Ethosomes as topical drug delivery system

[1]Shrutika Harpale, [2]Trusha Shangrapawar, [3]Dr. Ashok Bhosle

[1]Student, [2]Assistant Professor, [3]Principal
[1]Pharmaceutics,
[1]P.D.E.A.'s Shankarrao Ursal College of Pharmaceutical Sciences and Research Centre, Pune, India

[1]shrutikaharpale@gmail.com , [2]trusha.shan@gmail.com

Abstract—

Ethosomes are an advanced lipid-based vesicular system designed to enhance the delivery of drugs through the skin. Composed primarily of phospholipids, high concentrations of ethanol (20-45%), and water, ethosomes are known for their deformable and elastic nature, which allows them to penetrate the tough stratum corneum barrier more effectively than traditional liposomes. The ethanol present in ethosomes plays a dual role: it increases the fluidity of the vesicle membrane and disrupts the skin's lipid layers, thereby facilitating deeper penetration of both hydrophilic and lipophilic drugs into the systemic circulation. Ethosomal systems have proven to be effective in improving the bioavailability of drugs, sustaining drug release, reducing systemic side effects, and enhancing patient compliance. These vesicles are utilized for the transdermal delivery of a wide variety of therapeutic agents, including antimicrobial, antifungal, antiviral, anti-inflammatory, and neuroactive drugs, as well as large biomolecules such as peptides and proteins. Ethosomes offer several advantages over conventional transdermal delivery systems, such as non-invasiveness, ease of preparation, and the ability to improve drug permeation across the skin. Due to their promising characteristics, ethosomes hold significant potential in pharmaceutical, cosmetic, and biomedical applications, making them a valuable innovation in topical drug delivery technology.

Key words: Ethosomes, TDDS, Phospholipids, Ethanol, Skin Permeation, Vesicular System, Hydrophilic Drugs, Lipophilic Drugs, Non-invasive Delivery, Vesicle Stability, Targeted Drug Delivery.

INTRODUCTION

The skin is one of the largest organs in the human body with a surface area of roughly 2 m2. it is composed of multiple layers and plays a crucial role in blood circulation. The skin is a very complex organ since it contains a wide variety of cell types. Because of its thinness, the skin acts as a barrier against all microbial, bacterial, and human threats, shielding the underlying blood supply network from the outside world. It regulates blood pressure, acts as a thermostat to regulate body temperature, and shields the body from the damaging effects of uv radiation(1)

The three layers of the skin—the epidermis, dermis, and hypodermis each have unique physical features and purposes (2)show in fig 1.

Epidermis:

The epidermis is a outermost layer of skin, is normally between 50 and 100 mm thick. Keratinocytes make up the majority of this layer, while melanocytes and Langerhans cells are found in lower amounts (Figure1). The keratinocyte population in the epidermis is entirely replaced roughly every 30 days.

The integrity of the permeability barrier, which keeps the delicate balance between clinically normal and dry skin, greatly influences the metabolic activity of the epidermis, a dynamic system. This role is found in the stratum corneum, the outermost layer of the epidermis. A dynamic, metabolically active tissue, the stratum corneum is composed of roughly 60% structural proteins, 20% water, and 20% lipids. Its lipid composition—mainly cholesterol, ceramides, and free fatty acids—determines its integrity.
The stratum corneum is susceptible to disruption of barrier function due to its dynamic nature. When the moisture level drops below 10%, the stratum corneum loses its suppleness and starts to crack or fissure, indicating clinically dry skin. Dehydration may cause a decreased ability to prevent harmful microorganisms from entering.

Dermis:

The dermis, which is 2 to 3 mm thick and primarily made up of blood vessels and connective tissue, makes up the majority of the skin (Figure 1) and serves to support and connect the epidermis to the hypodermis. Elastin and collagen are found in dermal connective tissue; elastin fibres add elasticity and resilience, while collagen fibres make up the majority of the skin's volume and tensile strength. In addition, the dermis contains nerve fibres, sensory receptors, supporting glycosaminoglycans (GAG), and hyaluronic acid, which is responsible for the dermis's normal turgor due to its exceptional water-holding ability.

Hypodermis:

The hypodermis, a layer of loose connective tissue that connects the skin to internal organs, is located underneath the dermis. This layer connects the dermis to internal organs and provides stability, cushioning, and thermoregulation. It also contains subcutaneous fat and adipose tissue.(3)

One of the largest and most accessible organs in the human body is the skin. When used as a drug delivery system, the skin can provide a number of benefits over conventional methods, such as reduced variations in plasma drug levels, prevention of gastrointestinal issues and first-pass drug metabolism, and increased patient compliance. The skin's limited permeability, which restricts the number of medications that may be administered this way, is one of the biggest drawbacks to transdermal drug delivery. With the exception of lipophilic and low molecular weight medications, the stratum corneum is the most formidable barrier to the passage of most pharmaceuticals, making the skin an excellent barrier for molecular transport.(4)

Transdermal Drug Delivery System

In order to accomplish the goal of systemic treatment through topical application to the intact skin surface, transdermal drug delivery system have recently been developed [5]. Transdermal therapy systems are self-contained, discrete dose forms that release the medicine to the systemic circulation at a controlled rate through the skin when applied to unbroken skin [6]. There are several benefits to transdermal distribution, such as increased safety, better patient compliance, and increased efficacy. This method of drug delivery increases patient compliance while avoiding the risks and pain of parenteral therapy [7]. Given its convenience and safety, the transdermal route is an intriguing choice in this regard [8].

Because the transdermal drug delivery method faces the stratum corneum's barrier qualities, only lipophilic medicines with molecular weights less than 500 Da can pass through it [9]. Other therapeutic advantages of TDD include the ability to bypass the first pass metabolism effect for drugs with low oral bioavailability and sustained drug delivery that produces a steady state plasma profile and fewer systemic side effects, potentially improving patient compliance [10]. These days, vesicular and non-invasive drug delivery methods such liposomes, niosomes, transferosomes, and ethosomes are employed to improve drug penetration into the stratum corneum [11].

Ethosomes

Ethanolic vesicles are called ethosomes. Because ethanol is present in the vesicular structure, Touitou created a novel vesicular system that he called ethosomes. These days, the most researched method for transdermal drug delivery is the vesicular system [12].

Elastic nanovesicles made from phospholipids, containing a high concentration of ethanol (20-45%), are known as ethosomes. These are noninvasive drug delivery systems that enable medications to penetrate deep into the skin layers and reach the bloodstream. Ethosomes are soft, flexible vesicles specifically designed to deliver active ingredients more efficiently. Vesicles are widely recognized for their role in cell communication and transporting particles[13]. The high ethanol content makes ethosomes distinct, as ethanol disrupts the structure of the skin's lipid layers. This allows ethosomes to encapsulate drug molecules with a range of physical and chemical properties—whether they are water-soluble (hydrophilic), fat-soluble (lipophilic), or both (amphiphilic). Ethosomes can vary in size from a few nanometers to several micrometers, providing versatility in drug delivery[14].

Ethosomes consist of various phospholipid structures, water, and a high concentration of ethanol, which makes the vesicle membrane more flexible. The lipids in ethosomes are more fluid compared to those in liposomes with similar components but without ethanol. This makes ethanol a "blending" agent for lipid vesicles, giving them flexibility that allows them to distribute more effectively across different layers of the skin [13] Show in fig 2.

Type of Ethosomes

Ethosomes can be divided into several types based on their composition: classical ethosomes, binary ethosomes, transethosomes, composite phospholipid ethosomes, and active targeting ethosomes. Each type has unique characteristics and benefits, making them suitable for different applications [15].

1. **Classical Ethosomes**
   These are modified ethosomes composed of phospholipids, water, and a high concentration of ethanol—up to 45% w/v. Due to their small size and negative zeta potential, traditional ethosomes have shown better entrapment efficiency compared to classical liposomes. They are effective in encapsulating drugs with molecular weights ranging from 130.077 Da to 24 kDa. Additionally, traditional ethosomes demonstrate superior skin penetration and stability compared to conventional liposomes [9,13] Show in fig 3.

2. **Binary Ethosomes**
   Binary ethosomes are created by improving classical ethosomes, using a combination of propylene glycol and ethanol during preparation instead of just ethanol alone. This reduces the ethanol content and its volatility, which increases drug solubility, enhances formulation stability, and promotes better drug penetration [16,17]. Researchers have developed binary ethosomes to boost formulation stability and skin absorption. For terbinafine hydrochloride. Their findings showed that binary ethosomes with a 7:3 (w/w) ethanol to propylene glycol ratio were the most effective for enhancing drug penetration through the skin. Moreover, rhodamine B demonstrated deeper penetration and higher fluorescence intensity when delivered through binary ethosomes compared to standard ethosomes and transfersomes [18]. In another study, Akhtar and colleagues prepared gels using binary ethosomes with soy lecithin and a 1:1 binary mixture containing triamcinolone. These vesicles displayed greater zeta potential, entrapment efficiency, and improved penetration of rhodamine B compared to the reference ethosomal gel [15,19].

3. **Transethosomes**
   Transethosomes are an advanced version of ethosomal systems. They are made up of the core ingredients found in traditional ethosomes, with the addition of a penetration enhancer or an edge activator (like a surfactant). These innovative vesicles were designed to merge the benefits of classic ethosomes and transfersomes into a single formula, resulting in the creation of transethosomes [13,20].

4. **Composite Phospholipid Ethosomes (CE)**
   Compared to traditional ethosomes, CE (Composite Ethosomes) contains both saturated and unsaturated phospholipids, such as soybean lecithin, phosphatidylcholine (PC), and hydrogenated lecithin. This combination effectively prevents the oxidation of unsaturated phospholipids [21]. For instance, Chen and colleagues developed a curcumin-loaded CE to enhance the stability and skin absorption of standard ethosomes. The curcumin-loaded CE, made with a 1:1 ratio of PC to hydrogenated phosphatidylcholine (HPC), showed better vesicle stability and flexibility compared to regular ethosomes. The increased stability is due to the interaction between saturated and unsaturated phospholipids, which helps reduce the oxidation of unsaturated phospholipids [15,22].

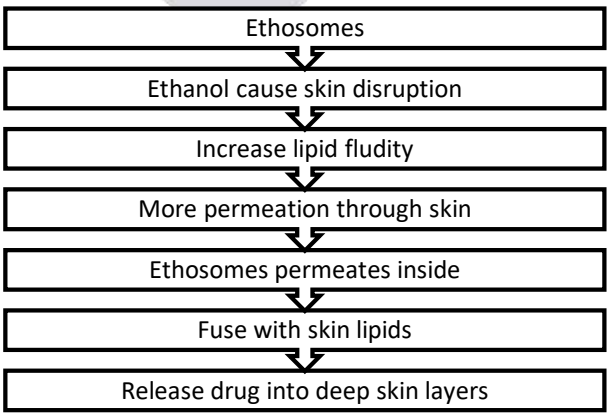
5. **Actively Targeted Ethosomes**
   Actively targeted ethosomes are created by attaching specific ligands, such as galactosylated chitosan, hyaluronic acid (HA), polyethyleneimine, and sodium cholate, to the surface of regular ethosomes [23]. Zhang and colleagues developed HA-modified ethosomes to improve their therapeutic effectiveness by specifically targeting the CD44 protein, which is known to be elevated in inflamed psoriatic skin [24]. In another study, Sagar and co-researchers formulated ethosomes carrying the hepatitis B surface antigen (HBsAg). Their results showed that these ethosomes had better internalization abilities and greater immunogenicity compared to elastic liposomes[15,25,26] Show in Table 1.

Mechanism of penetration

The main benefit of ethosomes compared to liposomes is their enhanced drug penetration. The exact mechanism by which ethosomes move into and through the skin remains unclear. Nevertheless, it is believed that drug absorption occurs in two distinct phases.[13]

1. **Ethanol Effect:**
   In the first approach, ethosomal formulations include ethanol, which interacts with lipid molecules in the polar head region between cells. This interaction increases fluidity and reduces the density of the lipid layers, leading to greater membrane permeability [28,29,30,31] Show in fig 4.

2. **Ethosomes Effect:**
   The ethanol in ethosomes increases the fluidity of cell membrane lipids, which enhances skin permeability. As a result, ethosomes can easily penetrate into the deeper layers of the skin, where they merge with skin lipids and release the drugs into the deeper layers [27,32,33].



Composition of Ethosomes

1. **Ethanol**
   An effective penetration booster is ethanol[37]. By providing the vesicles with unique dimensional features like as size, ζ-Potential, stability, clogging prevention, and improved skin permeability, it plays a significant role in ethosomal systems. Ethanol concentrations in ethosomal systems have been observed to range from around 10% to 50%[27,38].Numerous researches came to the conclusion that the ethosomes' size would decrease as the ethanol concentration rose. However, elevating the ethanol concentration over the ideal level would make the bilayer leaky, which would increase the size of the vesicles slightly and significantly reduce their ability to be trapped.

Additionally, increasing the ethanol concentration would cause the vesicles to become more soluble. One crucial factor that can influence vesicular characteristics like stability and skin vesicle contact is vesicular load. High ethanol concentrations shift the vesicular charge from positive to negative, as ethanol contributes a negative charge to the vesicle surface. This negative charge helps prevent vesicle aggregation due to electrostatic repulsion, thereby stabilizing the vesicles. Ethanol also enhances trapping efficiency, with increased concentrations generally improving this property, although excessive ethanol can reduce overall system efficacy[36,39,40,45].

2. **Phospholipids**

The ethosomal system was developed using phospholipids from several sources. When producing an ethosomal system, the type and concentration of phospholipids used in the formulation are crucial since they will impact the system's stability, penetration, ζ-Potential vesicular characteristics, size, and trapping efficiency. DPPG (1,2-dipalmitoylsn-glycero-3-phosphatidylglycerol) was added to the ethosomal formulation to create highly negatively charged vesicles, whereas a cationic lipid, like DOTAP (1,2dioleoyl-3-trimethylammonium-propane [chloride salt]), was used to create cationic ethosomal vesicles. Phospholipid concentrations in an ethosomal formulation typically vary from 0.5% to 5%. A slight or moderate increase in vesicular size may result from rising phospholipid content, but the effectiveness of trapping will be significantly enhanced. However, the link only lasts as long as there is a specific level of concentration[41,40].

The different types of phospholipids used in the preparation of ethosomal systems are summarized in Table 2[45].

3. **Cholesterol** As a stable steroid molecule, cholesterol improves the stability and clogging efficacy of medications when it is incorporated into ethosomal structures. This prevents leakage, lowers vesicular fusion, and permeability of the vesicles. Although it is typically employed at a concentration of 3%, in certain formulations it has been utilised up to 70% of the formulation's total phospholipid concentration. Numerous investigations have shown that cholesterol increased the ethosomal systems' vesicular size[39,42,43,45]**.**

4. **Dicetyl Phosphate**

Dicetyl phosphate is frequently used to increase formulation stability and prevent vesicle aggregation. In the ethosomal formulation, it is utilised at concentrations ranging from 8% to 20% of the total phospholipid concentration. Dicetyl phosphate's effect on other ethosomal system characteristics is still unknown [44,45].

5. **Stearylamine**

Stearylamine is an agent with a positive charge. Stearylamine significantly increased vesicular size and decreased entrapment when added to the ethosomal formulation. Stearylamine's reduced molecular weight (296.5 Da) allows it to easily enter the skin [45].

6. **Other Alcohols**

Binary ethosomes are created using ethanol as well as specific alcohols like PG and IPA. efficiency, and a shift from a negative to a positive ζ-potential charge, which causes the vesicles to aggregate in a week.[45]

1. **Propylene Glycol**

One popular penetration enhancer is PG. It has been discovered to affect the ethosomal characteristics of size, trapping capacity, permeation, and stability when utilised at concentrations between 5% and 20% in the creation of binary ethosomes. When PG is included into ethosomal systems, particle size will decrease more than in systems without PG. The particle size decreased significantly from 103.7±0.9 nm to 76.3±0.5 nm when the PG concentration was increased from 0% to 20%v/v.It is proposed that via improving the viscosity and antihydrolysis properties, PG improves ethosome stability.

2. **Isopropyl alcohol**

Dave et al. investigated how IPA affected an ethosomal system loaded with diclofenac's entrapment efficiency and skin penetration. Three different formulations have been made: a vesicular system with 40% IPA, binary ethosomes with roughly 20% IPA and 20% ethanol, and classical ethosomes with 40% ethanol. It was discovered that the vesicular device with 40% IPA had better trapping performance (95%) than the binary ethosomes (83.8%).

7. **Adge activators or Penetration Enhancers**

Since they significantly alter the characteristics of the ethosomal system, choosing the right edge activator or penetration enhancer is an essential step in the creation of transethosomes [35,45].

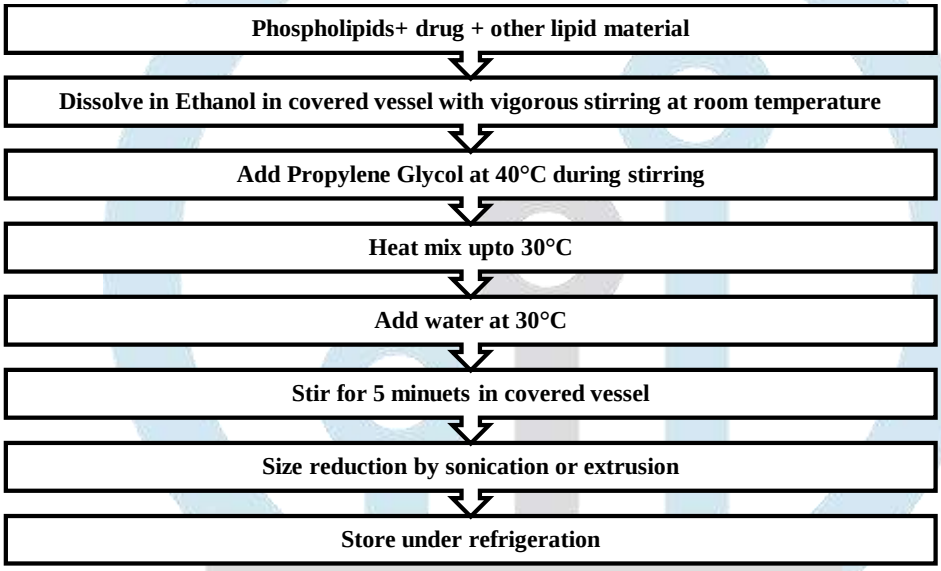
1. N-Decylmethyl sulfoxide and Dimethyl sulfoxide
2. Tweens and Spans
3. Oleic acid
4. L-Menthol
5. Sodium stearate
6. Bile acids and salts
7. Polyethylene glycol 4000
8. Hexadecyltrimethylammonium bromide
9. Cremophor
10. Skin-penetrating and cell-entering (SPACE) peptide
11. Sodium dodecyl sulfate

Different Additives Employed in Formulation of Ethosomes Show in Table 3.

Method of Preparation

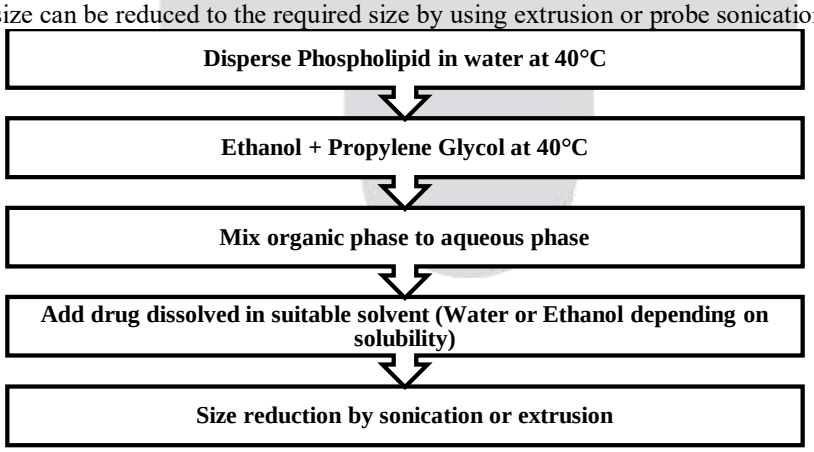
1. **Cold Method**

This is the most used method for creating ethosomal formulation. Using a mixer and vigorous agitation, phospholipids, medications, and other lipid molecules are dissolved in ethanol in a covered jar at room temperature. Another polyol, such as propylene glycol, is added while stirring. This mixture is cooked to 30°C in a water bath. The combination is stirred for five minutes in a covered jar after the water has been heated to 30°C in a different vessel. An ethosomal formulation's vesicle size can be decreased to the required degree by using the sonication or extrusion techniques. For maturation, the ethosomal solution was maintained at 4 °C for the entire night. Lastly, the mixture needs to be stored in a refrigerator [13,59].



2. **Hot Method**

Phospholipid is dissolved in water by heating it to 40°C in a water bath until a colloidal solution is formed. Propylene glycol and ethanol should be properly combined in a different vessel and heated to 40°C. Mix the aqueous and organic phases. Dissolve the medication in either ethanol or water, depending on its solubility. An ethosomal formulation's vesicle size can be reduced to the required size by using extrusion or probe sonication [13,56,57].



3. **Classic Mechanical Dispersion Method**

Soy phosphatidylcholine is dissolved in a 3:1 solution of methanol and chloroform in a round-bottom flask. A thin lipid coating forms on the flask wall as a result of the organic solvents being evaporated at temperatures higher than the lipid transition temperature using a rotating vacuum evaporator. Lastly, remnants of the solvent mixture are eliminated from the formed lipid coating by vacuuming the contents for an entire night. By utilising different amounts of a hydroethanolic mixture containing medication and spinning the flask at the right temperature, hydration is achieved [13,58].

4. **The ethanol injection-sonication method**

Using a 200-flow syringe system, the organic phase containing the phospholipid dissolved in ethanol is delivered into the aqueous phase at a rate of 38 µl per minute. An ultrasonic probe is then used to homogenise the mixture for five minutes [45].

5. **Classic Method**

In a water bath, the medication and phospholipid are dissolved in ethanol and heated to 30°C±1°C. In a closed vessel, the lipid mixture is continuously stirred at 700 rpm while a fine stream of double-distilled water is introduced. Using a

hand extruder for three cycles, the resultant vesicle suspension is homogenised by passing over a polycarbonate membrane [4].

Advantages

1. Ethosomes improve the way that medications penetrate the skin.
2. for intracellular, transdermal, and cutaneous administration.
3. Provide a range of molecules with distinct physicochemical characteristics, including proteins, peptides, hydrophilic and lipophilic compounds, and other macromolecules.
4. The ethosomes' constituents are permitted for use in pharmaceutical and cosmetic applications, are generally recognised as safe (GRAS), and are non-toxic.
5. Low risk profile: Since ethosome feature toxicity profiles are well-established in the scientific literature, there is no significant risk associated with ethosome structure in drug development.
6. The ethosomal system is appropriate for instant marketing because it is non-invasive and passive.
7. The pharmaceutical, biotechnology, veterinary, cosmetic, and nutraceutical industries can all benefit greatly from the use of ethosomal drug delivery systems.
8. High patient compliance: High patient compliance results from the semi-solid delivery of the ethosomal medication (gel or cream).
9. A straightforward approach to medication delivery as opposed to more complex techniques like sonophoresis and iontophoresis.
10. Industrial scale-up ease: Ethosomes production requires no complex technical investments and is comparatively easy to create. It is convenient to prepare multiliter volumes for ethosomal formulation.
11. Drugs can more effectively penetrate the skin thanks to ethosomes, which helps them get to the intended location in the skin or the blood.
12. It is evident that medicines have higher entrapment efficiency than liposomes.
13. It has outstanding stability over extended periods of time.
14. The ethosomes inherent preservative, alcohol, eliminates the need for additional preservatives.
15. Ethosomes are incredibly inexpensive to manufacture.
16. Drug distribution through the skin is independent of concentration[45,60,61].

Disadvantages

1. Precipitation can occur when ethosomes with poor shelling group together.
2. The drug needs to be soluble in both lipophilic and aqueous solutions in order to penetrate the cutaneous microcirculation and gain entry to the systemic circulation.
3. Skin irritation or dermatitis is brought on by excipients and enhancers in drug delivery systems.
4. Ethosomal administration is frequently intended to administer medication in a steady, sustained manner as opposed to a rapid bolus.
5. Only potent medications are permitted; those that require elevated blood levels cannot be administered.
6. When ethosomes are moved from the organic to the aqueous layer, product is lost.
7. For percutaneous absorption, the drug's molecular size should be appropriate.
8. The practical yield is low. It might not be economical [12,13].

Characterization of Ethosomes [32]

1. **Vesicle Shape**
   The ethosomal vesicles surface morphology is examined using scanning electron microscopy (SEM) and transmission electron microscopy (TEM). Mount the ethosomes onto double-sided tape that has been previously fastened to copper stubs and coated with platinum before doing an analysis at various magnifications.

2. **Vesicle size and Zeta potential**
   The two techniques utilised to evaluate the particle size and zeta potential of manufactured ethosomes are photon correlation spectroscopy (PCS) and dynamic light scattering (DLS) with a computerised inspection system.

3. **PH Measurement**
   Using a pH meter, the formulation's pH was measured by fully submerging the glass electrode into the colloidal suspension formulation to cover it [13].

4. **Transition Temperature**
   Vesicular lipids' transition temperature (T) is measured twice using DSC in an aluminium pan with a steady nitrogen stream and a heating rate of 10°C per minute.

5. **Entrapment Efficiency**
   The most popular method for determining an ethosome's entrapment efficiency is ultracentrifugation. In a high-speed cooling centrifuge, the vesicles are separated for 90 minutes at 20,000 rpm while being kept at 4°C. Determine the amount of drug in the sediment by lysing the vesicles with methanol and separating the sediment and supernatant liquids. Use the following formula to calculate the entrapment efficiency based on this: Efficiency of entrapment = DE /DT x 100
   where DE is the drug's amount in the ethosomal sediment.

DT stands for the theoretical amount of drug utilised to make the formulation, which is equal to the amount of drug in the sediment and the liquid supernatant.

6. **Skin Permeation Study**
The depth of penetration from ethosomes is ascertained using the confocal laser scanning microscopy (CLSM) approach. Ethosomes exhibit noticeably greater skin deposition, which may be the result of the combined action of phospholipid and ethanol, offering a delivery method for both dermal and transdermal application.

7. **Surface Tension Measurement**
It makes use of the Du Nouy ring tensiometer. To determine a drug's surface tension activity in an aqueous solution, the ring approach is utilised

8. **Vesicle Stability**
Ethosomal preparations' drug-retentive behaviour can be evaluated by storing them at various temperatures, such as room temperature (RT) at 25 ± 2°C, 37 ± 2°C, and 45 ± 2°C, over varying lengths of time (1, 20, 40, 60, 80, and 120 days). After being flushed with nitrogen, the ethosomal preparations were stored in sealed vials with a capacity of 10 ml. By employing DLS and TEM to track the vesicles' size and shape, the stability of ethosomes was also quantitatively assessed.

9. **Drug Content**
A modified high performance liquid chromatographic and UV Spectroscopy method can be used to quantify the drug.

Evaluation Test

1. **Filter Membrane-Vesicle Interaction Study by Scanning Electron Microscopy**
A filter membrane with a pore size of 50 nm was coated with vesicle suspension (0.2 mL) and put in diffusion cells. While the lower side of the filter was in touch with PBS (phosphate buffer saline solution), which has a pH of 6.5, the upper side was left open to the air. After an hour, the filters were taken out and fixed for the night at 4°C in Karnovsky's fixative before being dehydrated using graded ethanol solutions (30%, 50%, 70%, 90%, 95%, and 100% vol/vol in water) in order to prepare them for SEM investigations. After applying a gold coating, the filters were inspected using a SEM (Leica, Bensheim, Germany)[62].

2. **Vesicle-Skin Interaction Study by TEM and SEM**
Ultra-thin animal sections were cut using Ultracut (Vienna, Austria), gathered on grids covered with Formvar, and viewed under a transmission electron microscope. Following dehydration, the skin slices were adhered to stubs using adhesive tape and coated with gold palladium alloy using a fine coat ion sputter coater in preparation for SEM analysis. A scanning electron microscope was used to investigate the slices[12].

3. **Vesicle-Skin Interaction Study by Fluorescence Microscopy**
Fluorescence microscopy was performed in accordance with the TEM and SEM study methodology. A microtome (Erma optical works, Tokyo, Japan) was used to cut 5-µm thick sections of paraffin blocks, which were then inspected under a fluorescence microscope. micro Cytotoxicity Assay Dulbecco's modified Eagle media (HIMEDIA, Mumbai, India) was used to cultivate MT-2 cells, which are T-lymphoid cell lines.It is kept at 37°C with 5% CO2 and contains 10% foetal calf serum, 100 U/mL penicillin, 100 mg/mL streptomycin, and 2 mmol/L L-glutamine.The cytotoxic dosage 50 (CD50) that caused a 50% decrease in absorbance at 540 nm was used to express cytotoxicity[63].

4. **Skin Permeation Study**
Using a pair of scissors, the test animals' (rats') hair was meticulously cut short (less than 2 mm), and a knife was used to detach the abdomen skin from the underlying connective tissue. After the skin was removed, it was laid out on aluminium foil, and the dermal side was carefully scraped off to check for any remaining fat or subcutaneous tissue. Diffusion cell and receptor cell volumes had effective permeation areas of 1.0 cm2 and 10 mL, respectively. A constant temperature of 32°C ± 1°C was maintained. There was phosphate buffer saline solution (10 mL of pH 6.5) in the receptor compartment. 1.0 mL of the ethosomal formulation was applied to the skin's epidermis. At 1, 2, 4, 8, 12, 16, 20, and 24 hour intervals, samples (0.5 mL) were extracted via the diffusion cell's sampling port and subjected to high performance liquid chromatography analysis[64].

5. **Drug Uptake Study**
100 µL of RPMI media was applied to 24-well plates (Corning Inc.) to test the drug's absorption into MT-2 cells (1×106 cells/mL). Drug uptake was assessed by analysing the drug content using an HPLC assay after cells were treated with 100 µL of the drug solution in PBS (pH 7.4), ethosomal formulation, or marketed formulation.[43]

6. **HPLC Assay**
Using methanol, an HPLC assay was used to measure the amount of drug that penetrated the receptor compartment in both MT-2 cells and in vitro skin penetration experiments: The LC 10AT vp pump (Shimadzu, Kyoto, Japan) delivers a distilled-water:acetonitrile (70:20:10 vol/vol) combination as the mobile phase at a rate of 1 mL/min[12].

Factors Affecting Properties of Ethosomes[15]

1. **Effect of Ethanol**
   Because it gives the vesicles unique properties like size, zeta potential, stability, encapsulation efficiency (EE), and skin permeability, ethanol plays a crucial role in ethosomal systems. According to reports, ethosomal systems normally have an ethanol content of 20% to 45% (w/w). The size of the vesicles in these systems has been found to increase when the ethanol concentration falls [71]. Ethanol also negatively charges the vesicles to increase their stability and prevent static energy from building up within the vesicle system. In addition to making the medicine more soluble in the stratum corneum of the skin, a high ethanol concentration can facilitate the drug's penetration. Ethosomes have the ability to deform during transdermal penetration, go through cell gaps smaller than their own particle size, and enter the skin's deep layer. Because of this, ethosomes have a deeper penetration depth than liposomes, which greatly increases the effectiveness of drug transdermal penetration. Elevated ethanol concentrations may cause the vesicle membrane layer to thin, which would affect the EE.

2. **Effect of Phospholipids**
   In an ethosomal formulation, the concentration of phospholipids ranges from 0.5% to 5%. It might affect the ethosomes' EE and size. An increase in phospholipid concentration was accompanied by a minor or moderate increase in vesicle size, and a first increase and subsequent drop in ethosome EE. Additionally, the phospholipids are crucial to the transdermal penetration process. The medication could be released from the ethosomes and enter the skin in a free state if the phospholipids fuse with the lipids in the stratum corneum of the skin, disrupting its dense and orderly structure.

3. **Effect of Propylene Glycol**
   Propylene glycol was discovered to have an impact on the ethosomes' stability, EE, size, and skin penetration. The particle size of the vesicles may be further decreased by adding propylene glycol to the ethosomal systems. Propylene glycol may help improve the ethanol's skin irritation and volatility while also making the vesicles more stable. Ethosomes including ethanol and propylene glycol can also improve the drug's distribution within the vesicle and raise its EE.

4. **Effect of Cholesterol**
   By encouraging membrane fluidity, cholesterol has been shown to increase the ethosomes' EE while simultaneously increasing liposomes' capacity to penetrate the skin. Vesicles that contain too much cholesterol will be less able to encapsulate medications. Additionally, cholesterol can improve the ethosomal system's stability, which lowers particle size and vesicle aggregation.

5. **Effect of Adge Activator**
   Since it greatly affects the properties of the ethosomal system, the careful selection of an appropriate edge activator or penetration enhancer is an essential component in the formulation of ethosomes. Following its insertion into the phospholipid bilayer, the edge activator can lengthen the phospholipid molecules' distance from one another, disrupt the phospholipid phthalide chain's sequence, and make the ethosomes more fluid. The ethosomes distort and penetrate the stratum corneum when the skin is hydrated, which facilitates the drug's transdermal absorption. Prior research has demonstrated that adding Tween 80 to ethosomal formulations reduces vesicle size, improves system stability, and improves skin permeability properties.

Application of Ethosomes

1. **Delivery of Antibacterial Drug**
   One easy way to boost the therapeutic effectiveness of antibiotics is to apply them topically. Many immunological reactions, adverse consequences, and poor therapeutic efficacy have been associated with the widespread oral administration of antibiotics. Traditional topical antibiotic treatments are ineffective because they do not penetrate deeply into the skin or subdermal tissues. By supplying the deeper layers of the skin with the right amount of antibiotics, ethosomes can be beneficial. Ethosomes can readily pass through the epidermis, releasing a large number of medications into the deeper layers of the skin and preventing infection at its source. As a result, Godin and Touitou were able to create erythromycin and bacitracin with ethosomal genetic improvements for intracellular and cutaneous distribution. They gave an example of how to create an ethosomal-structured antibiotic formulation.

2. **Delivery of Antifungal Drug**
   Ethosomes have demonstrated remarkable potential as a vesicular carrier system to enhance transdermal penetration of ketoconazole. Ethosomes minimise side effects while offering the advantages of rapid onset and maximum drug release. Furthermore, medications are absorbed through the intact skin into the systemic circulation since ethosomes do not harm the structure of the skin. Ethosomal fluconazole gel formulation improves disease remission and shortens therapy time for individuals with candidiasis.

3. **Delivery of Anti-Parkinsonism Agent**
   Trihexyphenidyl hydrochloride (THP), a psychoactive ingredient, was prepared ethosomal by Dayan and Touitou, who then contrasted it with conventional liposomal formulations. Parkinson's disease is treated with THP, an antagonist of M1 muscarinic receptors. The findings showed that the ethosomal-THP formulation could assist manage Parkinson's disease and had a greater potential for skin penetration.

4. **Delivery of Anti-Arthritis Drug**
   The best option for targeted medicine distribution to the right location over an extended period of time is topical anti-arthritis medication administration. The findings show that it has much improved skin penetration and, consequently, action.

5. **Delivery of Antiviral Drug**
   Zidovudine and acyclovir are two potent antiviral medications. The second is frequently applied topically to treat Herpes labialis, whereas the first targets the human immunodeficiency virus. It has been demonstrated that ethosomes improve the transport of these two antiviral medications.

6. **In the Treatment of Herpetic Infection**
   The ethosomal preparation of 5% acyclovir shows a significant improvement in the treatment of herpes infections in comparison to 5% acyclovir cream. It also has an improved pharmacodynamic profile and greater skin permeability.

7. **Ethosomes are used in Pilosebaceous Targeting**
   Since the ethosomal vesicle contains a significant amount of ethanol, it can enter the skin deeply. Because it enhances dermal deposition, intercellular transport, and bioavailability, this vesicle seems to be a promising choice for transdermal drug delivery of hydrophilic and impermeable medicines across the skin. Minoxidil, a lipid-soluble medication used to treat baldness, can be used for pilosebaceous targeting for improved clinical efficacy because it accumulates two to seven times greater in the skin of naked mice.

8. **Transdermal Delivery of Hormones**
   Issues with oral hormone therapy include poor oral bioavailability, first-pass metabolism, and a range of dose-dependent side effects. To prevent this, transdermal administration is used. The likelihood of treatment failure rises with each missed dosage.

9. **Transcellular Delivery**
   Ethosomes seem to be a promising anti-HIV treatment option when compared to the commercial formulation. As a result, transdermal flow is enhanced, drug toxicity is decreased, and medicine activity is prolonged. According to Touitou et al., erythromycin, DNA, and bacitracin intracellular absorption in a variety of cell lines can be enhanced by CLSM and FACS techniques. Compared to the existing formulation of anti-HIV medication, ethosomes might present a more attractive clinical approach.

10. **Delivery of Problematic Drug Molecules**
    Transdermal distribution is a better choice because the gastrointestinal tract breaks down bigger biogenic substances like peptides or proteins, as well as insulin. However, the penetration of conventional transdermal formulations of insulin and biogenic agents such as proteins or peptides is poor. Transforming these substances into ethosomes significantly improves therapeutic efficacy and penetration. Show in Fig 5.

Conclusion

A novel area of vesicular research in transdermal drug delivery has emerged with ethosomes. These structures can be tailored to enhance the skin penetration of active compounds and are recognized for their simplicity of production, safety, and efficacy. Ethosomes have the unique ability to effectively bypass the epidermal barrier, a major challenge in transdermal drug delivery systems. They have been proven capable of encapsulating proteins, peptides, hydrophilic drugs, and cationic agents. Consequently, ethosomal formulations hold great promise for the efficient transdermal delivery of bioactive compounds.

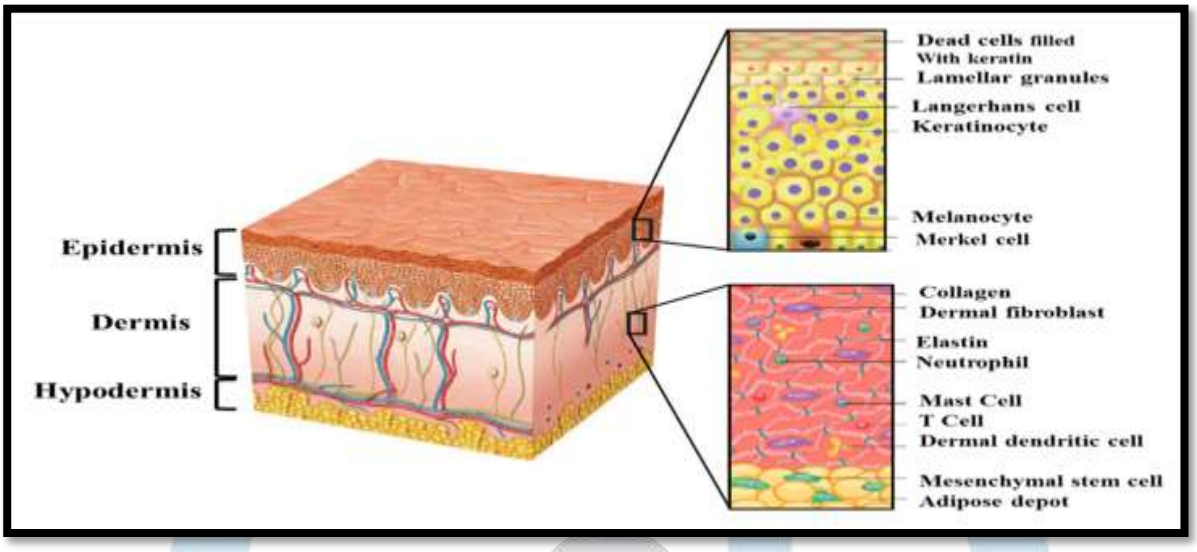


Figure 1 Structure of Skin

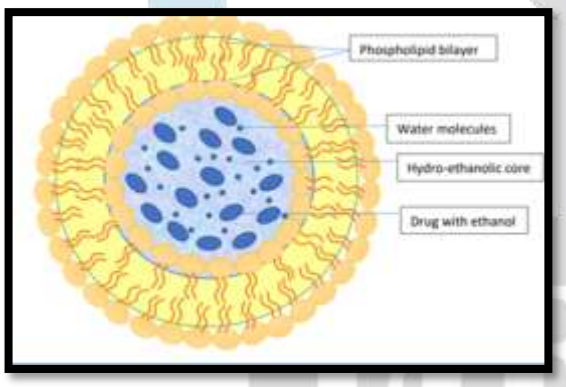


Figure 2 Structure of Ethosome.

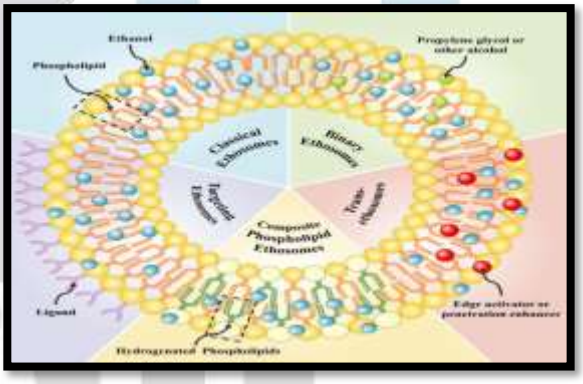


Figure 3 Structure of Different Ethosomes

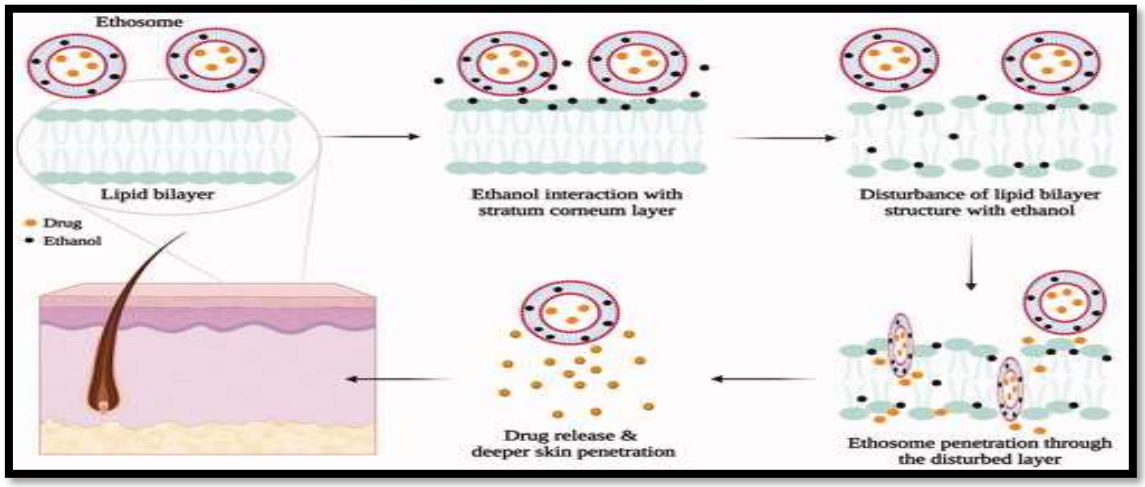


Figure 4 Mechanism of Ethosomal Drug Penetration through the Skin.

Figure 5 Application of Ethosomes

Table 1 Difference Between Various Ethosomes for Transdermal Drug Delivery [34]

Sr.No	Parameter	Classical Ethosomes	Binary Ethosomes	Transethosomes
1	Composition	1. Phospholipids 2. Ethanol 3. Stabilizer 4. Charge Inducer 5. Water 6. Drug/Agent	1. Phospholipids 2. Ethanol 3. Propylene Glycol 4. Charge Inducer 5. Water 6. Drug/Agent	1. Phospholipids 2. Ethanol 3. Edge Activator 4. Charge Inducer 5. Water 6. Drug/Agent
2	Morphology	Spherical	Spherical	Regular or Irregular Spherical Shape
3	Size	Smaller than Classical Liposomes	Smaller than Classical Ethosomes	Size based on type and Concentration of Penetration Enhancer
4	Entrapment Efficiency	Superior than Traditional Liposomes	Often Higher than Traditional Ethosomes	Higher than the Majority of Typical Ethosomes
5	Skin Permeation	Usually Greater than Traditional Liposomes	Usually Superior to Traditional Ethosomes	Higher than Traditional Ethosomes
6	Stability	More Robust than Traditional Liposomes	Stable than Classical Ethosomes	There was no Clear Trend Found

Table 2 Different Type of Phospholipids

Phospholipids name / brands	Composition and source	references
Phospholipon 90G	Phosphatidylcholine from soybean (90%), granules.	46
Phospholipon 90H	Hydrogenated phosphatidylcholine from soybean (90%), powder.	47
Phospholipon 80H	Hydrogenated phospholipids from soybean with 70% phosphatidylcholine, powder.	48
NAT 8539	Contained phosphatidylcholine (73%–79%), lysophosphatidylcholine (up to 6%), cephalin (up to 4%), and phosphatidic acid (up to 6%) of the dry residue; natural oils and sterol up to 6%; and ethanol (23%–27%).	49
Dipalmitoylphosphatidylcholine(DPPC)	1,2-Dipalmitoyl-rac-glycero-3-phosphocholine, ~99%, powder.	50
Lipoid S100	Phosphatidylcholine from soybean, agglomerates.	51
Lipoid S75-3	Phosphatidylcholine content (70%–75%), from soybean.	52
Lipoid S75	Phosphatidylcholine content (68%–73%), from soybean.	53
Lipoid E80	Phosphatidylcholine content (81.7%), from egg yolk, agglomerates.	54
Phosphatidylethanolamine (PE)	3-sn-Phosphatidylethanolamine, $98%, from bovine/sheep brain, lyophilized powder.	34
l-α-Phosphatidylcholine (PC)	1,2-Diacyl-sn-glycero-3-phosphocholine, $99%, from soybean/egg yolk, lyophilized powder.	42
POPC (1-palmitoyl-2-oleoyl-sn-glycero-3-phosphocholine)	1-Hexadecanoyl-2-(9Z-octadecenoyl)-sn-glycero-3-phosphocholine, .99%, synthetic, powder.	34
DPPG (1,2-dipalmitoyl-sn-glycero-3-phosphatidylglycerol)	1,2-Dipalmitoyl-sn-glycero-3-phospho-(1′-rac-glycerol) (sodium salt), powder.	45
Coatsome FE-608 ISU5 POPE-NHS	N-(Succinimidyloxy-glutaryl)-l-α-phosphatidylethanolamine, 1-palmitoyl-2-oleoyl.	41
DOTAP (1,2-dioleoyl-3-trimethylammonium-propane [chloride salt])	1,2-Dioleoyl-3-trimethylammonium-propane (chloride salt), powder or ethanol solution.	41
Phospholipon 50	Lecithin from soy purified phosphatidylcholine, concentration 45%, rich in linoleic acid (65%) and palmitic acid (~20%), solid wax.	34
SPC50	Phosphatidylcholine content (50.3%), from soybean	55

Table 3 Different Additives Employed in Formulation of Ethosomes

Class	Example	Uses
Phospholipids	Soya phosphatidylcholine, Dipalmityl phosphatidylcholine, Egg phosphatidylcholine	Membrane forming agent
Dye	Rhodamine red, Rhodamine-123, 6-Carboxy fluorescence, Fluorescence Isothiocyanate (FITC)	For characterization
Cholesterol	Cholesterol	For providing the stability to the vesicle membrane
Vehicle	Carbapol D934	As a gel former
Polyglycol	Propylene glycol, transcutol RTM	As a skin penetration
alcohol	Isopropyl alcohol, ethanol	For providing the softness for the vesicle membrane As a penetration enhancer

Reference:

[1] Dol H. Design and Development of Ethosomes of Transdermal Delivery of Poorly Soluble Drug.2020 Oct
[2] Tavakoli S, Klar AS. Advanced hydrogels as wound dressings. Biomolecules. 2020 Aug 11;10(8):1169.
[3] Farage MA, Miller KW, Elsner P, Maibach HI. Structural characteristics of the aging skin: a review. Cutaneous and ocular toxicology. 2007 Jan 1;26(4):343-57.
[4] Nandure HP, Puranik P, Giram P, Lone V. Ethosome: A Novel Drug Carrier. International journal of pharmaceutical research & allied sciences. 2013 Jul 1;2(3).
[5] Chien Yiew: Novel Drug Delivery System. Revised And Expanded Informa Healthcare. 2009;50:301-3022.
[6] Jain NK. Controlled and Novel Drug Delivery. 1sted. CBS Publisher & Distributors 1997;1:100- 101.
[7] Vyas SP. Khar Roop.K. Controlled Drug Delivery Concepts and Advances. 1sted. Vallabh Prakashan 2002;1:412-413.
[8] Jain NK. Advance in Controlled and Novel Drug Delivery. 1sted. CBS Publishers &Distributors 2001;1:426.
[9] Satnam S, Sandeep K. Ethosomes: A new pathway for novel drug delivery. Indo American Journal of Pharmaceutical Sciences. 2018 Aug 1;5(8):7552-9.
[10] Viswanad Vidya, PG Anju, Kumar Gopika S, Nair Sivapriya G. Formulation development and in- vitro characterisation of ethosomes for the enhanced transdermal delivery of clotrimazole. International Journal of Research in Pharmaceutical Sciences 2019;10:874-882.
[11] Kumar Ananda.Ch, Dutt Dr. Rajeswar. Ethosomes a novel transdermal drug delivery system. World Journal of Pharmaceutical Research 2014;3:3740-3750.
[12] Kumar N, Dubey A, Mishra A, Tiwari P. Ethosomes: A Novel Approach in Transdermal Drug Delivery System. International journal of pharmacy & life sciences. 2020 May 1;11(5).
[13] Thavare S, Deshmukh T, Rayjade R. Ethosomal Gel: As Novel Drug Carrier For Transdermal Drug Delivery System. European Journal of Pharmaceutical and Medical Research. 2022,9(6),160-166.
[14] Indora N, Kaushik D. Design, development and evaluation of ethosomal gel of fluconazole for topical fungal infection. International journal of engineering science invention research & development. 2015;1(8):280-306.
[15] Zhan B, Wang J, Li H, Xiao K, Fang X, Shi Y, Jia Y. Ethosomes: A Promising Drug Delivery Platform for Transdermal Application. Chemistry. 2024 Sep 13;6(5):993-1019.
[16] Zhou Y, Wei Y, Liu H, Zhang G, Wu XA. Preparation and in vitro evaluation of ethosomal total alkaloids of Sophora alopecuroides loaded by a transmembrane pH-gradient method. Aaps Pharmscitech. 2010 Sep;11:1350-8.
[17] Elsayed MM, Abdallah OY, Naggar VF, Khalafallah NM. Deformable liposomes and ethosomes: mechanism of enhanced skin delivery. International journal of pharmaceutics. 2006 Sep 28;322(1-2):60-6.
[18] Zhang JP, Wei YH, Zhou Y, Li YQ, Wu XA. Ethosomes, binary ethosomes and transfersomes of terbinafine hydrochloride: a comparative study. Archives of pharmacal research. 2012 Jan;35:109-17.
[19] Akhtar N, Verma A, Pathak K. Feasibility of binary composition in development of nanoethosomal glycolic vesicles of triamcinolone acetonide using Box-behnken design: in vitro and ex vivo characterization. Artificial cells, nanomedicine, and biotechnology. 2017 Aug 18;45(6):1123-31.
[20] Kumar L, Verma S, Singh K, Prasad DN, and Jain AK. Ethanol-based vesicular carriers in transdermal drug delivery: nanoliposomes and transethosomes. Nanoworld journal, 2016; 2(3): 41-51.
[21] Yang L, Wu L, Wu D, Shi D, Wang T, Zhu X. Mechanism of transdermal permeation promotion of lipophilic drugs by ethosomes. International journal of nanomedicine. 2017 Apr 26:3357-64.
[22] Li Y, Xu F, Li X, Chen SY, Huang LY, Bian YY, Wang J, Shu YT, Yan GJ, Dong J, Yin SP. Development of curcumin-loaded composite phospholipid ethosomes for enhanced skin permeability and vesicle stability. International journal of pharmaceutics. 2021 Jan 5;592:119936.
[23] Ma L, Wang X, Wu J, Zhang D, Zhang L, Song X, Hong H, He C, Mo X, Wu S, Kai G. Polyethylenimine and sodium cholate-modified ethosomes complex as multidrug carriers for the treatment of melanoma through transdermal delivery. Nanomedicine. 2019 Sep;14(18):2395-408.

[24] Zhang Y, Xia Q, Li Y, He Z, Li Z, Guo T, Wu Z, Feng N. CD44 assists the topical anti-psoriatic efficacy of curcumin-loaded hyaluronan-modified ethosomes: a new strategy for clustering drug in inflammatory skin. Theranostics. 2019;9(1):48.
[25] Mishra D, Mishra PK, Dubey V, Nahar M, Dabadghao S, Jain NK. Systemic and mucosal immune response induced by transcutaneous immunization using Hepatitis B surface antigen-loaded modified liposomes. european journal of pharmaceutical sciences. 2008 Apr 23;33(4-5):424-33.
[26] Rattanapak T, Young K, Rades T, Hook S. Comparative study of liposomes, transfersomes, ethosomes and cubosomes for transcutaneous immunisation: characterisation and in vitro skin penetration. Journal of Pharmacy and Pharmacology. 2012 Nov;64(11):1560-9.
[27] Touitou E, Dayan N, Bergelson L, Godin B, Eliaz M. Ethosomes—novel vesicular carriers for enhanced delivery: characterization and skin penetration properties. Journal of controlled release. 2000 Apr 3;65(3):403-18.
[28] Agarwal S, Gautam G, Rajpoot N. A review on ethosomes: an advanced approach to transdermal drug delivery system. The International Research Journal of Pharmacy. 2019 Apr 4;10(4):14-20.
[29] Satnam S, Sandeep K. Ethosomes: A new pathway for novel drug delivery. Indo American Journal of Pharmaceutical Sciences. 2018 Aug 1;5(8):7552-9.
[30] Shelke O, Kulkarni A. Formulation, development and evaluation of nano ethosomal gel of tramadol hydrochloride. J Nanomed Nanotechnol. 2018;9(514):2
[31] Satyam G, Shivani S, Garima G. Ethosomes: A novel tool for drug delivery through the skin. J Pharm Res. 2010 Apr;3(4):688-91.
[32] Aute PP, Kamble MS, Chaudhari PD, Bhosale AV. A comprehensive review on ethosomes. International journal of research and development in pharmacy & life sciences. 2012 Jan 15;2(1):218-24.
[33] Jafari A, Daneshamouz S, Ghasemiyeh P, Mohammadi-Samani S. Ethosomes as dermal/transdermal drug delivery systems: applications, preparation and characterization. Journal of liposome research. 2023 Jan 2;33(1):34-52.
[34] Abdulbaqi IM, Darwis Y, Khan NA, Assi RA, Khan AA. Ethosomal nanocarriers: the impact of constituents and formulation techniques on ethosomal properties, in vivo studies, and clinical trials. International journal of nanomedicine. 2016 May 25:2279-304.
[35] Jadhav PU, Gujare SG, Shende MA. Ethosomes: A novel tool for vesicular drug delivery. Asian Journal of Pharmaceutical Research. 2024;14(1):45-52.
[36] Li G, Fan Y, Fan C, Li X, Wang X, Li M, Liu Y. Tacrolimus-loaded ethosomes: physicochemical characterization and in vivo evaluation. European journal of pharmaceutics and biopharmaceutics. 2012 Sep 1;82(1):49-57.
[37] Finnin BC, Morgan TM. Transdermal penetration enhancers: applications, limitations, and potential. Journal of pharmaceutical sciences. 1999 Oct 1;88(10):955-8.
[38] Puri R, Jain S. Ethogel topical formulation for increasing the local bioavailability of 5-fluorouracil: a mechanistic study. Anti-cancer drugs. 2012 Oct 1;23(9):923-34.
[39] Lopez-Pinto JM, Gonzalez-Rodriguez ML, Rabasco AM. Effect of cholesterol and ethanol on dermal delivery from DPPC liposomes. International journal of pharmaceutics. 2005 Jul 14;298(1):1-2.
[40] Zhaowu Z, Xiaoli W, Yangde Z, Nianfeng L. Preparation of matrine ethosome, its percutaneous permeation in vitro and anti-inflammatory activity in vivo in rats. Journal of liposome research. 2009 Jun 1;19(2):155-62.
[41] Chen M, Zakrewsky M, Gupta V, Anselmo AC, Slee DH, Muraski JA, Mitragotri S. Topical delivery of siRNA into skin using SPACE-peptide carriers. Journal of controlled release. 2014 Apr 10;179:33-41.
[42] Limsuwan T, Amnuaikit T. Development of ethosomes containing mycophenolic acid. Procedia chemistry. 2012 Jan 1;4:328-35.
[43] Jain S, Tiwary AK, Sapra B, Jain NK. Formulation and evaluation of ethosomes for transdermal delivery of lamivudine. Aaps Pharmscitech. 2007 Oct;8:249-57.
[44] Maestrelli F, Capasso G, González-Rodríguez ML, Rabasco AM, Ghelardini C, Mura P. Effect of preparation technique on the properties and in vivo efficacy of benzocaine-loaded ethosomes. Journal of liposome research. 2009 Dec 1;19(4):253-60.
[45] Munde G, Satpute V. Ethosomes and trans Ethosomal drug delivery system. International journal of pharmaceutical sciences.2024:206-220.
[46] Ahad A, Aqil M, Kohli K, Sultana Y, Mujeeb M. Enhanced transdermal delivery of an anti-hypertensive agent via nanoethosomes: statistical optimization, characterization and pharmacokinetic assessment. International journal of pharmaceutics. 2013 Feb 25;443(1-2):26-38.
[47] Bhosale SS, Avachat AM. Design and development of ethosomal transdermal drug delivery system of valsartan with preclinical assessment in Wistar albino rats. Journal of liposome research. 2013 Jun 1;23(2):119-25.
[48] Prasanthi D, Lakshmi PK. Development of ethosomes with taguchi robust design-based studies for transdermal delivery of alfuzosin hydrochloride. International current pharmaceutical journal. 2012 Oct 3;1(11):370-5.
[49] Verma DD, Fahr A. Synergistic penetration enhancement effect of ethanol and phospholipids on the topical delivery of cyclosporin A. Journal of controlled release. 2004 May 31;97(1):55-66.
[50] Bodade SS, Shaikh KS, Kamble MS, Chaudhari PD. A study on ethosomes as mode for transdermal delivery of an antidiabetic drug. Drug delivery. 2013 Jan 1;20(1):40-6.
[51] Ascenso A, Pinho S, Eleutério C, Praça FG, Bentley MV, Oliveira H, Santos C, Silva O, Simões S. Lycopene from tomatoes: vesicular nanocarrier formulations for dermal delivery. Journal of agricultural and food chemistry. 2013 Jul 31;61(30):7284-9
[52] Choi JH, Cho SH, Yun JJ, Yu YB, Cho CW. Ethosomes and transfersomes for topical delivery of ginsenoside Rh1 from red ginseng: characterization and in vitro evaluation. Journal of nanoscience and nanotechnology. 2015 Aug 1;15(8):5660-2.
[53] Shen LN, Zhang YT, Wang Q, Xu L, Feng NP. Enhanced in vitro and in vivo skin deposition of apigenin delivered using ethosomes. International journal of pharmaceutics. 2014 Jan 2;460(1-2):280-8.

[54] Fathi-Azarbayjani A, Ng KX, Chan YW, Chan SY. Lipid vesicles for the skin delivery of diclofenac: cerosomes vs. other lipid suspensions. Advanced pharmaceutical bulletin. 2015 Mar;5(1):25.
[55] Shen S, Liu SZ, Zhang YS, Du MB, Liang AH, Song LH, Ye ZG. Compound antimalarial ethosomal cataplasm: preparation, evaluation, and mechanism of penetration enhancement. International journal of nanomedicine. 2015 Jun 30:4239-53.
[56] Barupal AK, Gupta V, Ramteke S. Preparation and characterization of ethosomes for topical delivery of aceclofenac. Indian journal of pharmaceutical sciences. 2010 Sep;72(5):582.
[57] Acharya A, Ahmed MG, Rao BD. Development and evaluation of ethosomal gel of lornoxicam for transdermal delivery: in-vitro and in-vivo evaluation. Manipal journal of pharmaceutical sciences. 2016;2(1):3.
[58] Ibrahim TM, Abdallah MH, El-Megrab NA, El-Nahas HM. Transdermal ethosomal gel nanocarriers; a promising strategy for enhancement of anti-hypertensive effect of carvedilol. Journal of liposome research. 2019 Jul 3;29(3):215-28.
[59] Agarwal S, Gautam G. Formulation, Development and Evaluation of Atorvastatin Ethosomal Gel. International Journal of Pharmaceutical Investigation. 2020 Oct 1;10(4).
[60] Pawar P, Kalamkar R, Jain A, Amberkar S. Ethosomes: a novel tool for herbal drug delivery. IJPPR Human. 2015;3:191-202.
[61] Aggarwal D, Nautiyal U. Ethosomes: A review. Int J pharm med res. 2016 Aug 10;4(4):354-63.
[62] Parashar T, Sachan R, Singh V, Singh G, Tyagi S, Patel C, Gupta A. Ethosomes: a recent vesicle of transdermal drug delivery system. Int. J. Res. Dev. Pharm. Life Sci. 2013 Feb;2(2):285-92.
[63] Ankush BK, Sushil Gaikwad S, Ayushi P. Somwanshi. Ethanolic vesicles a novel approach to enhance transdermal drug delivery. Indo American Journal of Pharmaceutical Research. 2017;7:7440-50.
[64] Ramakrishna GA, Manohar SD, Bhanudas SR. Ethosomes: carrier for enhanced transdermal drug delivery system. Journal of Advanced Pharmacy Education and Research. 2014;4(4-2014):380-7.