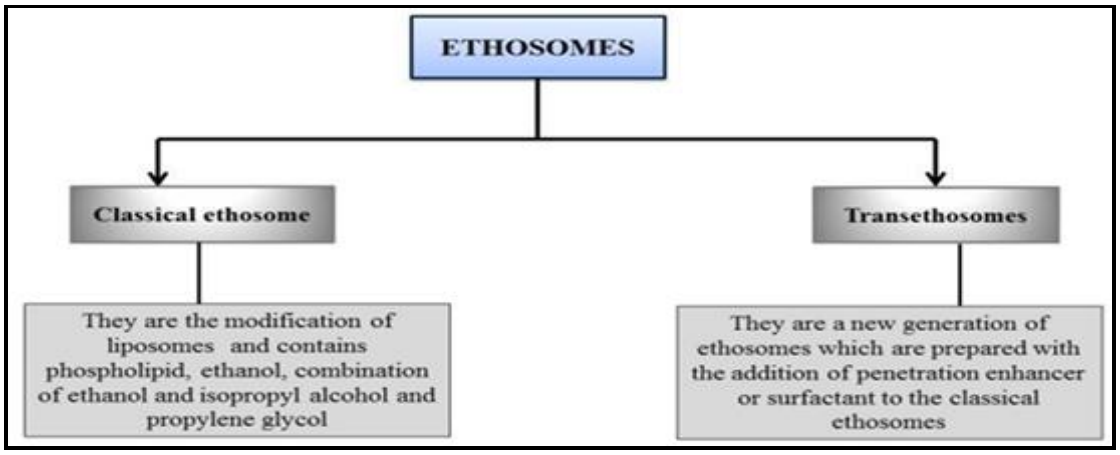


Figure No.1: Categories of Ethosomes

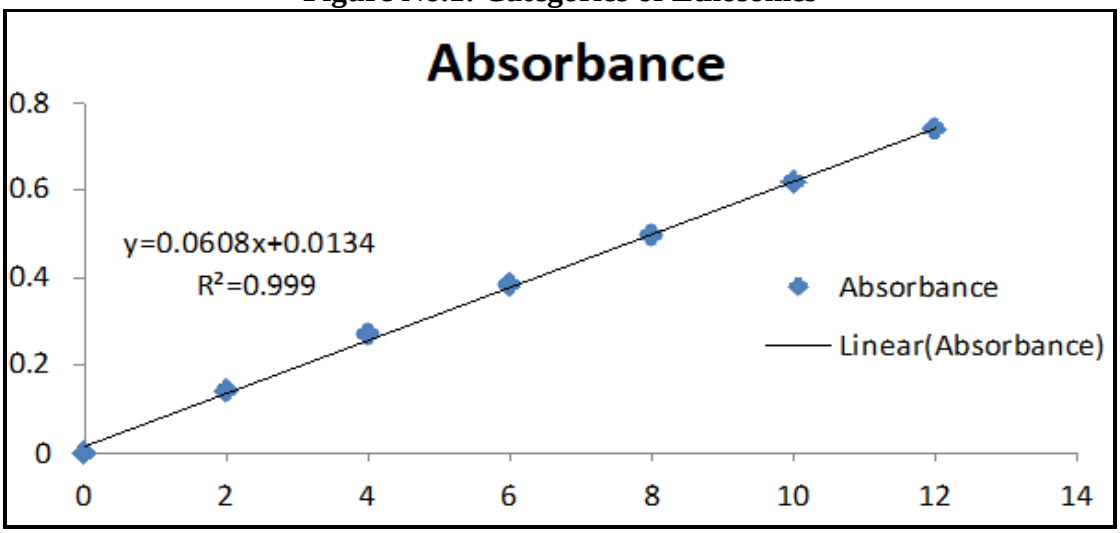


Figure No.2: Standard graph of Ketoconazole

Response No.1: Entrapment Efficiency

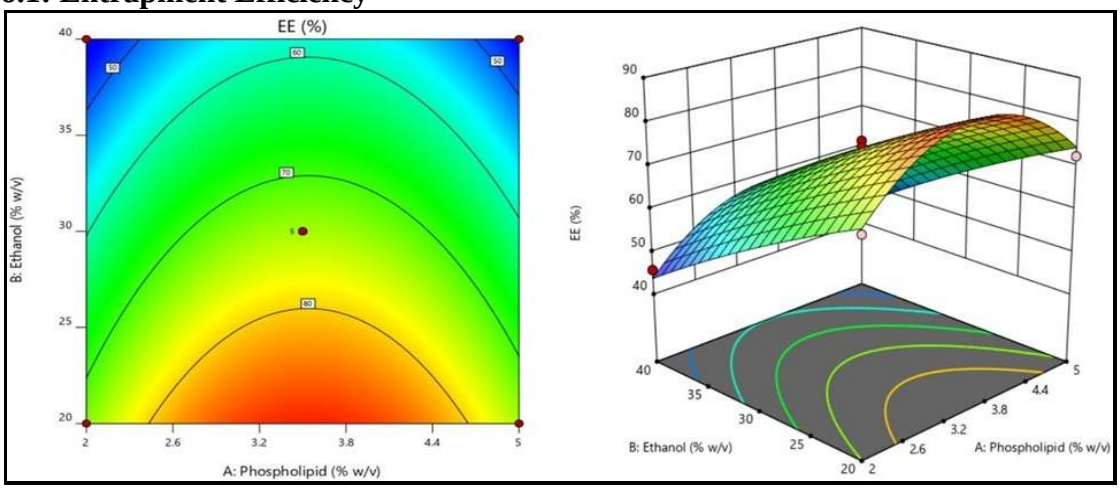


Figure No.3: Counter and RS Counter Effect of Independent Variables on Total Electrical Energy Consumption as a Percentage (%) Plot

Response No.2: Vesicle Size

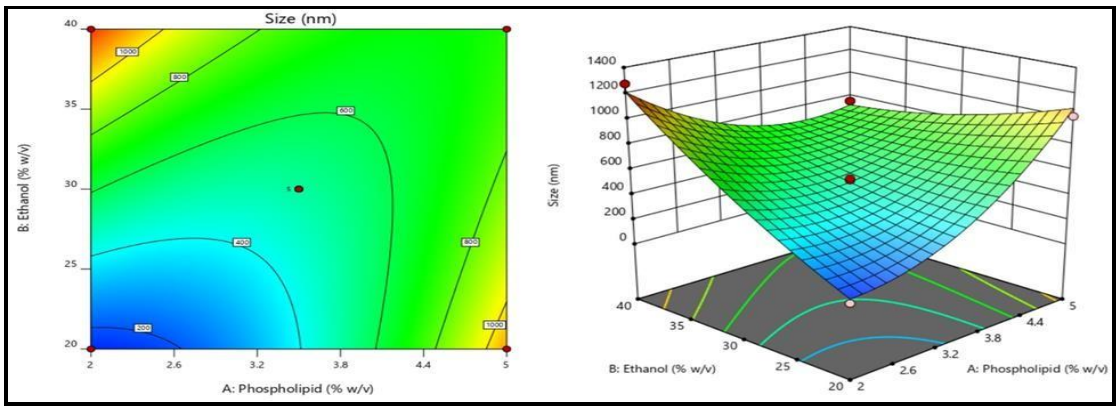


Figure No.4: 3D counter and a response board Influence of Independent Variables on: Size, Location and Time (3-D Plot)

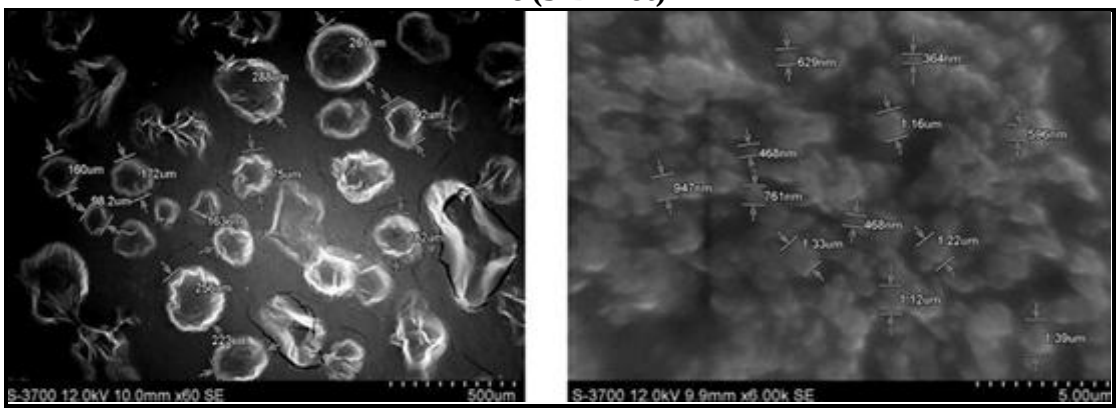
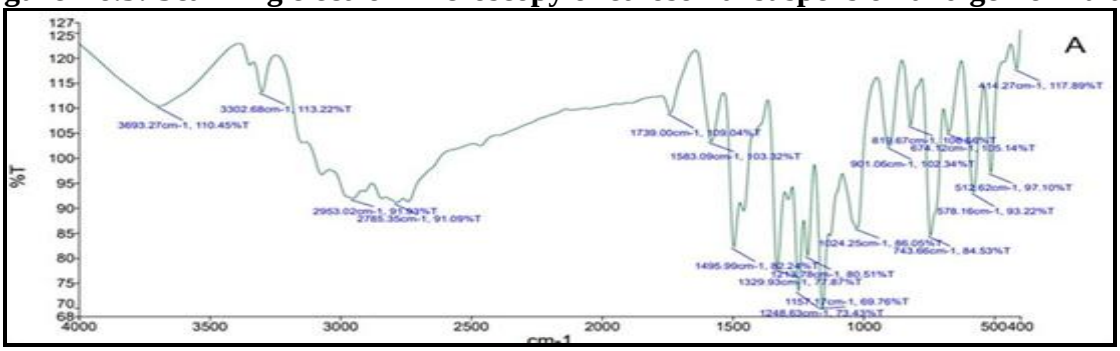
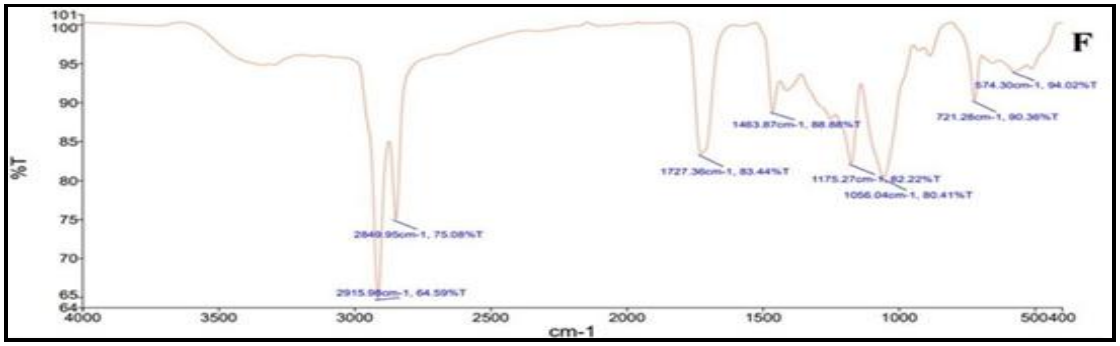


Figure No.5: Scanning electron microscopy of ethosomal suspension and gel formulation





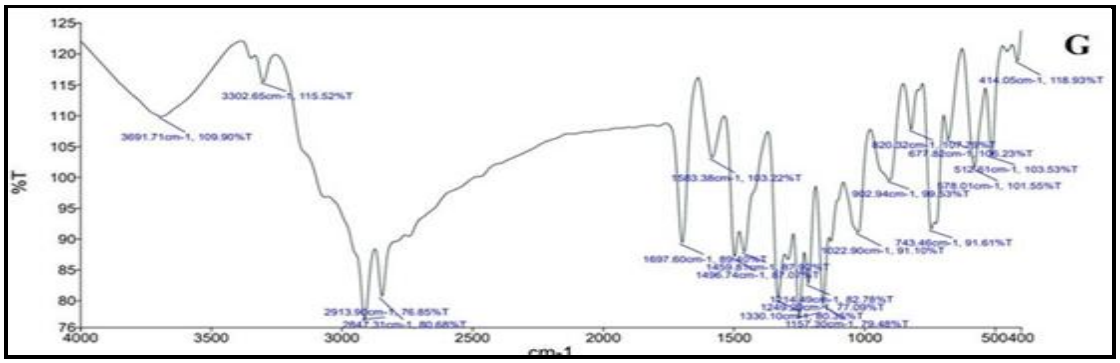


Figure No.6: A. Ethosomal suspension, F. Ethosomal gel, and G. Ethosomal gel with optimization FTIR spectral investigations

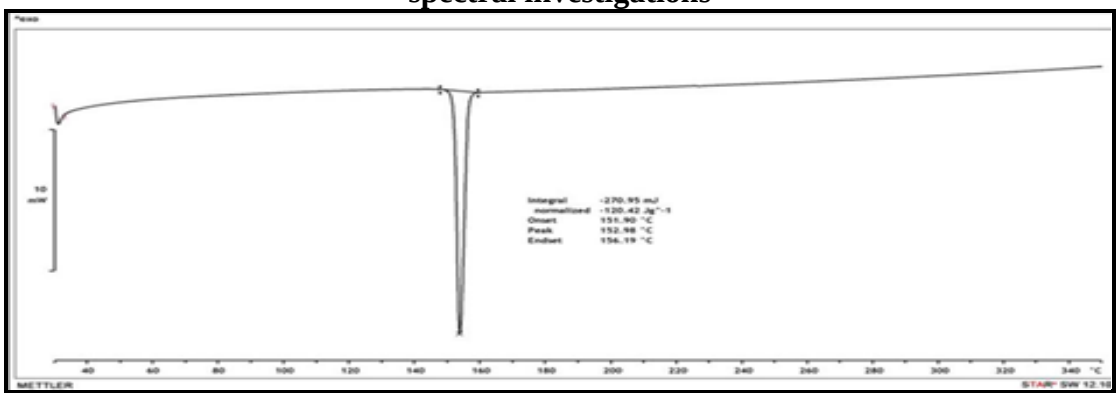


Figure No.7: DSC thermogram of Pure drug (Ketoconazole)

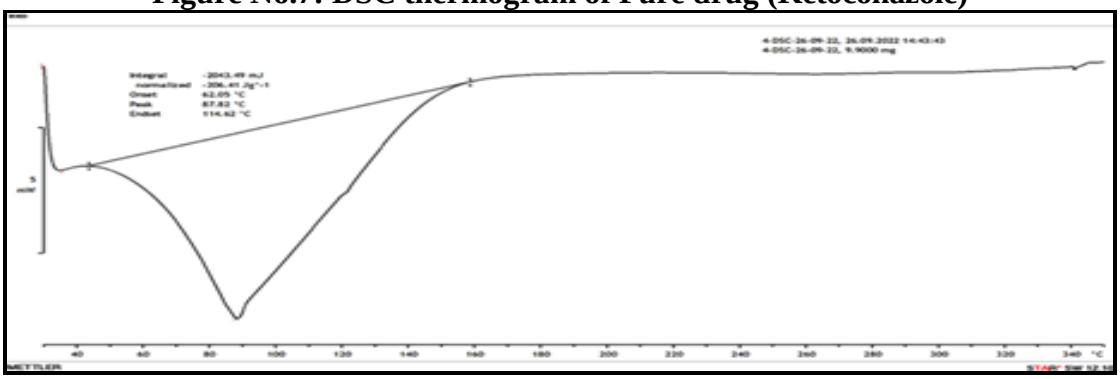


Figure No.8: Differential Scanning Calorimetry Thermogram of Ethmosomal Suspension Formulation

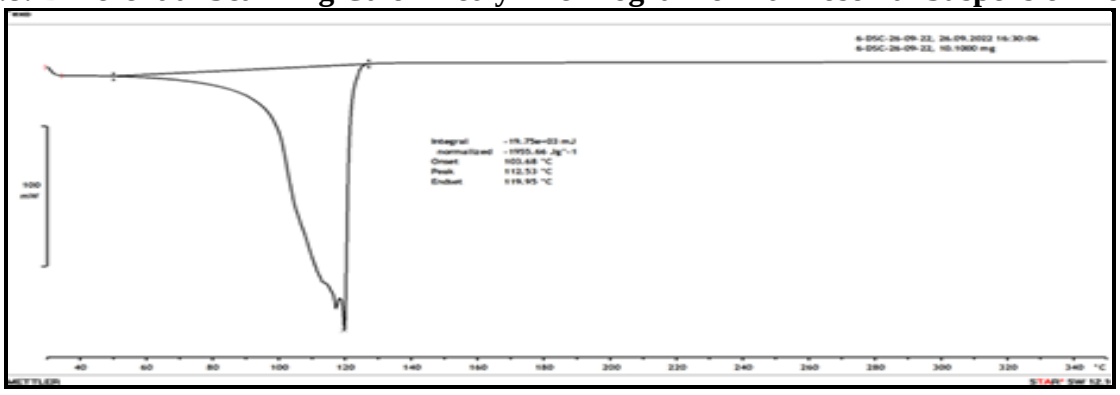


Figure No.9: DSC thermogram showing the development of ethosomalgel

pH measurement

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 periodic technological developments aimed at mitigating 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, antiviral, anti-rheumatoid, cosmetics, veterinary, etc). These, however, call for efficient transdermal administration and provide the ground work for investigating other routes into the body.

This study used a statistical strategy to the development of ethosomes of a BCS Class-II medication, ketoconazole, for transdermal administration. Drug integration into the new ethosome was characterized and validated using FTIR and DSC analyses.

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.

Vesicular system (liposomes and niosomes) seems as future development while enhancing topical medication delivery. Gel method demonstrated superior topical administration with increased transdermal flux and skin deposition, making it a tempting choice due to its many benefits.

These tests verified that the medicine was well integrated into the lipid and that it was compatible with the other excipients. In order to verify the binding posture and binding affinity of pharmaceuticals, an in-silico molecular docking research was carried out between the drug and the excipient. The research showed that, at the molecular level, the three medicines had strong binding energy when they were stuck in the polar head of the core cavity. Ultimately, after weighing the pros and cons of Ketoconazole ethosome formulations made using the cold technique, the ethanol injection method and the TFH approach, the latter was chosen for manufacture. The TFH strategy performed well, thus it was put to use in the construction of a statistical technique. Optimized ketoconazole ethosomes through Box-Behnken full factorial.

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CONFLICT OF INTEREST

We declare that we have no conflict of interest.

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