Formulation and Evaluation of Ethosomal Gel For Sertaconazole Nitrate (Anti-Fungal Agent)

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Abstract:

The purpose of the present research was developing transdermal drug delivery of sertaconazole nitrate for treatment of cutaneous candidiasis. The main barrier of the skin is located within its uppermost layer, the stratum corneum (SC).Several approaches have been developed to weaken this skin barrier.one of the approaches for increasing the skin penetration of the drug use of vascular system such as, Ethosomes, Ethosomes are phospholipid based elastic nanovesicle, prepared by Hot method, containing soya phosphatidylcholine 3%,and high concentration of ethanol(40%), Ethanol is known as an efficient penetration enhancer and added in a vascular system to prepared elastic nanovesicle. It can interact with the polar head region of the lipid molecule, resulting in the reducing of the melting point of the stratum corneum lipid, thereby increasing lipid fluidity and cell membrane permeability. The optimized ethosomal formulation F5 Show highest EE% (90%),smallest particle size(155.3 ± 1.2), and maximum zeta potential(-42±0.3) was incorporate with Carbopol 934 a gelling agent, to produced ethosomal gel formation. Transmission electron microscopy, differential light scattering, and a vitro release profile, viscosity, spradability, pH determination, Antifungal activity were characterization the optimized formulation no F5. The EE% was found 90 %, show the in vitro skin permeability 67% within 80 min, The gel formulation showed 219.69 gm.cm/sec, it was noticed that ethosomes would improve transdermal flus, prolong release release, and proved an appealing that long term distribution of sertaconazole nitrate, and that the ethosomal delivery method is a successful design for topical drug delivery with improve bioavailability and patient enforcement.

1. Introduction:

The primary cause of fungal infections is tiny organisms that are easily found in the environment and can infiltrate epithelial tissue. Yeast, molds, rusts, and mushrooms are examples of fungi that may grow readily on mammals and absorb nutrition from their surroundings.**[1].**While the majority of fungi are beneficial to the environment and biodegrade well, a small number of them can cause infections when they enter the human body through cuts, wounds, or the nose passage.**[2**]The fungus known as superficial candida speciesusually impacts the layers of skin, nails, and hair, but it can also spread to deeper tissues . Human skin is frequently home to a wide variety of microorganisms, including fungus and bacteria (normal flora).**[3**].Any of these common flora members can become overgrown due to a variety of possible underlying medical issues. A yeast called Candida albicans usually resides on the folds of skin in the mouth, vagina, digestive tract, and armpits. [**4]**A Candida albicans skin infection, often known as candidiasis, can be extremely itchy and red[5].The primary causes of candidiasis include warm temperatures, tight clothes, poor personal cleanliness, obesity, the use of antibiotics and corticosteroids, and a compromised immune system**.[6]**

In medicine, oral formulations of sertaconazole nitrate, such as liquid preparation and tablets, as well as venous formulations, are famous and often utilized. Recently, Sertaconazole nitrate topical antifungal properties have come under increased scrutiny due to concerns about toxicity associated with systemic use. Numerous papers have demonstrated the effectiveness of this delivery method**. [.7, 8]**

The medication delivery mechanism of conventional topical treatments, including lotions, tablets, ointments, and creams, is being hindered by various problematic aspects. These include the drug's high molecular

weight, the skin's barrier qualities, and limited lipid solubility. The stratum corneum, the outermost layer of the epidermis, acts as a barrier to prevent topical antifungal medicine penetration.

Skin permeation enhancers or changes to the drug formulation can both improve skin permeability. Drug delivery methods based on nanotechnology provide efficient medication delivery to solve these issues.

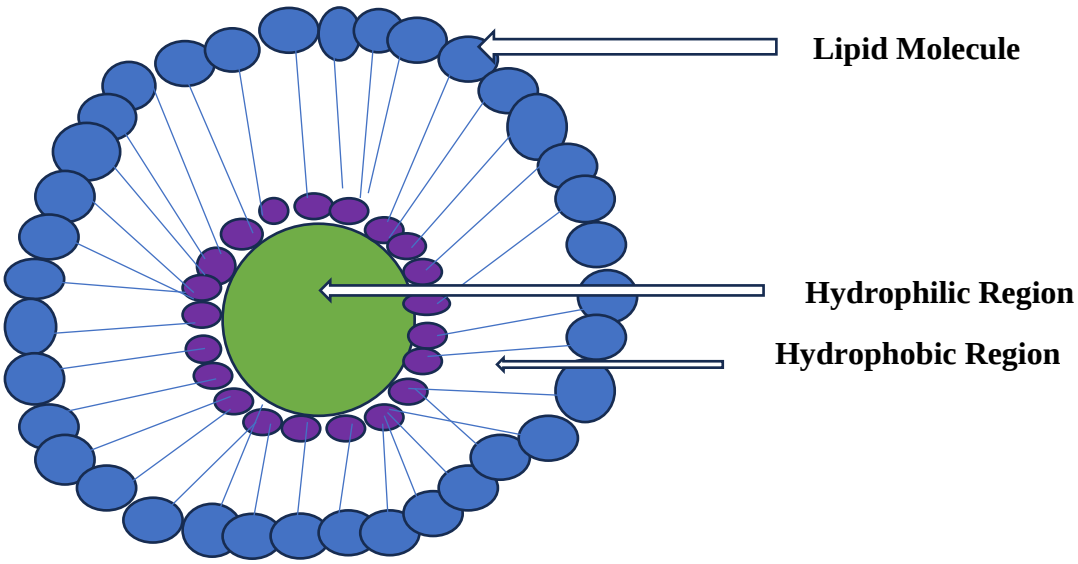
By manipulating size and shape at the nanoscale, nanotechnology is a set of methods that can be used to design, synthesize, characterize, and apply structures, materials, devices, and systems. This technology can solve a number of problems related to medication delivery...[**9].**

1.1. ETHOSOMES:

Nanoparticles known as etherosomes are composed of phosphatidylcholine lipids with a high concentration of water and ethanol. These particles offer unique properties and advantages.These are perfect for the topical delivery of a variety of therapeutic agents because of their excellent deformability, high drug entrapment efficacy, and enhanced rate of drug penetration in the skin...**Tarek.A.et.al.2016[10].**

Touitou et al., 1997 created etherosomes, which are additional unique lipid carriers made of phospholipids, water, and ethanol. Many medications are said to be better delivered via the skin thanks to them. [11]Drugs can be delivered non-invasively via etherosomes to the subcutaneous layers of the skin and/or the systemic circulation.Because ethanol is known to create disruptions in the organization of the skin's lipid bilayer, the ethosomes stand out due to their high concentration of ethanol. Consequently, when a vesicle is integrated into the stratum corneum's membrane, it permits the vesicle to pass through. Furthermore, despite being equally stable, the stratum corneum lipids' high ethanol content causes the lipid barrier to be packed less securely. This makes the structure more adaptable and improves the medication's distribution potential...**Verma.et.al.2010[12]**

Figure 1.1: **Ethosomes with inner polymer core congaing drug molecule surrounded by lipid**



1.2. Advantages of ethosomes:

1. Transdermal and dermal administration of the medication are improved by etheromethanes.
2. Many different types of drugs are delivered by phagosomes (peptides, protein molecules).
3. Ethosomal methods are significantly more effective at delivering a fluorescent probe (quantum) to the skin in terms of both number and depth.
4. Low risk profile: There is no risk involved in using the technique for large-scale drug development because the toxicological profiles of the ethosome components are well-documented in the scientific literature.

5. Excellent adherence from patients: Because the ethosome medications are administered as a gel or cream, they are semisolid, which results in great patient compliance. Conversely, the use of iontophoresis and phonophoresis is more difficult, which will have an impact on patient compliance.
6. Products with unique technology have a high level of commercial appeal. Ethamome production involves minimal technical investments and is quite easy to create.
7. The ethosomes system may be commercialized right now and is both passive and non-passive.
8. A range of uses in the veterinary, cosmetic, and pharmaceutical industries**[13]**

1.3. Disadvantages of ethosomes:

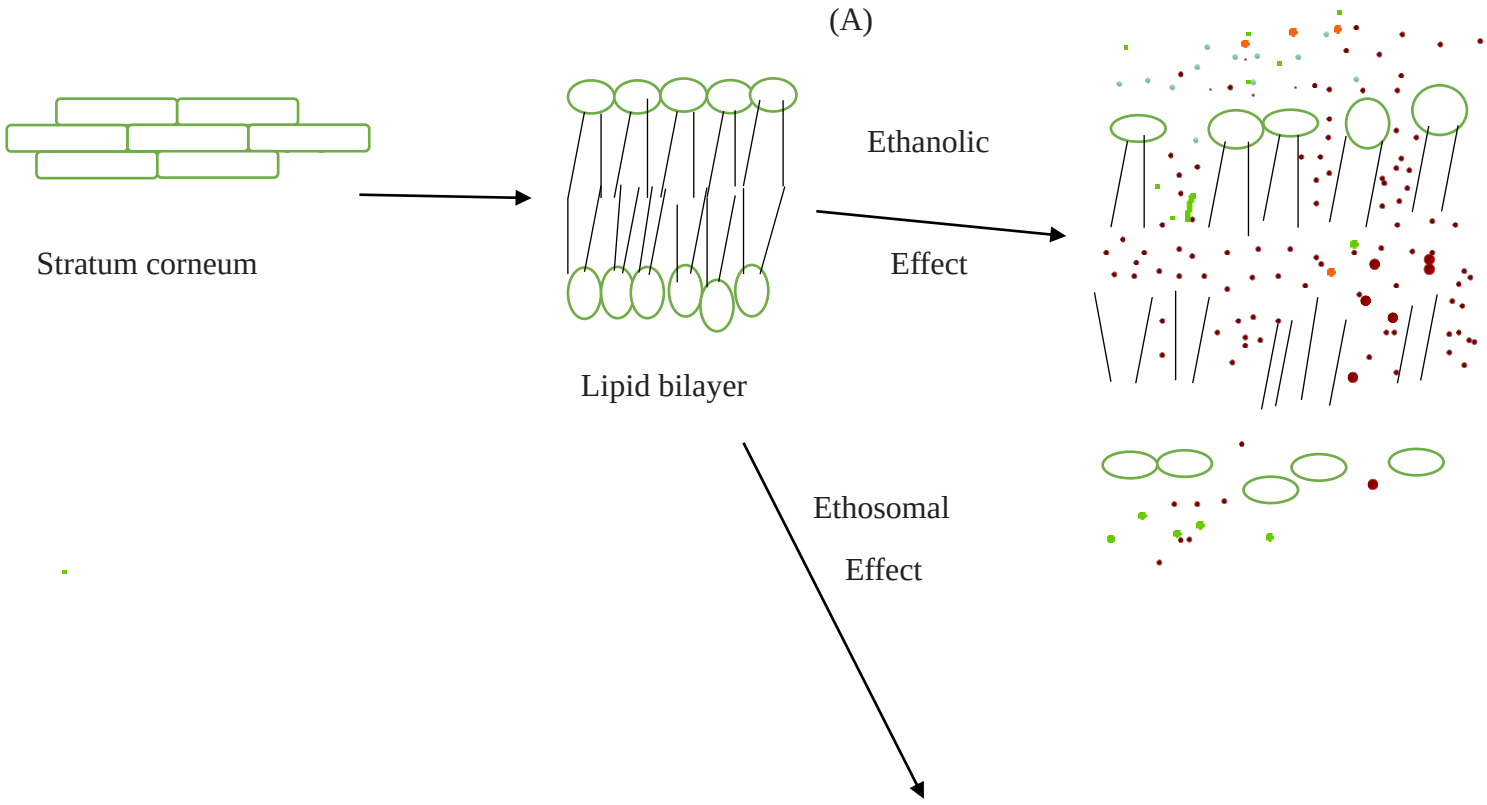
1. Iontophoresis and phonophoresis are very difficult in use, affecting thus patient compliance.
2. Difficult to manufacture in large scale wise.
3. Difficulty in obtaining physical and chemical stability.
4. Production cost is pretty high
5. Dose dumping
6. Leaking problem**[14]**

1.4. Mechanism of action of ethosomes:

The object of ethosome preparation ended liposome preparation is the enhanced drug permeation on transdermal delivery. The mechanism of ethosomes drug delivery system are not identify. The mechanism are follow two step:

1. The impact of ethanol
2. The ethosomes impact

- **Ethanol effect**: CH3OH goes about as a dissemination enhancer over the transdermal medication conveyance framework. Ethanol infiltrates into profound layer of skin and expands the ease of the corneal film of skin and reduction the thickness of lipid multi-facet of skin.
- **Ethosomes effect**: Ethanol are improving fluidity on cell membrane, These ethosomal ethosomes are enhance the skin permeability. Then the ethosomes diffused easily subcutaneous layer[15]



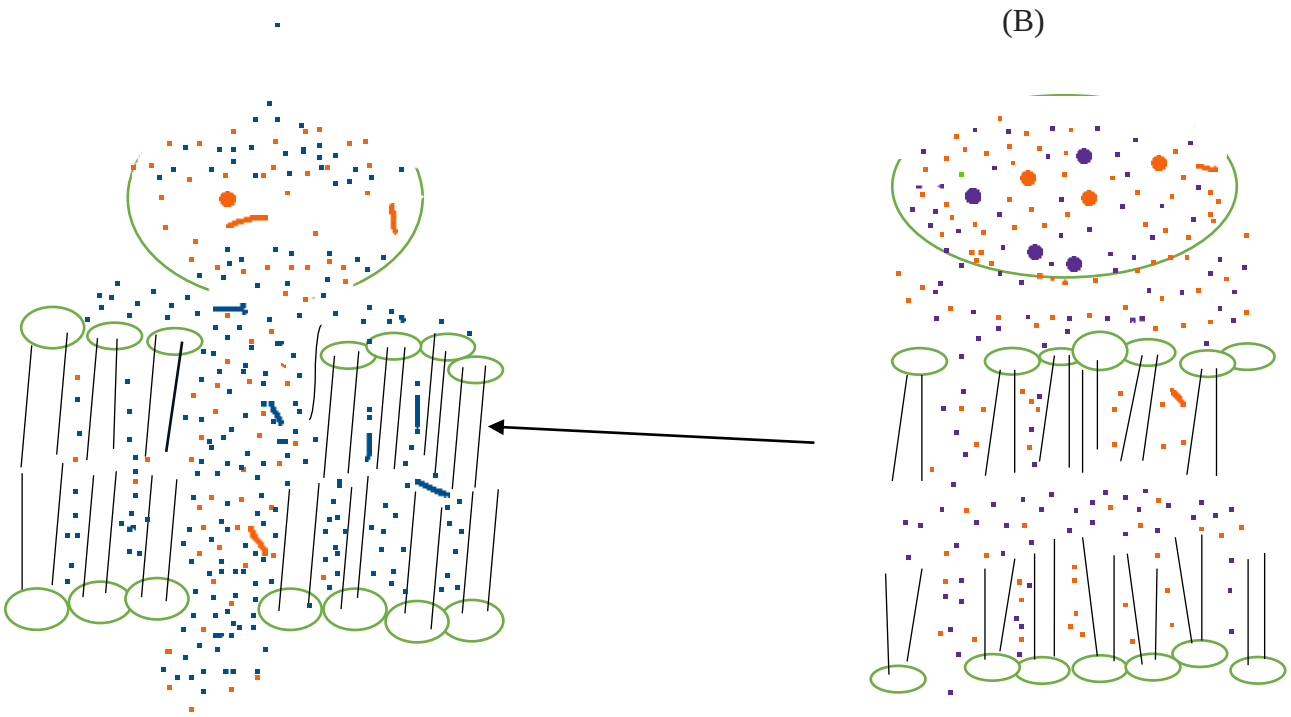


Figure 1.2 Mechanism of penetration of ethosomes

Table 1.1 Composition of ethosomes:[16]

CLASS	EXAMPLE	USES
Phospholipid	Phosphatidylcholine from soy, Egg phosphatidylcholine, Dipalmitylphostidylcholine, Distearylphosphatidyl choline.	part of the vesicles formation.
Alcohol	C_2H_5OH,Alcohol isopropyl.	in order to give the vesicle membrane its suppleness. In order to improve penetration.
Polyglycol	Transcutol RTM, ethylene glycol, Diethylene glycol monomethyl ether, glycerin	as an increase of skin penetration.
Lipid	Fat[Cholesterol]	To give the vesicle membrane stability [emollient and stabilizing agent]
Dye	Rhodamine-123, Rhodamine red fluorescence Isothiocyanate {FITC} 6-carboxy fluorescence.	Regarding the characterisation analysis .

Vehicle	Carbopol934	As a gel former. SSSS

Table 1.2. There are numerous ethosome-based products on the market.[17]

Sr. No	**Name of product**	**Uses**	**Manufacturer**
1	Supravir cream	The herpes virus treatment acyclovir has a long shelf life and no stability issues; at 25°C, it can remain stable for up to three years. Research on the cream's potential to improve skin penetration revealed that its effects lasted for three years...	Trima, Israe
2	Noicellex	topical cellulite reducing cream	Novel Therapeutic Technologies, Israel
3	Cellutight EF	**Strong chemicals that accelerate metabolism and break down fat are found in topical cellulite creams.**	Hampden Health, USA
4	Nanominox	**A potent blend of chemicals found in topical cellulite creams helps to break down fat and speed up metabolism.**	Sinere, Germany
5	Decorin cream	Anti-aging cream that lessens the appearance of wrinkles, sagging skin, age spots, and hyperpigmentation in the skin	Genome Cosmetics, Pennsylvania, US

Table 1.3.Use of ethosomes:[18]

Diseases/ Disorder	**Drug name**
Hormones delivery	Testosterone
Anti-parkinsonian drug delivery	trihexyphenidyl hydrochloride [THP]
Delivery of a medication for arthritis	Cannabidol,
Delivery of antifungal drugs	Ketoconazole, Itraconazole
Antiviral drug delivery	Acyclovir and zidovudine
Topical delivery of DNA	uptake of DNA in skin cells
Antibiotics delivery	erythromycin
Pilosebaceous targeting	Minoxidil
Delivery of problematic drug molecules	peptides and proteins

1.5.Human skin:[19]

The human skin is an intricate and dynamic organ that performs a number of vital tasks. An outline of the composition and purposes of human skin may be found here.

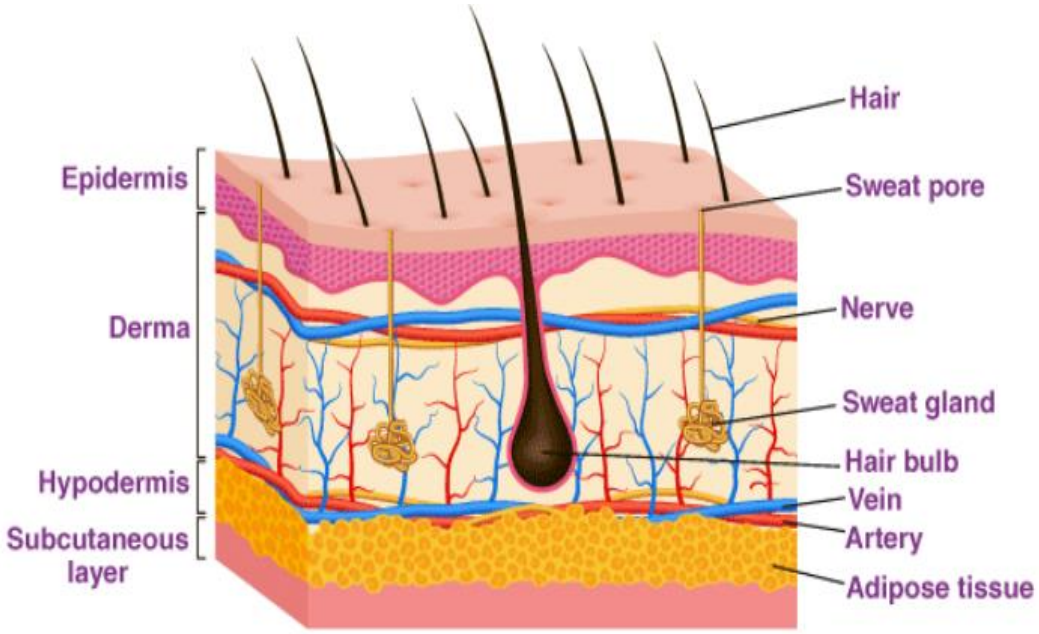


Figure 1.3- Human Skin

❖ **Epidermis:**
Keratinocytes make up the majority of the skin's outermost layer. offers a shield of defense against external influences.

❖ **Dermis:**is made up of blood vessels, nerves, connective tissue, and appendages that are located beneath the epidermis, such as sweat glands and hair follicles. increases the skin's strength, suppleness, and resilience.
❖ **Hypodermis (Subcutaneous Tissue):**made up of connective tissue and adipose (fat) tissue. offers energy storage, cushioning, and insulation.

1.6 Fungal infection: Today's higher rate of fungal infection has greatly increased the number of immunocompromised patients with HIV, hematologic diseases, cancer, and other illnesses, as well as those on immunosuppressive medications for the treatment of autoimmune diseases or organ transplants.**(Limper et al., 2011)[20].**Candida species is the most common cause of fungal infections in persons with compromised immune systems. Systemic candidiasis can be lethal due to deeper tissue invasion by Candida**.(Verma and Pathak, 2012)[21].**sertaconazole nitrate (STZL), an imidazole antifungal medication, inhibits the production of ergosterol, an essential component of fungal cell walls. Among other superficial skin mycoses, it can be used to treat cutaneous candidiasis, dermatophytosis, and seborrheic dermatitis of the scalp**(Croxtall and Plosker,2009)[22].**The hydrophobic drug sertaconazole nitrate has a low skin penetration rate, with a partition value of about 6.23.. **(Lopez-montero et al., 2009)[23]**Because the effectiveness of topical antifungal treatment depends on the drug's ability to penetrate the stratum corneum and reach deeper skin layers, especially viable epidermis, this poses a challenge for the conventional topical therapy of deep skin infections. (**Güngör et al., 2013; Lee and Maibach, 2006)[24]**

1.7. Sertaconazole Nitrate: [25]
Triazoles, or azoles, are also fungistatic antifungals. When treating dermatotic against cutaneous candidiasis species, itraconazole exhibits potent fungicidal activity, while sertaconazole nitrate functions similarly to voriconazole. Topical imidazole antifungal sertaconazole nitrate inhibits ergosterol, a crucial component of the cell wall.

A relatively new medication choice for fungal infections that manifest topically is sertaconazole. The current treatment has a very limited ability to penetrate the skin. can result in the requirement for prolonged, recurrent medication to fully cure the fungal condition.

Structure of sertaconazole nitrate:

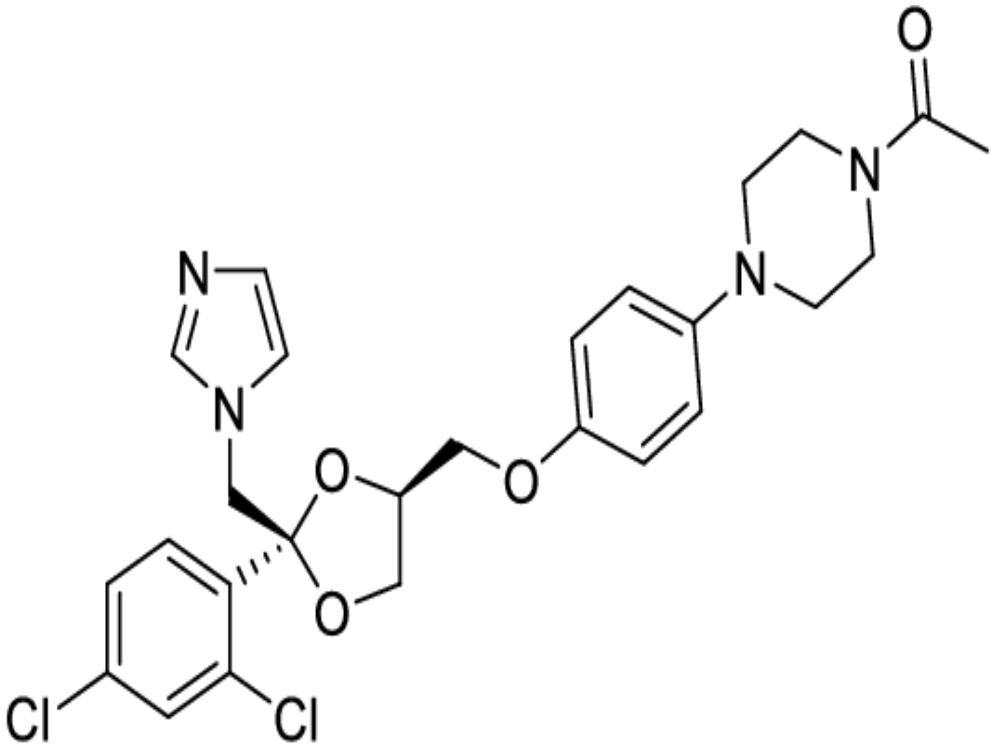


Figure1.4- Structure of sertaconazole nitrate:

Description of Drug [sertaconazole nitrate]:

Category	**Anti-fungal drug**
Molecular formula	$C_{20}H_{15}Cl_3N_2OS$
Molecular mass	437.76gmol.
Melting point	110-140^0**c**
Absorption	Bioavailability is negligible
Volume of distribution	Not Available
Protein binding	99% [in vitro, plasma protein binding
Metabolism	Hepatic
Route of elimination	Feces
Half life	60 hours
Toxicity	Hepatotoxicity, LD50=86 mg/kg[orally in rate
Affected organisms	Fungi
Solubility	Non aqueous solubility

Formulations	Tablet, Shampoo, Cream, for topical use
Therapeutic uses	Treatment of **cutaneous Candidiasis** trat tinea pedis other fungal infection
Adverse effect	Commansise effect are itching ,and blistering swelling oozing or irritation

1.8. Mechanism of action of sertaconazole[pharmacology of drug]: Synthetic antifungal drugs like sertaconazole block the cytochrome p450 enzyme 14-alfa demethylase, which aids in the conversion of lanosterol to ergosterol.Ergosterol is a crucial component of the cell wall; when it is deficient, the cell becomes more permeable, which causes the cell membrane to lyse and inhibits endogenous respiration.interact with the phospholipid found in the membrane, preventing yeast from becoming mycelial forms, which prevent purine uptake and promote the phospholipid, triglyceride biosynthesis.[26]

2. Material and Method:

2.1. Material: Sertaconazole nitrate was obtained as a gift sample from **Aagya Biotech Private Limited, Mangler Roorkee, [Haridwar].** Soya lecithin {Phosphatidylcholine} and Carbopol are purchased by R.K. Enterprises [H.O-139, Jain MohallaBaghpat, B.O-D-9, Dev Park, Opposite Millenium Public School, Baghpat Road Meerut-250002. And Ethanol, Span 80, Propylene glycol are provide collage facility.

2.2 Preformulation study: -

- **Organoleptic Characteristics:**Sertaconazole was physically described based on its color, odour look. Each of these physiological markers was noted and its value was compared to existing research
- **Melting Point:** The resulting sample's melting point was determined because it is a reliable indicator of purity. Using the capillary method, the melting points of both medications were found Table no
- **Solubility:**The medications were dissolved into a saturated solution at pH 1.2, 6.8, and 7.4. The vials were then shaken for 24 hours at 37^0 C in a mechanical shaker. The medium was examined using a UV visible spectrophotometer after being filtered for 24 hours using Whatman filter paper. Then, using a formula, the solubility of the medicines in mcg/ml in a particular medium was determined. Table no

  Dissolved amount (mg/ml) = [Absorbance x slope = intercept] x bath volume /1000

2.3.Analytical Characterization:

Construction of calibration Curve of Setaconazole nitrate:[27]
Sertaconazole's λ max was ascertained by measuring the medication solution's absorbance in an ultraviolet spectrophotometer against a blank wavelength of 240 nm, and then running the spectrum from 200 to 400 nm. **Graph 4.1**

Preparation of drug solution:
Preparation of Standard solution: After carefully measuring and transferring a 10 mg equivalent of oxiconazole nitrate to a 10 ml volumetric flask, The capacity was filled up with ethanol, bringing the final concentration to 1 mg/ml.

Sample solution [stocks solution]: The standard stock solution was pipetted at 0.2, 0.4, 0.6, 0.8, 1.0, 1.2, and 1.4 mL to create aliquots that were diluted with the same ethanol to obtain concentrations of 2, 4, 6, 8, 10, 12, and 14µg/mL**. Table 4.7**

Figure 2.1. Stocks solution of sertaconazole nitrate

2.4. FTIR Spectra of sertaconazole nitrate and other excipients:[29]There are two methods to operate with infrared radiation: either the radiation passes through the sample or it is absorbed by it. The molecular absorption and transmission of the sample are represented by the FT-IR measurements, which provide a fingerprint image of the material. Thus, it carries distinct spectra and offers the sample's blueprint for various samples. For the analysis of various samples, this characterisation is highly helpful [123]. KBr pellets are manufactured for the FTIR measurement, and the sample has been studied in the wave number range of 4000-400 cm-1. After the sample and KBr are combined, the pellet is created and ready for FTIR analysis. Because of its excellent transparency, The most typically used alkali halide in the pellets is potassium bromide (KBr). FT-IR analysis can be used for the identification of unknown substances, sample quality evaluation, and sample compatibility evaluation in a mixture**. Graph no.4.2**

2.5.FORMULATION DESIGN: Factorial design 2^3 DESIGNED BY DESIGN EXPERT 10.

Table 2.1 Formulation design

PC(mg)	ETHANOL(g)	Span-80(g)	q. s-Water:PG	Vesicle size	EE	ZP
500	4	1	2.5:2	178.3 ± 2.4	87.00%	-29.5 ± 0.6
500	2	1	2.5:2	172.6 ± 3	88.51%	-30.3 ± 1.5
1000	4	2	2.5:2	166.2 ± 4.6	82.96%	-32.5 ± 0.8

500	2	2	2.5:2	162.7 ± 5.3	90.01%	-37.5 ± 0.5
1000	2	1	2.5:2	**155.3 ± 1.2**	**90.74%**	**-42.2 ± O.3**
1000	4	1	2.5:2	178.6 ± 4.1	84.00%	-27.4 ± 0.5
1000	2	2	2.5:2	182.8 ± 2.7	82.00%	-31.3 ± 0.4
500	4	2	2.5:2	189.3 ± 5.3	87.00%	-38.5 ± 0.7

2.6.Ethamome preparation technique: : followed by hot method method

Hot Method:[30]

In collage labs, we make use of the hot approach when facilities are available. A fixed medication concentration of 0.05% w/w was used. The concentrations of ethanol and soy lecithin (phospholipid) were 20–40% w/w and 1–4% w/w, respectively. A precisely measured amount of medication was dissolved in ethanol and then mixed with propylene glycol. In a another beaker, the soy lecithin was dissolved in water at 40 C. After that, the medication solution was gradually added to a soy lecithin water dispersion in a closed vessel that was being agitated for 30 minutes at 40 C and 1,700 rpm. The last preparation underwent an hour-long, 30-minute cycle of ultrasonication. The ethosomal vesicle was produced and distributed equally.

1. Phospholipid are dissolved in water at 40 degree Celsius on water bath in round bottle vessel. We obtain a colloidal solution.
2. In a another jar, ethanol and propylene glycol are combined at 40 degrees Celsius.
3. Add organic phase into aqueous phase
4. Add drug in a mixture
5. Stirring at 1100 rpm
6. Sonicator at 30 min
7. Stored in cold temp. 4-degree cels.

3. Characterization of ethosomes:

3.1An assessment of the polydispersity, zeta potential, and particle size of the ethosomal formulation Indux[PDI]:[33]

Using dynamic light scattering (DLS), the size of the particles was estimated. The obtained ethosomal formulations were tested for their polydispersity index, and their particle sizes varied from 155.3 ± 1.2 to 196.4 ± 2.4 nm. The magnitude of charge at the boundary between the dispersion medium and the nanoparticle is known as the zeta potential. The greater the zeta potential, the more stable the dispersion is physically. An indicator of the homogeneity or heterogeneity of particle size is the PDI value. Before each ethosomal formulation sample was assessed by Zetasizer at 25 °C, it was diluted to a concentration of 1%. A 90° angle was used for the measurement, which was made using the dynamic light scattering method.

3.2Efficiency of drug entrapment:[34]

Efficiency of drug entrapment was computed using the. method of ultracentrifugation Making use of centrist tubes, the formulation was ultracentrifuged for one hour at 5000 rpm and 4 degrees Celsius (Remi Electrotechnics Limited). After centrifugation, the liquid supernatant was diluted with ethanol. Create a solution using stocks. With the use of UV visible spectroscopy operating at 240 nm, the amount of TH included in the formulation was determined. The provided equation was used to determine the drug entrapment percentage. Table no.

$$\text{Entrapment efficiency [\%]} = \frac{\{\text{Entrapment drug- Supernatant Drug}\}}{\text{Entrapment drug}} \times 100$$

Slope intercept from y= mx + c

Slope{m} = 0.012

Intercept{c} = 0.0074

Concentration{x}=? {Supernatant Drug}

Absorption = Table no…

0.038=0.012x+0.0074

X= 2.55mg {0.025 microgram]

$$\text{DEE\%} = \frac{0.27-0.025}{0.27} \times 100 = \text{DEE\%=90\%}$$

Table 2.2. - Absorbance Data of Entrapment efficiency

Formulation	Absorbance[nm]
F_1	0.050
F_2	0.045
F_3	0.063
F_4	0.040
F_5	**0.038**
F_6	0.058
F_7	0.061
F_8	0.048

3.3 Optimization of the best Formulation of ethosomal*:*

Further research was decided to pursue with the ethosomal formulation that proved to be the most successful. The ideal formulation was selected based on the highest EE%, smallest particle size, and maximum zeta potential. To create optimized equations with the best potential responses, the optimization procedure made use of Design Expert software version 10.

3.4 Formulation of gel: At small concentrations, carbopol 934 creates a translucent gel with very good uniformity. By dispersing 1g of carbopol934 in 90ml of warm purified water, to which 1 ml of glycerol had

already been added, 1% carbopol gel base was created. Additionally, a precise weighted quantity of propyl and methyl paraben was added. After swirling the liquid until it thickened, 50% [w/w] triethanolamine was added dropwise to neutralize the mixture and produce a translucent gel.

3.5 Incorporation of ethosomes in gel: For one hour, carbopol 934K, 1% w/v, was dissolved slowly and gradually in a tiny amount of distilled water while being continuously stirred with a magnetic stirrer. As a result, the polymer was given time to swell before being supplemented with 5 milliliters of ethosomal solution and 15 milliliters of Carbopol gel while being continuously stirred. In a sealed vessel, the mixture was continuously stirred at 700 rpm and 30°C, respectively, until a uniform texture resembling ethosomal gel was achieved. Additionally, a few drops of triethanol amine were added slowly while stirring to neutralize the pH and aid in the gel's development. Eight ethosomal formulations with varying concentrations were created using the aforementioned techniques, and they were then used for a number of evaluation criteria

3.6. Surface Morphology of sertaconazole nitrate loaded ethosome through Transmission Electron Microscopy[TEM]:[35]

With the use of TEM, the surface shape of the prepared, optimized F5 accumulation was represented in (Fig.). One drop of the ideal solution was placed to a copper grid that had been covered in collodion after it had been diluted with distilled water. The material was stained using a solution of uranyl acetate. and when the stain solution had dried at room temperature, a TEM image was taken. With a 10–100 k magnification power and an acceleration voltage of 100 kV, a TEM image was recorded by the camera. Images captured by TEM revealed that the ethosomes had a spherical form, were smooth, and lacked the drug's crystalline structure.
AFM pictures are shown in (Fig.). It is very difficult to extract detailed information from TEM images regarding the morphological characteristics, formulation behavior, and swelling dynamics that are provided by an AFM image. Furthermore, it provides justification for the outward morphology of the molecule with respect to its height, diameter, and area. The picture makes it quite evident that the diameter and height are within a reasonable range.

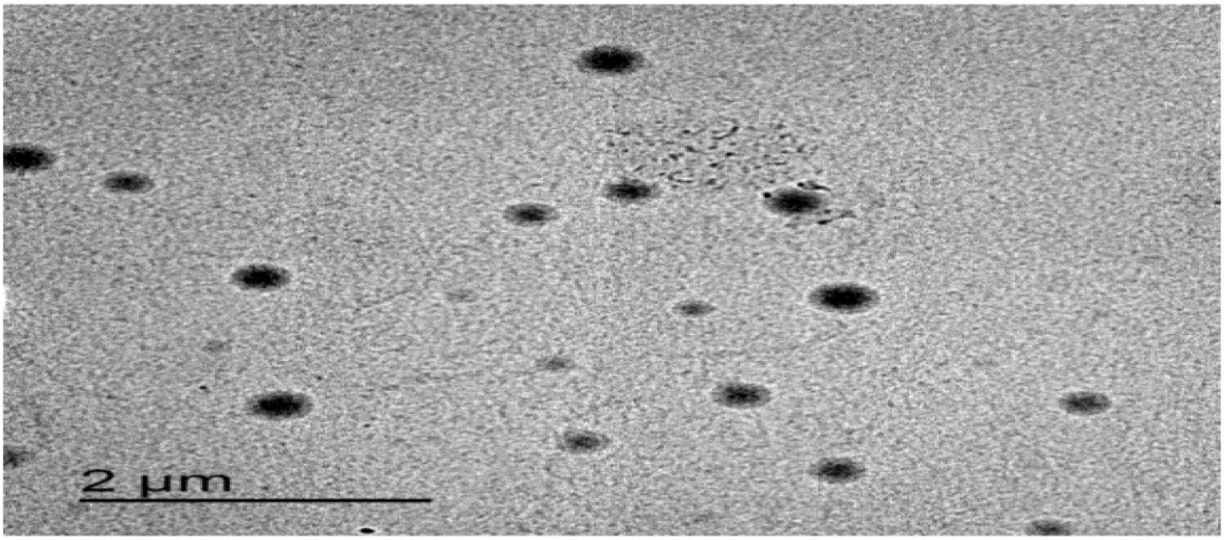


Figure 2.2-TEM image of ethosomes

3.7. Spreadability:[36]

After carefully weighing one gram of ethosomal gel, two 14-cm-long glass slides were positioned between them. The weight at which the glass slide moved and the amount of time required to draw the upper slide and push the gel to the lower slide were both noted. The pulley was fastened to a variety of weights. Following three measurements, the spreadability readings were computed using the formula below..

$$S=M^{*}L/T$$

S denotes spradability, M stands for mass, L for length, and T for time it takes for the top side to travel beneath the lower side.

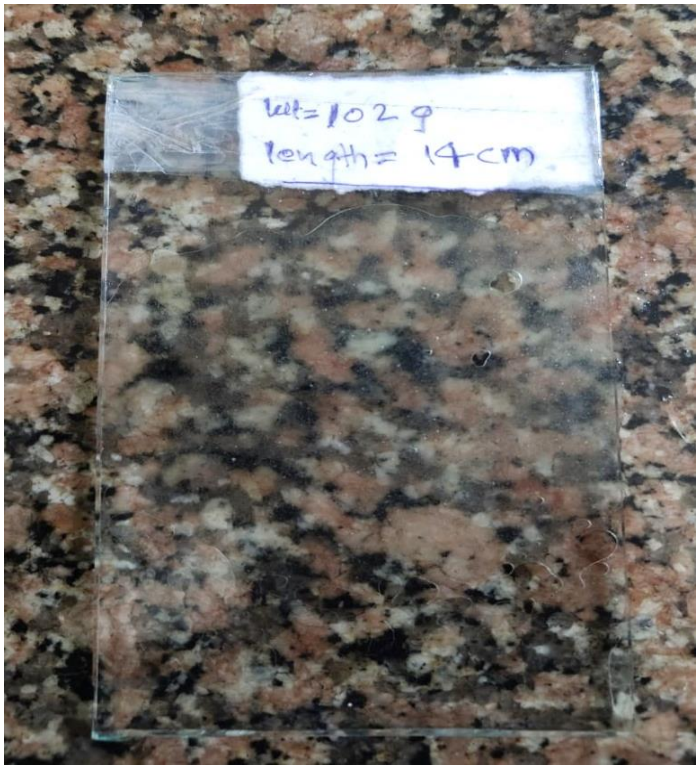


Figure 2.3 Glass slide

3.8. PH determination:[37]

Utilizing a digital pH meter, the composition was ascertained. After completely submerging the glass electrode in the ethosomal gel, the readings were recorded and shown

3.9. In vitro Antifungal activity:[28]

Anti-fungal activity was tested in order to gauge the improved formulation's effectiveness against the strains taken into account for the investigation. The study's results indicate that, when compared to the normal antibiotic formulation, the improved formulation (F5) was found to be more active and effective against the candidiasis strain. It is evident from Figs. and **Fig**. that the formulation F5 is more effective against species of candidiasis. And this was further demonstrated by the formulation's 2% w/w sertaconazole nitrate cream against the two strains. The optimal formulation of sertaconazole nitrate cream was found to be 15µg/ml and 20µg/ml against strains of candidiasis species, while the sertaconazole-loaded ethosomal gel was found to be 80µg/ml and 60µg/ml against strains of candidiasis. In summary, the study's findings clearly show that the optimized formulation F5, as opposed to the commercial formulation cream, is more effective against candidiasis. Because of this, commercial formulations containing 2% w/w sedrtaconazole nitrate cream are less efficient against the strain of candidiasis.

3.10. Stability study: [39]

A stability study was used to determine the ethosomal system's ability to adhere to the medication. For three days, the investigation was conducted constantly at various temperatures. The main problems with ethosomal formulation are drug agglomeration and drainage into/from the lipid bilayer. Stability testing was conducted on the optimized formulation F5, and the results are presented in Table. Two sets of Etosomal gel that had been lyophilized and ethosomal gel that had been sealed in a vial were kept at 4 °C/60 ± 5 RH (n =

3) and 25 °C/60 ± 5 RH when the research initially began. Following 7, 15, 30, 60, and 90 days of hoarding, sampling was done. Following lyophilization, the sample was redistributed using Millipore water and subjected to further tests, which included measurements of particle size, zeta potential, PDI, and percentage entrapment efficiency. The procedure was run three times in order to get exact findings, and the mean ± SD was

3.11. In vitro skin permeation study:[40]

For An egg membrane-equipped modified Franz diffusion cell was used for the in vitro permeation investigation. The saline phosphate buffer (pH 7.4) utilized in the study was. The formulation—which is comparable to 2.7 mg of medication—was applied to the donor section's top side of skin. The assembly's temperature was kept constant at 37±2º. Every ten minutes, samples were taken out of the receptor media using a sampling tube. The same volume of fresh receptor media was also provided simultaneously in order to establish a sink condition. In the extracted samples, the sertaconazole nitrate constant was determined using a UV/Vis spectrophotometer.

Table 2.3- Absorbance data of in vitro release of drug

Time (hour)	Absorbance
1	0.575
2	0.750
3	0.950
4	1.20
5	1.75
6	1.96
7	2.10
8	2.20

3.12. Viscosity:[41]

The produced ethosomal gel's viscosity was assessed using a Brook-field viscometer at 25°C and a spindle 96 in order to determine the formulation's viscosity. The ethosomal gel-containing beaker was dipped into the spindle 96 at varying intervals of 10, 15, and 20 rpm for lower, medium, and upper case.

3.13. Elasticity measurement:[42]

Using a 50 mm diameter stainless steel filter holder, the ethosomal formulation was extruded at a strain of 2.5 bar using a filter membrane with a porous diameter of 100 nm.In five minutes, the total of the vesicle suspension was computed **. [4.3]**

4. Result and discursion:

4.1. Organoleptic Characteristics of Sertaconazole: Sertaconazole nitrate were tested for organoleptic properties such as appearance, colour, odour, taste etc. Table 4.1

Table no.4.1 - Organoleptic Characteristics of Sertaconazole

DRUG	Properties	Observed Results
Sertaconazole nitrate	Appearance	Fine powder
	colour	White
	Odour	SlightOdour

4.2. Melting Point:

Using the capillary method, the melting points of both medications were found Table no.4.2

Table no. 4.2 - Melting Point of Sertaconazole nitrate

Drug	Observed value	Reported Value
Sertaconazole nitrate	156.0-161c	157.6-158c

4.3 Solubility of sertaconazole nitrate:

solubility of the medicines in mcg/ml in a particular medium was determined. Table no4.3, 4.4, 4.5

Table no.4.3- Solubility of sertaconazole nitrate in different solvent:

Solvent	Solubility
Water	Insoluble [Practically soluble]
Methanol	Soluble
6.8PBS	Insoluble
7.4 PBS	Soluble
Ethanol	Soluble[sparingly soluble 95 percent]

Table no.4.4 -Solubility study of sertaconazole nitrate in different oils:

Sr. No	Oils	Solubility
1	**Castor oil**	11.30
2	**Oleic acid**	13.00
3	**Almond oil**	29.01
4	**Paraffin in liquid form**	10.05
5	**Propyl myristate**	19.67

Table no.4.5 -Solubility study of sertaconazole in Surfactant and cosurfactant:

Sr.No	Surfactant	Solubility{mg/ml}
1	Tween 20	28.04
2	Span 20	3.06
3	Tween 80	37.29
4	Span 80	30.43
5	Propylene glycol	35.67

4.4. Micromeritic properties: The flow properties of drug and excipients are successfully determined in Table no 4.6

Table no. 4.6- Micromeritic properties Sertaconazole and Carbopol 940.

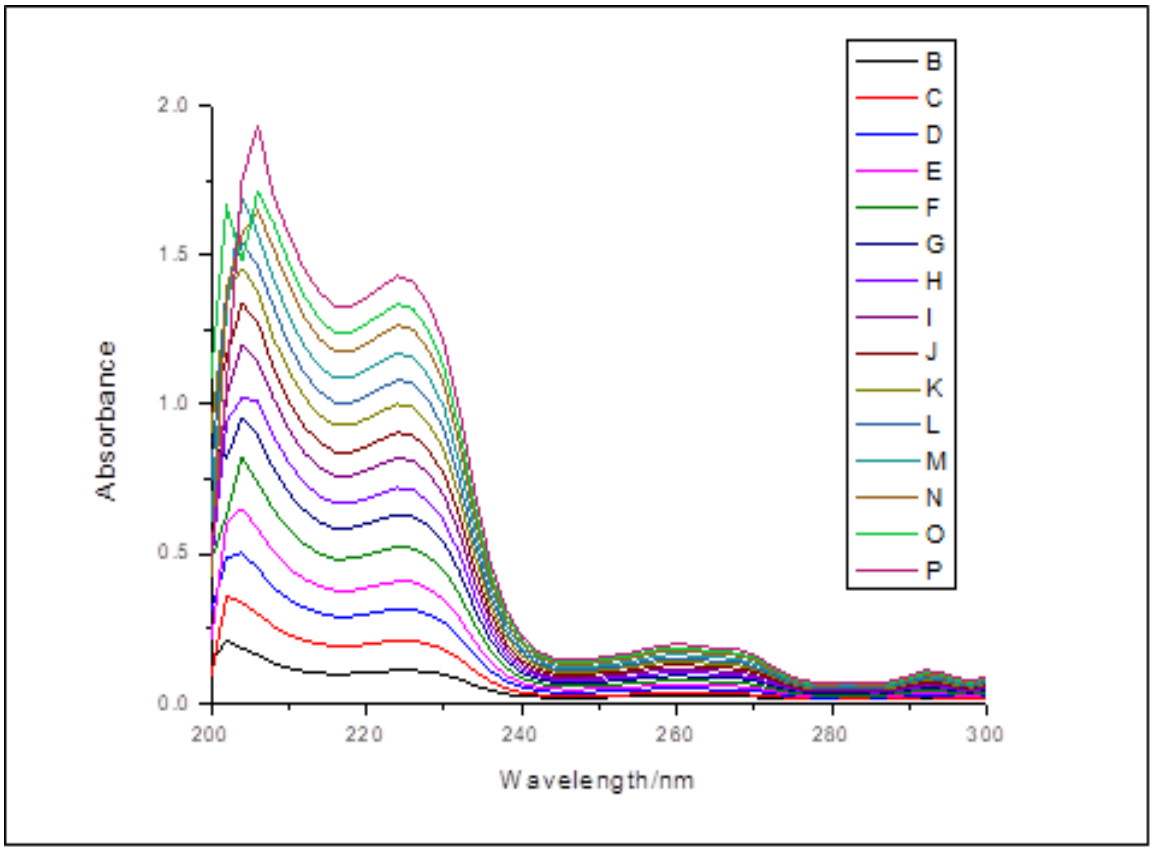
FLOW PROPERTIES	Bulk density	Tapped density	Carr's index	Hausner ratio	Angel of repose
Sertaconazole	15/35=0.428	15/24=0.625	31.52	1.460	0.56
Carbopol 940	0.28	0.318	.096	1.107	0.212

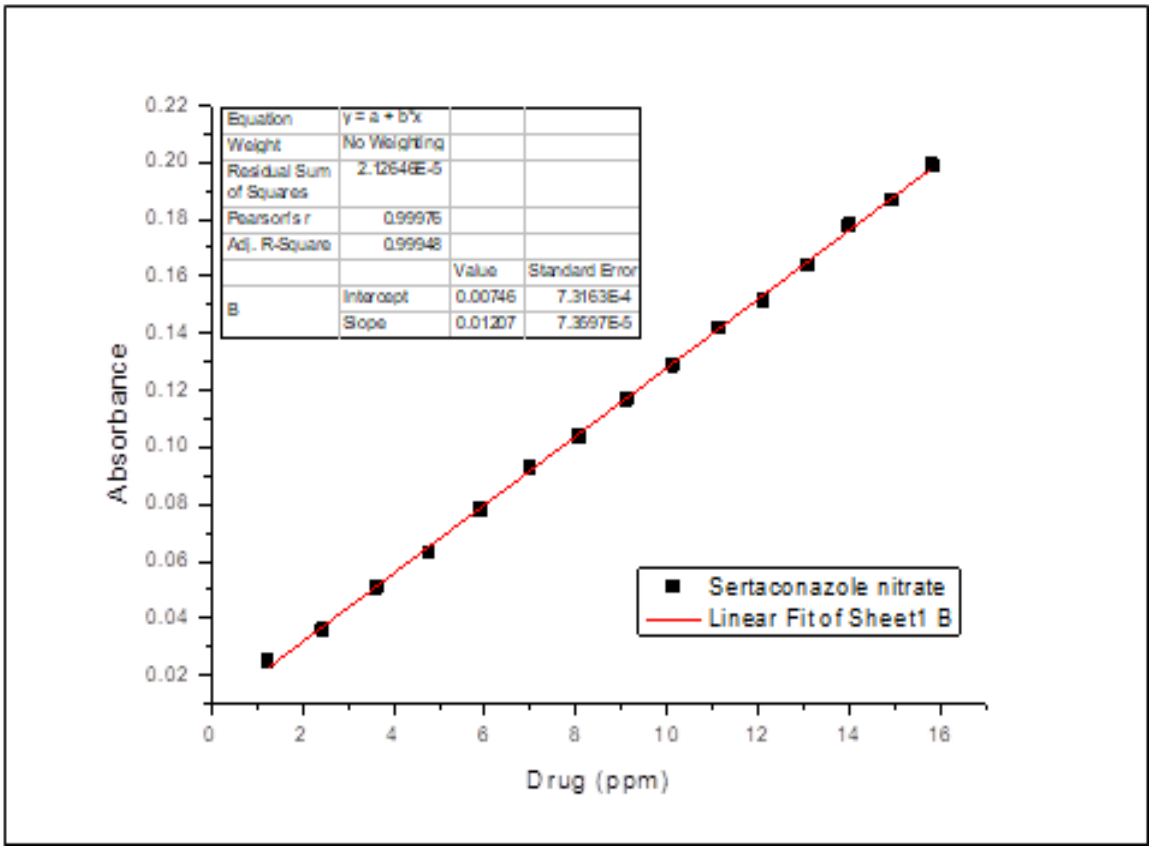
4.5. Calibration curve of sertaconazole nitrate:

Table no 4.7.Calibration curve of sertaconazole nitrate

Sr. No.	Volume	Total volume	Total solvent	Drug in ppm	Absorbance
1	0.1	0.1	0.8	1.234567	0.025
2	0.1	0.2	0.81	2.439024	0.036
3	0.1	0.3	0.82	3.614458	0.051
4	0.1	0.4	0.83	4.761905	0.063
5	0.1	0.5	0.84	5.882353	0.078
6	0.1	0.6	0.85	6.976744	0.093
7	0.1	0.7	0.86	8.045977	0.104
8	0.1	0.8	0.87	9.090909	0.117
9	0.1	0.9	0.88	10.11236	0.129
10	0.1	0.10	0.89	11.11111	0.142
11	0.1	0.11	0.9	12.08791	0.152
12	0.1	0.12	0.91	13.04348	0.164
13	0.1	0.13	0.92	13.97849	0.178
14	0.1	0.14	0.93	14.89362	0.187
15	0.1	0.15	0.94	15.78947	0.199

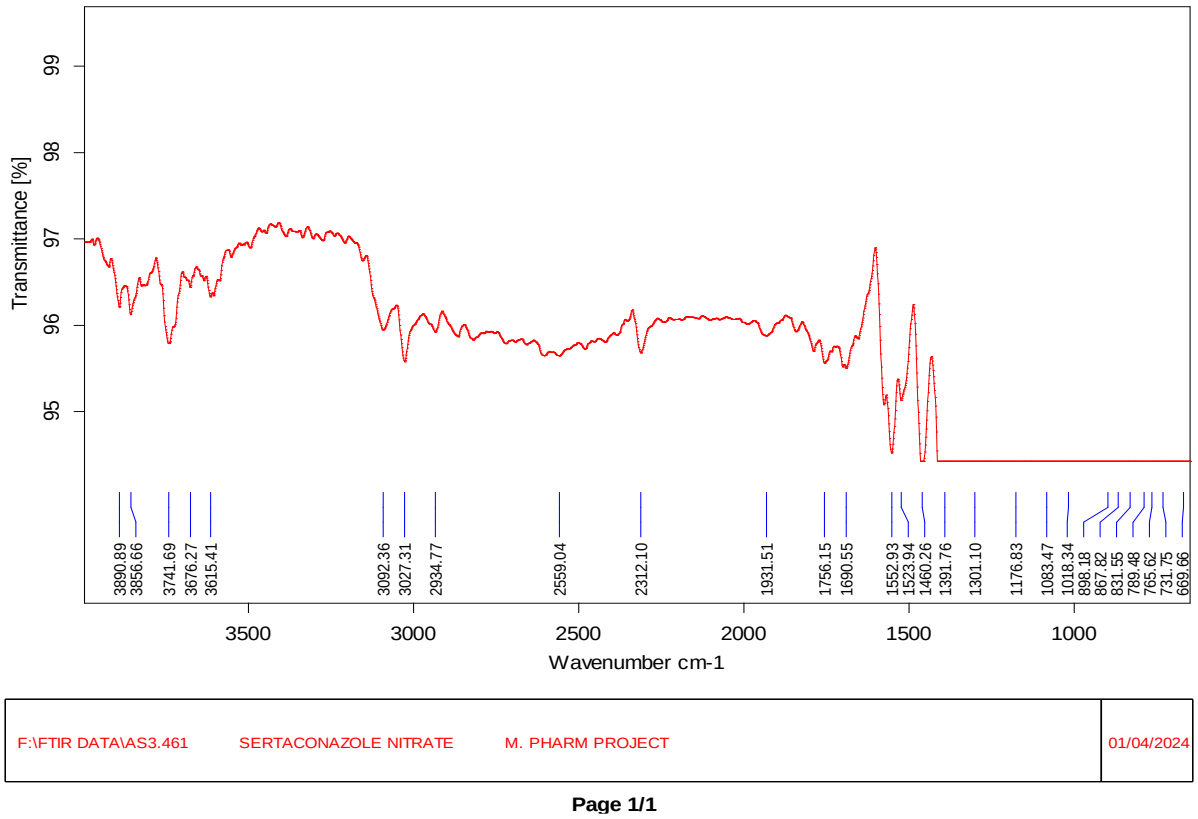
Graph no.4.1. Calibration curve of sertaconazole nitrate





4.6. FTIR Spectroscopy:

Figure4.2-FTIR Spectrum of pure drug sertaconazole nitrate



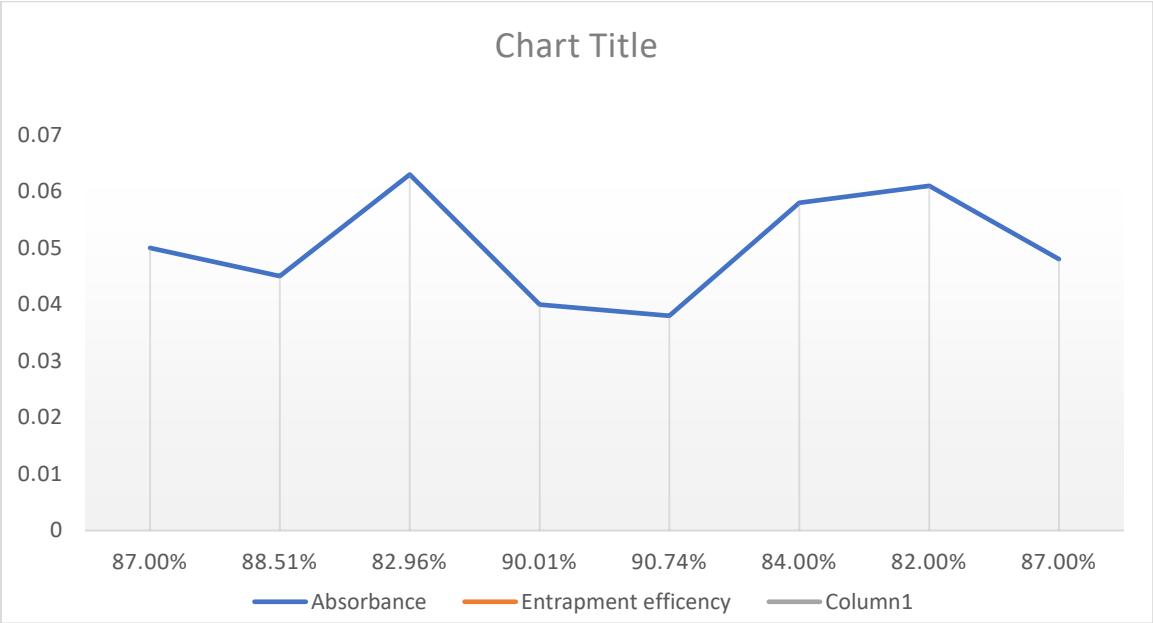
4.7. Measurement of the particle size, zeta potential, and polydispersityIndux[PDI] of ethosomal formulation: The optimized formulation F5 have smallest particle size(**155.3 ± 1.2**), and maximum zeta potential (**-42.2 ± O.3**), Show the table no.4.8

Table no.4.8 -Characterization of Particle size, polydespersity index, zeta potential

Formulation code	Particle size(nm)	PDI	Zeta potential
F_1	178.3 ± 2.4	0.468 ± 0.02	-29.5 ± 0.6
F_2	172.6 ± 3	0.379 ± 0.01	-30.3 ± 1.5
F_3	166.2 ± 4.6	0.470 ± 0.01	-32.5 ± 0.8
F_4	162.7 ± 5.3	0.400 ± 0.02	-37.5 ± 0.5
F_5	**155.3 ± 1.2**	0.426 ± 0.01	**-42.2 ± O.3**
F_6	178.6 ± 4.1	0.468 ± 0.03	-27.4 ± 0.5
F_7	182.8 ± 2.7	0.318 ± 0.02	-31.3 ± 0.4
F_8	189.3 ± 5.3	0.350± 0.02	-38.5 ± 0.7

4.8. Entrapment efficiency: Drug Entrapment efficiency: The ultracentrifugation method was used calculate the drug EE%. Optimized formulation F4 and F5 shoe the maximum drug Entrapment efficiency. Show Graph 4.3

Graph no.4.3. -Entrapment Efficiency (%)



4.9. Gel formation:

Ingredients	**Concentration**
Carbopol 934	1%
Glycerol	5%
Methyl Paraben	0.02%
Propyl Paraben	0.01%
purified water	Upto 100%
Triethanolamine	Few drops

Table no.4.10– Composition of gel forming agents

4.10. Spreadability:

A single gram of ethosomal gel was precisely weighed and sandwiched between two 14-cm-long glass slides. The pulley was attached to various weights, and the weight at which the glass slide moved was noted, along with the amount of time required to draw the upper slide and cause the gel to extend further to the lower slide. After taking the measurement three times, the spreadability average readings **222.54 gm.cm/sec** are found. Table no4.11

Table no. 4.11 -Spreadability Measurement

Reading	**Spread ability [gm.cm/sec]**
Reading 1	**204**
Reading 2	**238**
Reading 3	**225.63**

4.11. pH determination:

A digital pH meter was used to determine the composition. We take a three reading optimized F5 formulation. Average **reading 4.943** are calculated. Table 4.12

Table no. 4.12. -Ph Measurement

Reading of pH metter	**pH**
Reading 1	**4.94**
Reading 2	**4.93**
Reading 3	**4.96**

4.12. Anti-microbial study of sertaconazole

The study's results indicate that, when compared to the normal antibiotic formulation, the improved formulation (F5) was found to be more active and effective against the candidiasis strain compare to marketed formulation 2%w/w sertaconazole nitrate cream(Standard). Show table no.4.13

Table no.4.13 - Composition of nutrients ager powder:

Composition	**Concentration**
Agar	**15%**
Peptone	**5%**
Beef extract	**3%**
NaCl	**5%**
Water	**1000ml**
pH	**7.4+/-0.2**

Table no 4.14-Zone of inhibition

Fungi	The diameter of the Zone of Inhibition in mm		
	Control	Standard	Test-F_5
Candida albicans	0	6.4	11.4
Aspergillusniger	0	6.7	10.5

4.13. Stability study of optimized formulation ethosomal gel[F_5]

Table no.4.15- Ethosomal gel [F_5], At 4^0C/60± 5 RH [n=3]

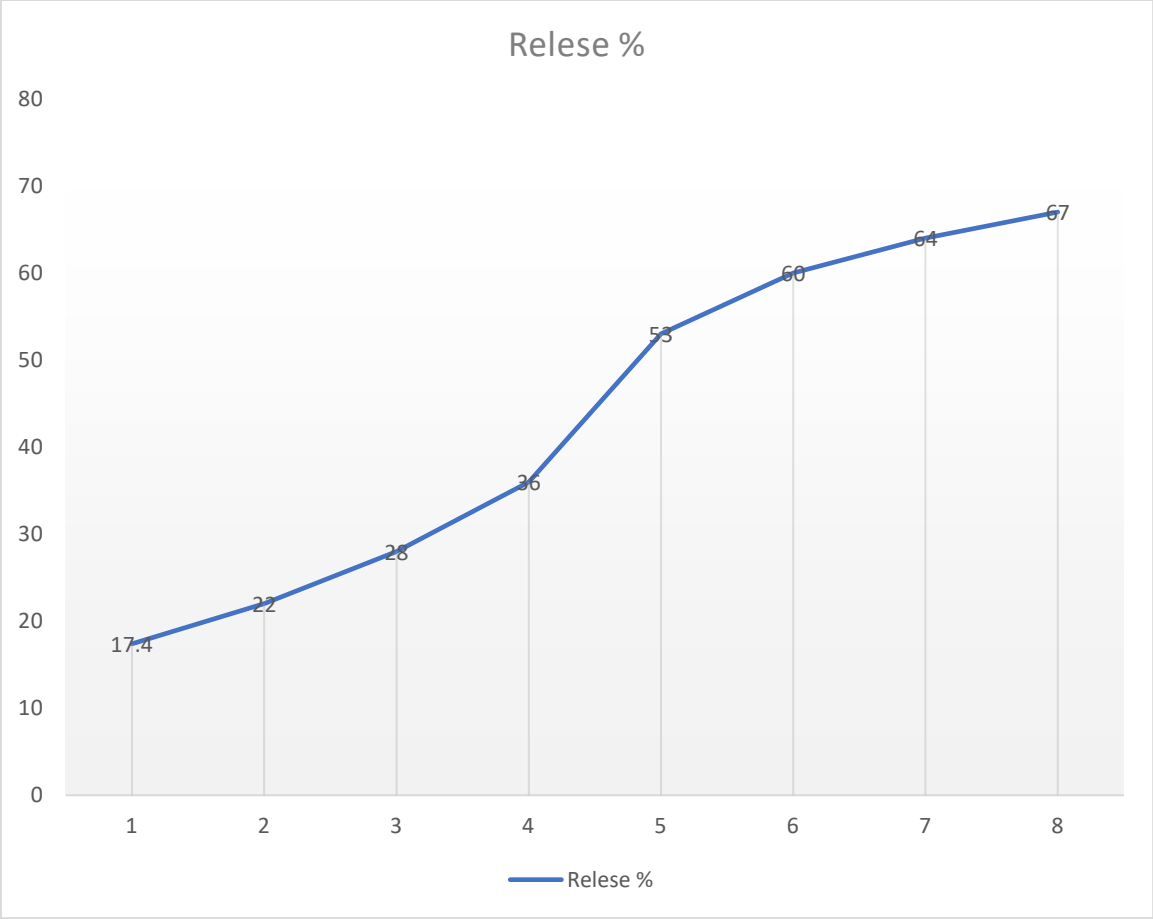
Time [days]	Microscopic evaluation	% Entrapment efficiency	pH
Initial	Smooth spherical vesicle	96 ± 2.9	5.02
7	Smooth spherical vesicle	94 ± 1.2	5.02
15	Smooth spherical vesicle	94 ± 1.0	5.05
30	Smooth spherical vesicle	90 ± 2.6	5.07
60	Smooth spherical vesicle	84 ± 1.7	5.10
90	Rough spherical vesicle	78 ± 2.1	5.20

Table no4.16 -Ethosomal gel [F_5] At 25 °C/60 ± 5 RH (n=3)

Time [days]	Microscopic evaluation	% Entrapment efficiency	pH
Initial	Smooth spherical vesicle	96 ± 1.0	5.02
7	Smooth spherical vesicle	92 ± 1.5	5.10
15	Smooth spherical vesicle	88 ± 2.0	5.20
30	Smooth spherical vesicle	81 ± 2.4	5.30
60	Rough spherical vesicle	67 ± 2.0	5.40
90	Agglomerate	61 ± 1.2	5.60

4.14. In- Vitro release

Graph no. 4.4 -In vitro drug release



4.15. Viscosity

To estimate the viscosity of the formulation, the manufactured ethosomal gel's viscosity was measured using a Brook-field viscometer at 25°C and a spindle 96. We take a three reading calculate **average 7006.66** are found. Table 4.18

Table no. 4.18 -Data of Viscosity its formulation

Reading	**Viscosity[cps]**
Lower case	**6828+ -0.5**
Middle case	**7064+ -1.3**
Higher case	**7128 ± 0.5**

4.16. Elasticity measurement

Table no.4.19- Elasticity measurement

Formulation	**Elasticity ± SD**
F_1	**84.72 ± 5.46**
F_2	**80.93 ± 7.32**

F_3	**86.07 ± 5.2**
F_4	**86.4 ± 4.67**
F_5	**89.9 ± 5.77**
F_6	**84.68 ± 7.44**
F_7	**84.33 ± 3.22**
F_8	**83.91 ± 3.21**

5. CONCLUSION:

Novel penetration enhancer, ethosomes were prepared successfully by Hot method for prolonged as well as controlled release of Sertaconazole nitrate across SC. The prepared formulation were characterized for various evolutionary parameters like vesicle size, entrapment efficiencies, vesicles elasticity, in-vitro drug deposition study and rate of transdermal flux across stratum corneum and prepared formulation were also characterized for in-vitro release studies by using Egg membrane (semipermeable membrane).

From in-vitro drug release studies, it is concluded that by changing the ratio of Propylene glycol and ethanol, Sertaconazole nitrate release can be controlled for a prolonged period of time by reducing possible side effects occurred during conventional therapy. Ethosomes of different vesicle size and drug content could be obtained by varying the ratio of PLand ethanol.

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