

# Formulation and Evaluation of Fluconazole Loaded Ethosomes for Topical Drug Delivery

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## ABSTRACT

In recent years, ethosomes have been employed extensively as a transdermal drug delivery system due to their improved bioavailability, modified release characteristics, and compatibility as compared to topical formulations that are currently accessible. Because of their enhanced bioavailability, altered release properties, and compatibility with currently available topical formulations, ethosomes have been widely used as a transdermal drug delivery system in recent years. In this study, the cold technique was used to prepare fluconazole-entrapped ethosomes for topical administration. Size, percentage entrapment, pH, viscosity, in vitro drug release study, and interaction study were assessed for the prepared ethosome formulation. The optimal formulation was selected based on its size, entrapment effectiveness, and release profile. The findings of measuring the viscosity and pH of the fluconazole ethosomes trapped in gel were satisfactory, confirming that ethosomes could be an effective carrier for fluconazole topical delivery.

**Keywords:** Fluconazole, Ethosomes, Fungal infection, Phospholipid

## INTRODUCTION

Inhibiting ergosterol synthesis, a crucial component of fungal cell membranes, results in membrane disruption and fungal cell death. Fluconazole is a broad-spectrum antifungal medication that belongs to the triazole class and is commonly used to treat superficial fungal infections, including dermatophytosis, tinea infections, and candidiasis. The fungal cytochrome P450-dependent enzyme lanosterol 14- $\alpha$ -demethylase, which is necessary for the synthesis of ergosterol, a key structural element of the fungal cell membrane, is selectively inhibited by fluconazole. Blocking this enzyme prevents lanosterol from being converted to ergosterol, which results in an excess of ergosterol and the buildup of toxic, aberrant sterols inside the fungal cell. The cell membrane thus becomes weak and porous, making it difficult to sustain regular processes like growth and permeability control. In the end, this disruption results in the organism's death and prevention of fungal cell reproduction. Crucially, fluconazole is a safe and efficient antifungal drug due to its excellent selectivity for fungal enzymes and negligible impact on human sterol production. Worldwide, the

prevalence of fungal infections of the skin, hair, and nails has increased. A fungal infection can spread quickly and seriously. Numerous fungal species, particularly *Candida albicans*, are the primary cause of fungal infections. These species thrive and spread on the skin's surface, causing symptoms such as skin thickening, reddening, and itching. Transdermal medication delivery systems can treat important problems like vaginal infections and skin infections like onychomycosis. Most often, topical semisolid dose forms are used to treat fungal infections on the skin in order to prevent the GIT irritations, either systemic or otherwise. Targeting the infection site, reducing systemic side effects, and achieving high patient compliance are just a few advantages of topical treatment for fungal infections. Various topical antifungal compounds have been used to treat a variety of skin infections, but they are unable to deeply penetrate the stratum corneum layer and instead remain there, making this system a complete failure. The skin serves as both a major target and a barrier for the delivery of topical drugs. An inventive method for the topical administration of fluconazole is the use of ethosomal solutions filled with the drug. Phospholipids, ethanol, and water make up

**Relevant conflicts of interest/financial disclosures:** The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.



ethersomes, which are vesicular transporters. These formulations are intended to improve the drug's ability to pass through the skin, delivering a precise and regulated release of the active ingredient right at the infection site.

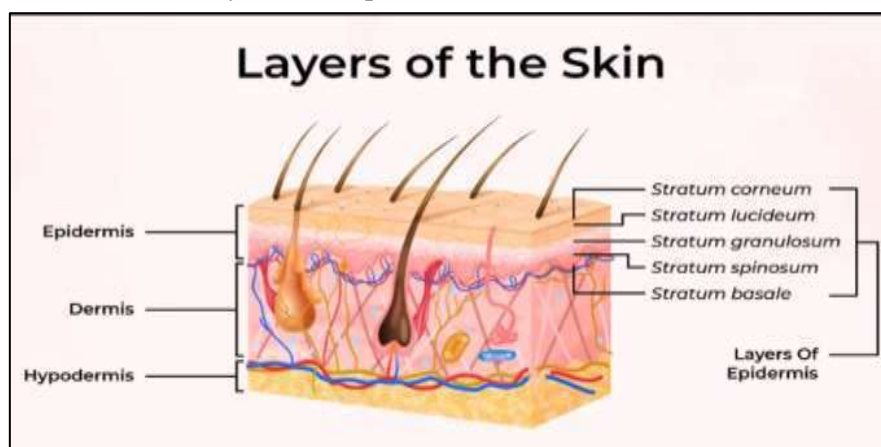
### Skin:

The primary components of skin are skin layers, which are made up of small wrinkles, hairs, and lipids on the skin's outermost surface. Additional components of skin include skin layers. The optical

behaviors of these components vary greatly depending on their structural makeup.

### Skin layers:

The most versatile and broad route for topical and systemic drug administration is the skin. When applied topically, the stratum corneum, the skin's outermost layer, limits the bioavailability of medications by acting as the skin's strongest barrier to drug penetration.



**Figure No. 01: Layer of skin**

### Epidermis:

The epidermis and dermis are the two distinct layers that make up the skin layer. The boundary between these two layers is clearly wavy, as seen in the image. The skin layer's thickness varies significantly according to a person's gender, age, body parts, etc. Additionally, skin conditions like water retention vary by location, age, and person. For example, because the NMF tends to decline with age, older people's skin layers have a reduced capacity to retain water. The stratum corneum, the outermost layer of the epidermis, has also been shown to become less transparent as its water content increases.

**Stratum spinosum [prinkle cell layer]:** refers to the ten to twenty layers that grow on top of the basal cells as a result of turnover, which flattens out their structure [multi-sided]. These cells, which are known as prickle cells, feature tiny spines on the membrane's outside. Usually, these sublayers range in thickness from 50 to 150  $\mu\text{m}$ .

**Stratum granulosum [granular cell layer]:** consists of two to four layers of granular cells. 3  $\mu\text{m}$  is the usual thickness. Cornification, also known as keratinization of keratinocytes, starts in this sublayer. Organelles like mitochondria and nuclei begin to resolve during this step. Compared to basal and prickle cell layers, cells have less moisture and are more densely packed with keratin fibers.

**Stratum lucidum [clear layer]:** is limited to the palms and soles. This sublayer has a high refractive index. During turnover, its cells became flatter and closer together.

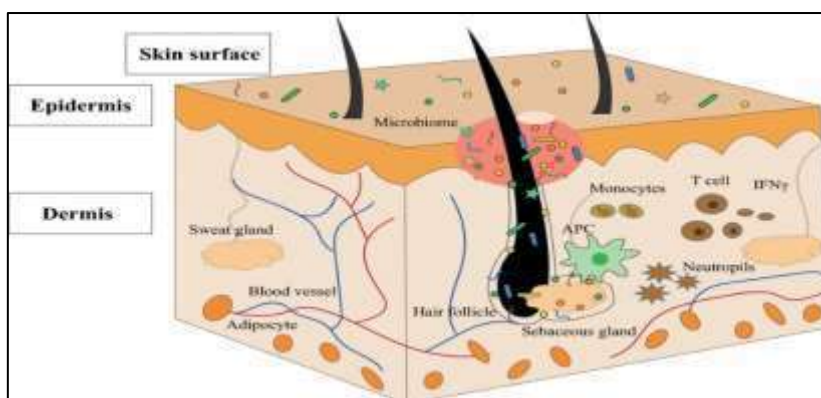
**Stratum corneum [horny cell layer]:** is the epidermis' outermost layer. It is between 8 and 15  $\mu\text{m}$  thick. This sublayer is made up of multiple layers of hard, flat, hexagon-shaped cells called corneocytes or horny cells. These are keratin-fiber-filled, dry, dead cells devoid of organelles.

### Fungal infection on skin:

When fungal spores come into contact with the skin's surface, the illness starts. Small abrasions, high

wetness, perspiration, or weakened skin barriers frequently make entry easier. Fungal growth thrives in places that stay warm and damp, like under tight garments, between the toes, and skin creases. When

the fungus gets to the skin, it sticks to the stratum corneum, the epidermis' outermost layer, which is rich in keratin, a protein that serves as dermatophytes' main source of nutrition.



**Figure No.02: Fungal infection on skin**

**Classification of Fungal Infections:** Mycoses, another name for fungal infections, are illnesses brought on by several types of fungi that infiltrate human tissues. The site, intensity, and pathogenic processes of these infections vary greatly. Fungal infections are categorized primarily according to the type of fungal agent involved, the mechanism of entry, and the extent of tissue involvement in order to better understand them. Systemic, cutaneous, subcutaneous, superficial, and opportunistic mycoses are the main categories.

**Superficial Mycoses:** The least invasive kind of fungal infection is superficial mycoses. They are limited to the stratum corneum, the outermost layer of the skin, and occasionally the hair shaft. The symptoms of these infections are typically moderate or entirely cosmetic since they rarely cause an immune response and do not infiltrate deeper tissues.

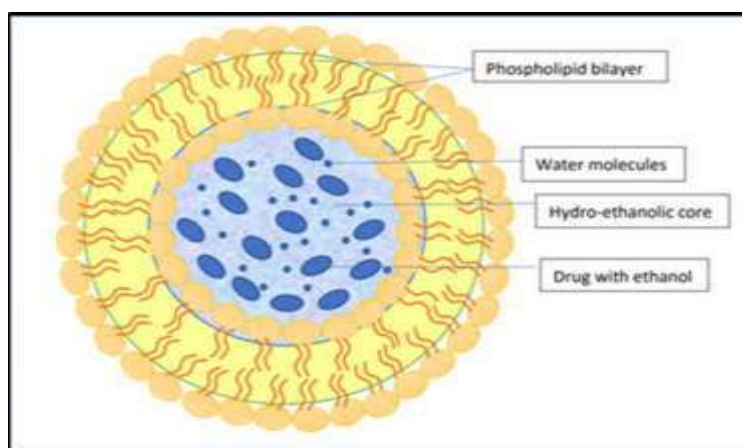
**Dermatophytosis:** Infection of keratinized tissues, including the skin, nails, and hair, is a feature of cutaneous mycoses. Trichophyton, Microsporum, and Epidermophyton are among the dermatophytes, a group of fungus that cause these illnesses.

**Subcutaneous Mycoses:** Chronic infections of the dermis, subcutaneous tissues, and muscles are known as subcutaneous mycoses. They typically happen when fungus spores penetrate the skin through wounds, thorns, or splinters.

**Systemic Mycoses:** After inhaling fungal spores, systemic mycoses affect deeper tissues, particularly the lungs, as well as internal organs. Serious, occasionally fatal problems can result from these infections, which can spread through the circulation.

**Ethosomes:**

Ethosomes are lipid-elastic vesicles that have a comparatively high alcohol content and phospholipids. Ethosomes are non-invasive delivery systems that deeply penetrate the epidermis. Ethosomes are pliable and flexible. Drugs with a high molecular weight and poor solubility are the primary candidates for ethersomes. Because of the combination of phospholipids and a high concentration of ethanol in vesicular formulations, ethosomes have a synergistic effect that allows for greater penetration and dispersion in the skin's lipid bilayer, which varies in size from nanometers to microns.



**Figure No.3: Structure of Ethosome**

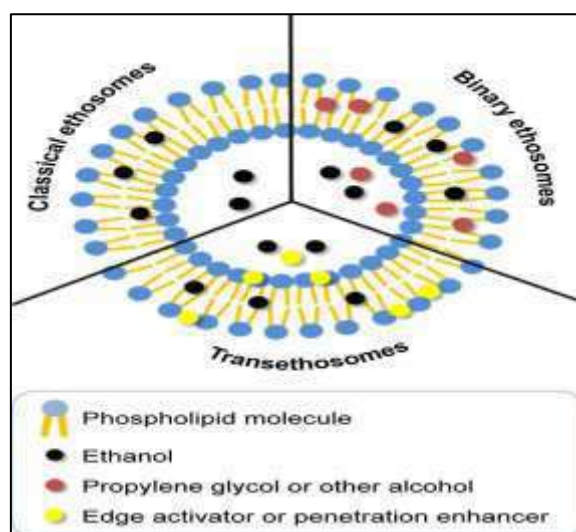
**Types of Ethosomal Systems:**

**1. Classical Ethosomes:** Phospholipids, water, and a high 40% ethanol content make up traditional ethosomes. It is possible for drugs with molecular weights between 130,077 Da and 24 kDa to become caught in typical ethosomes.

**2. Binary Ethosomes:** Another kind of alcohol can be added to the traditional ethosomes to create binary

ethosomes. The two most often utilized alcohols are isopropyl alcohol (IPA) and propylene glycol (PG).

**3. Transethosomes:** Along with a penetration enhancer (surfactant), it includes fundamental elements from classical ethosomes. created to create transethosomes by fusing the benefits of transferosomes and classical ethosomes. Because they combine the benefits of both standard ethosomes and transferosomes, transethosomes are a more sophisticated form of ethosome.



**Figure No. 04: Types of Ethosome**

**HOW do ethosomes work?** In ethosome function, vesicles, ethanol, and skin lipids work in concert. The dispersion of active substances is improved over liposomes by ethosomes and skin lipids because they interact more effectively. The transition temperature of the lipids in the stratum corneum is lowered when ethanol interacts with the lipid molecules in the polar head group region. These increase fluidity and

decrease lipid multilayer density, which allows the medicine to enter the deep layers of the skin.

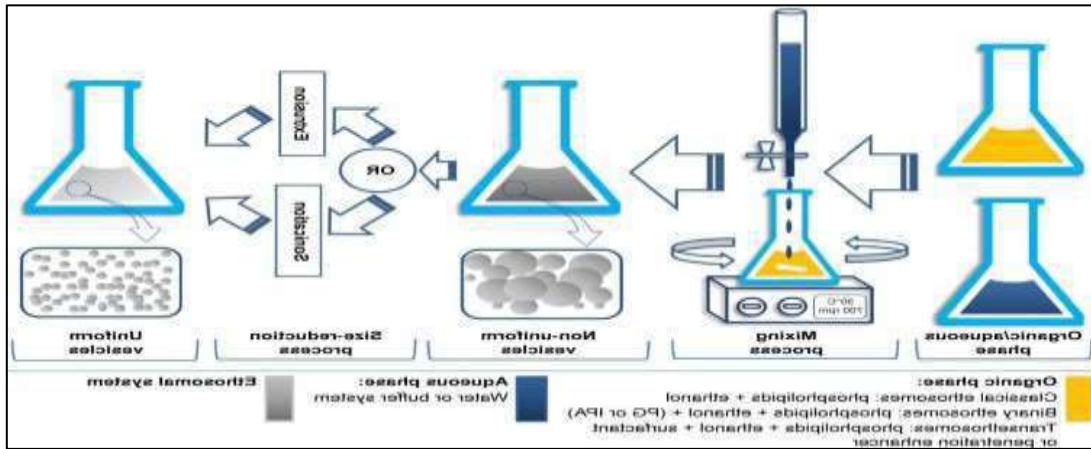
**METHOD:**

- Cold method
- Hot method
- Classic mechanical dispersion method
- Classic method

**Cold Method:**

PEG, ethanol, medication, cholesterol, and phospholipid were combined and heated to 40 °C. After five minutes of continuous stirring at 700 rpm,

distilled water was gradually added. The dispersion was allowed to cool at room temperature for half an hour. Lastly, a probe sonicator was used to sonicate it for five cycles (3 minutes each) at 4 °C.

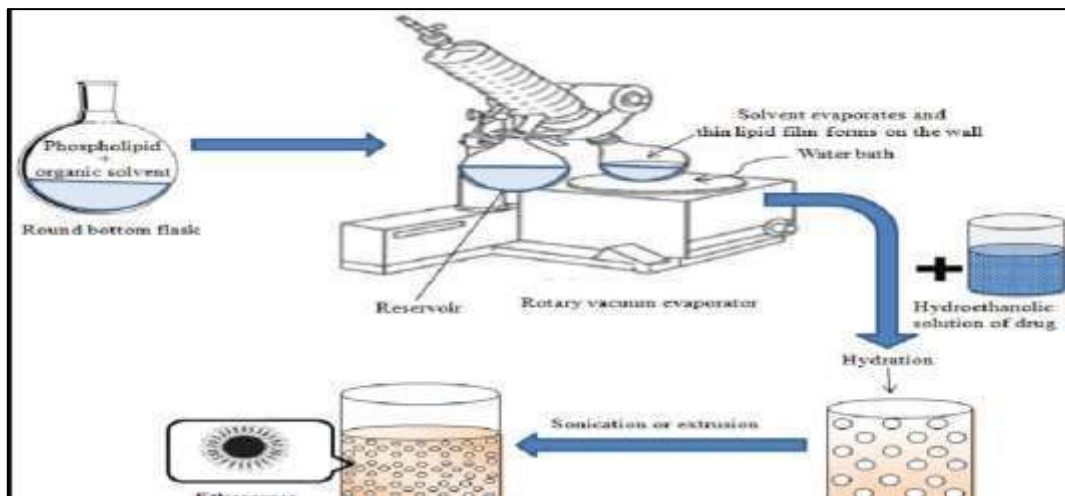


**Figure No. 5: Cold Method**

**Hot Method:**

To create a colloidal solution, phospholipids are dissolved in water and heated to 40 °C. Propylene glycol and ethanol are combined and heated to 40 °C

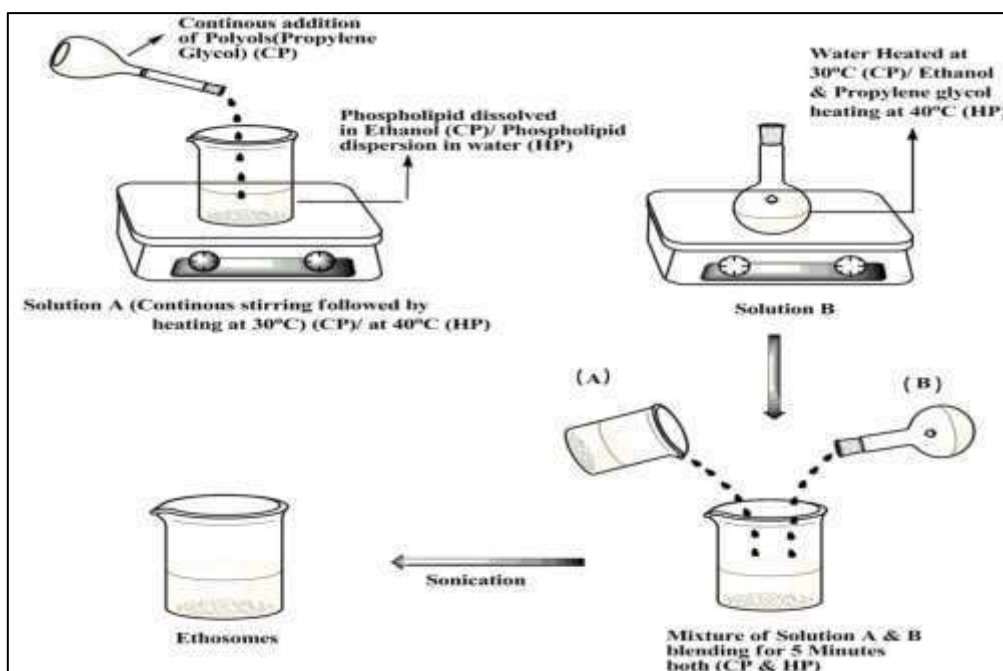
in a separate tank. The medicine is then dissolved according to its solubility after the organic phase is added to the aqueous phase at the same temperature. Finally, extrusion or probe sonication are used to minimize the size of the vesicle.



**Figure No.6: Hot Method**

**Classic mechanical dispersion method:** After being dissolved in ethanol, the medication and phospholipid are heated to 30 °C. After that, distilled water is gradually added to this combination while being

constantly stirred at 700 rpm. A closed vessel is used to collect the vesicle suspension that has developed. Lastly, a hand extruder is used to homogenize the dispersion by running it through a polycarbonate membrane three times.

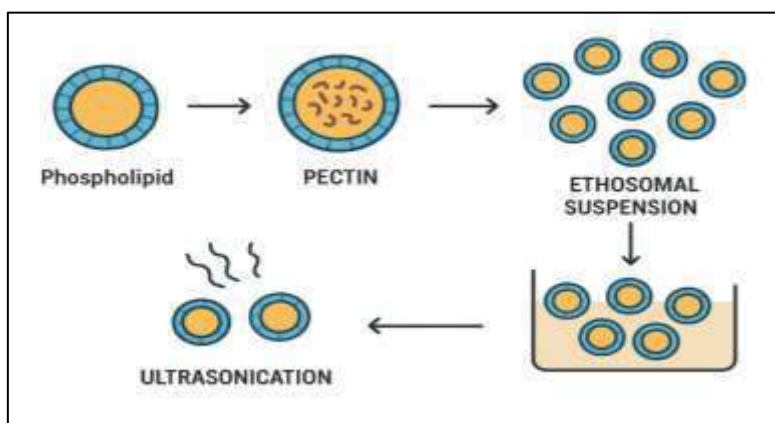


**Figure No.7: Classic mechanical dispersion method**

**Classic Method:**

In a round-bottom flask, soy phosphatidylcholine is dissolved in a 3:1 chloroform: methanol combination. To create a thin lipid layer, the solvents are removed using a rotating vacuum evaporator above the lipid

transition temperature. The flask is kept under vacuum for the entire night in order to eliminate any remaining solvent residues. The flask is then rotated at the proper temperature to hydrate the dry lipid film with a hydroethanolic drug solution.



**Figure No.8: Classic Method**

**MATERIAL AND METHOD**

**Material:** Span 80, Polyethylene Glycol, Fluconazole, Soya lecithin, Ethanol.

**Method:**

**Cold Method**

1. Weigh the required amount of fluconazole and phospholipid.

2. Dissolved both in ethanol under magnetic stirring at 30-40 degree Celsius.
3. Add span 80 and polyethylene glycol.
4. Heat the required amount of distilled water to the same temperature (30-40 degree Celsius).
5. Slowly add the aqueous phase into the ethanolic phase with continuous stirring to form ethosomes.
6. Stir the resulting dispersion for 30-40 minutes. Preparation was left to cool at room temperature for 30 min.

7. Then it was magnetic stirring 4 degree celcius for five cycles at 3 min, each with a min. rest between cycles using a magnetic stirrer.

### Formula For Ethosomes Formulation

Table no. 1

Sr. No	Ingredient	F1	F2
1	Fluconazole	50mg	50mg
2	Soya lecithin(Phospholipid)	250mg	250mg
3	Ethanol	20ml	25ml
4	Span 80	100mg	100mg
5	Polyethylene Glycol	10mg	10mg
6	Distilled Water	10ml	5ml

### Evaluation Parameter of Ethosome Suspension

#### 1. Particle shape analysis

The particle shape analyzed by using optical microscope.

#### 2. pH-

The pH of the formulation was determined by using digital pH meter. The measurement of pH of the formulation was done in triplicate and average values are calculated.

#### 3. Particle size & Zeta potential –

Particle size shows the average diameter of the ethosome vesicle. Zeta potential represents the surface charge of the vesicles, which helps to predict their stability. These parameters were measured using a Zetasizer instrument.

**4. Entrapment efficiency - EE** is defined as the ratio of drug molecules encapsulated into the ethosomal nanoparticles to the total used drug, and can be determined by the following equation.

$EE = \left( \frac{\text{Amount of trapped drug}}{\text{Total amount of initially added-drug}} \right) \times 100\%$  after preparing ethosomal dispersion untrapped drug is separated by dialysis, gel filtration and centrifugation.

#### Centrifugation

Centrifuge the sample at high speed (e.g., 15,000-20,000 rpm) for 60 minutes at 4°C. This separates the free (untrapped) drug in the supernatant from the entrapped drug in the ethosomal pellet.

#### Separation:

Carefully decant or pipette out the supernatant without disturbing the pellet.

### RESULT & DISCUSSION

#### 1. Particle Shape analysis-

The ethosomes prepared using fluconazole drug was studied under microscope to observe the formation of ethosomal vesicles. The shape of the ethosomes was observed spherical.



Figure no.9 Particle shape

2. pH-

The standard range of the pH of the ethosomal solution was 5-7 and the pH of the ethosomal solution was found to be 6.27 which is ideal pH.

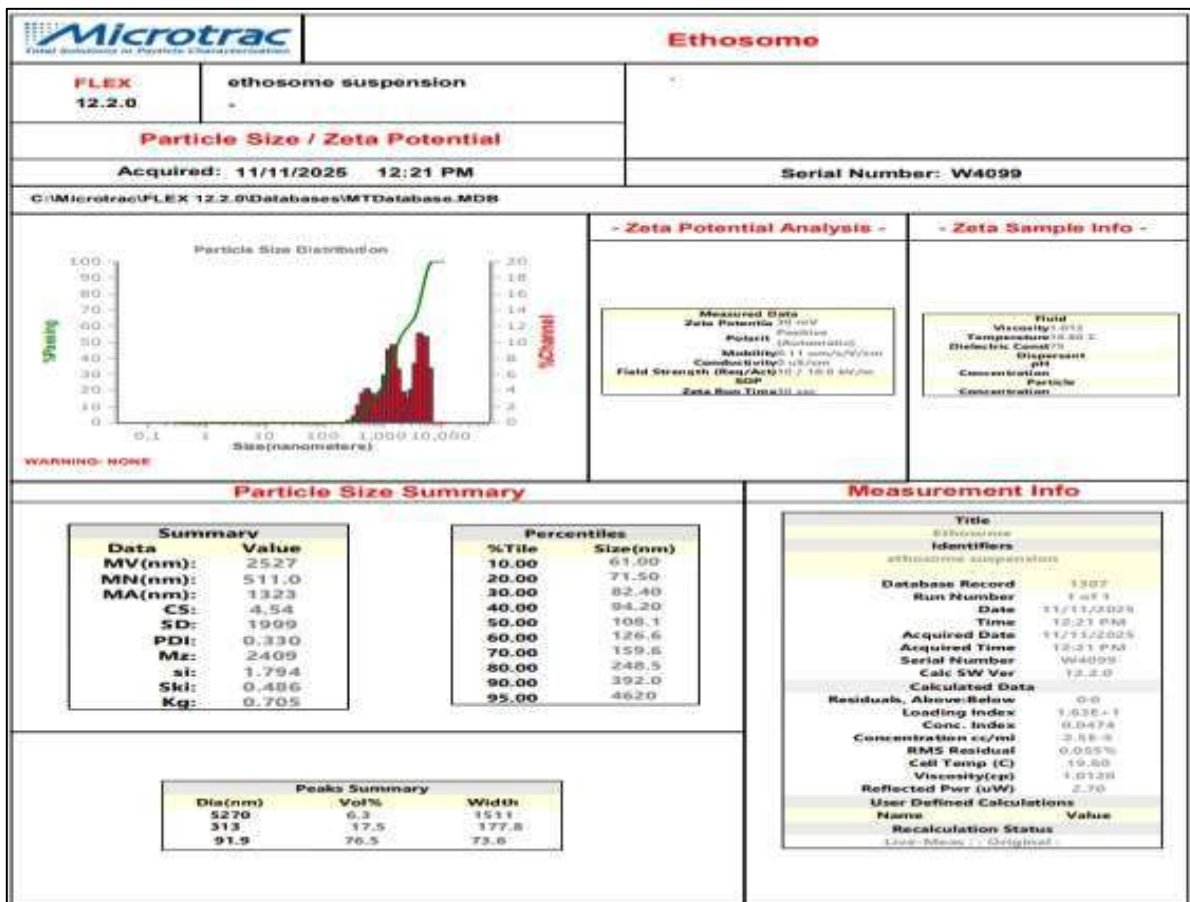
Sr. No	Solution	pH	Mean
1.	Ethosomal Suspension	6.20	6.27
2.		6.30	
3.		6.31	



Figure no .10 Determination of pH

3. Particle size & Zeta potential-The prepare fluconazole-loaded ethosomes show an average particle size of 313nm.The zeta potential was found to

be 30mV, confirming good stability of ethosomal suspension.



#### 4. Entrapment efficiency-

The entrapment efficiency of ethosome is of ethosome is 87.75 %.

#### CONCLUSION-

The study effectively made and assessed an ethosomal solution filled with fluconazole for topical medication administration. Traditional formulations for fungal infections frequently exhibit poor stability, reduced safety, and a limited therapeutic impact. Due to their difficulty passing through the epidermis, many topical antifungal medications are unable to deeply penetrate the skin's outermost protective layer, the stratum corneum. As a result, the medication loses its effectiveness and fails to adequately cure fungal infections. The ethosomal drug delivery technology can be used to address these issues. Because of their high ethanol concentration, ethosomes have greater flexibility and penetration capabilities than liposomes and transfersomes, which have trouble passing through the stratum corneum. This enhances the medication's therapeutic effect by enabling it to reach deeper skin layers.

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**HOW TO CITE:** Suvarna Sangale\*, Vaishnavi Dhage, Snehal Gondkar, Tanvir Pathan, Prerana Jamdhade, Gursal Kanchan, Formulation and Evaluation of Fluconazole Loaded Ethosomes for Topical Drug Delivery, *Int. J. Sci. R. Tech.*, 2026, 3 (3), 1-10. <https://doi.org/10.5281/zenodo.18851378>