Review Article

ETHOSOMES: A Novel Approach in Vesicular Drug Delivery Systems

Abstract:
Transdermal drug delivery is a dosage form applied to the intact skin, delivering the drug through the skin at an uncontrolled rate to the systematic circulation. Ethosomes are non-intrusive delivery systems that enable them deep into the skin layers or systemic circulation of phospholipids. Ethosomes are soft, flexible vesicles that let active medications get to where they need to go faster. Due to their unique structure, ethosomes can encapsulate and transport high lipophilic chemicals such as cannabis, testosterone, and minoxidil, as well as cationic medicines such as propranolol trihexaphenidyl and Cyclosporine through the skin. Ethosomes are becoming more common in drug delivery systems for topical and transdermal use due to their ability to permeate deep skin layers and systemic circulation.

Key Words: *Ethosomes, transdermal, phospholipids, skin permeation*

1. INTRODUCTION [1-3]

Ethosomes are preferred because they remove gastrointestinal interferences and first-pass metabolism of the medicine; transdermal drug delivery systems (TDDS) have shown promising outcomes compared to oral drug delivery systems. The fundamental disadvantage of TDDS is that it interacts with the Stratum Corneum's barrier characteristics, which means that it can only pass through lipophilic medicines with molecular weights less than 500 Da. Chemical and physical enhancers, including iontophoresis, sonophoresis, and other similar techniques, have all been studied to promote medication penetration through the skin. Drug permeation across the Stratum Corneum barrier has also been observed to be increased by liposomes, niosomes, transferosomes, and ethosomes. Permeation enhancers increase the skin's permeability, allowing drugs to flow through more efficiently. Ethosomes penetrate the skin layers faster and have a higher transdermal flow than traditional liposomes. Ethosomes have demonstrated excellent percutaneous medication delivery efficacy as a lipid carrier. They also have better pharmaceutical properties than conventional liposomes, such as room temp stability, high trap performance, and improved compatibility with the Stratum Corneum (SC), allowing for even more effective penetration of lipophilic and hydrophilic drugs into the skin's deep layers through the SC. The role of ethosomes in transdermal penetration and their effects on the skin are unknown. Some of the critical techniques used to study the transdermal process include Attenuated Total Reflectance (ATR-FTIR), Confocal Laser Scanning Microscopy (CLSM), Differential Scanning Calorimeter (DSC), Raman, Scanning Electronic Microscopy (SEM), Transmission Electron Microscopy (TEM), X-ray Photoelectron Spectroscopy (XPS), and Electron Spin Resonance (ESR). Vesicles have been recognized for their relevance in cellular communication and particle transport for many years. Researchers have identified a mechanism to enhance drug administration within vesicles' cavities while simultaneously marking the vesicles for cell selectivity using the structure of the vesicles. The discovery of ethosomes, and vesicle derivatives, was a crucial breakthrough in vesicle research.

2. ADVANTAGES AND DISADVANTAGES OF ETHOSOMES[4-7]

2.1 ADVANTAGES-

1. *Large molecules such as peptides and protein molecules can be delivered.*
2. *It is made up of non-toxic raw materials.*

3. *Increased drug absorption through the skin.*
4. *The composition is safe, and the ingredients have been approved for use in pharmaceuticals and cosmetics.*
5. *It has a low-risk profile.*
6. *Patient compliance is high.*
7. *The ethosomal system is non-invasive and passive, making it ideal for direct marketing.*
8. *Ethosomal drug delivery systems can be used in various industries, including pharmaceuticals, biotechnology, veterinary medicine, cosmetics, and nutraceuticals.*
9. *Compared to iontophoresis, phosphophoresis, and other complex processes, this is a simple drug delivery approach.*

2.2 DISADVANTAGES

1. *Rather than a fast bolus-type drug intake, ethosomal administration is designed to provide continuous, sustained pharmaceutical distribution.*
2. *Effective drug solubility in lipophilic and aqueous media for cutaneous microcirculation and systemic circulation.*
3. *The drug's molecular size must be adequate for percutaneous absorption.*
4. *Adhesive might not stick to all skin types.*
5. *It might not be cost-effective.*
6. *Allergy responses to ethanol or other ethosomal components can be identified.*
7. *Ethosomal carriers, in contrast to other carriers (solid lipid nanoparticles, polymeric nanoparticles, and so on), are only required for transdermal administration.*
8. *Because ethanol is flammable, more caution should be exercised when planning, applying, transporting, and storing it.*
9. *Product loss during the transition from organic to water medium*
10. *It's only for potent chemicals that require a daily dose of long or less.*
11. *Excipients and penetration enhancers in drug delivery systems might cause skin irritation or dermatitis.*

3. COMPOSITION OF ETHOSOMES [8-9]

Ethosomes comprise several main components, as seen in ***Figure 1****.*

- *Phospholipids (phosphatidylcholine, phosphatidylserine, phosphatidic acid), ethanol, and water make up the majority of ethosomes, with the nonaqueous phase ranging from 22 percent to 70%. The alcohol may be ethanol or isopropyl alcohol.*
- *Ethosomes are vesicular carriers of hydroalcoholic or hydro/alcoholic/glycolic phospholipids with a high alcohol content or a mixture of alcohols.*
- *The lipid membrane is also packed less tightly than in typical vesicles due to the high ethanol content. It retains the same stability, allowing for a more flexible structure and better drug distribution through stratum corneum lipids.*
- *Ethanol and isopropyl alcohol are examples of alcohols that can be employed. Propylene glycol and Transcutol are the most often utilized glycols. Non-ionic surfactants (PEG-alkyl ethers) can also be used with phospholipids in these formulations.*

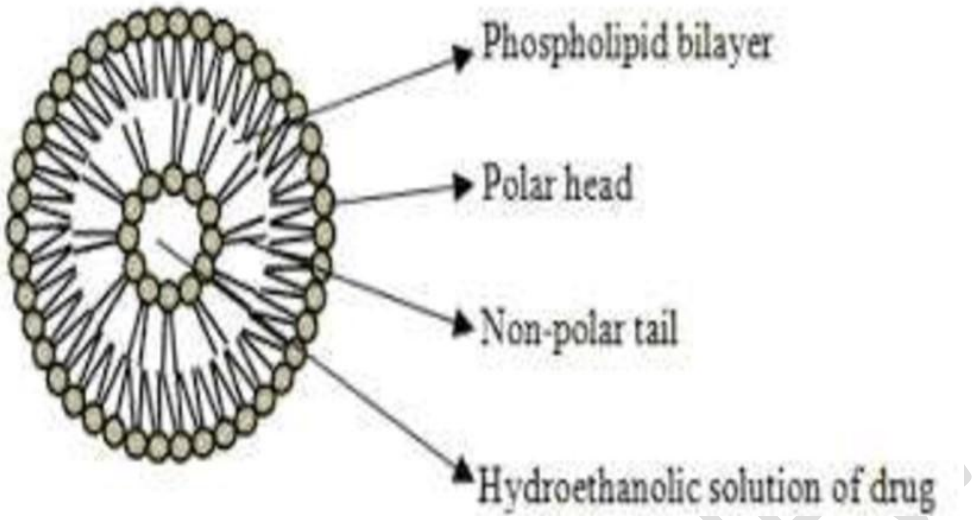

Figure 1: Structure of Ethosomes [8]

Class of Polymer	***Example***	***Uses***
Alcohol	*Ethanol, Isopropyl alcohol*	*Provides softness to the vesicle membrane and act as a penetration enhancer*
Polyglycol	*Propylene glycol*	*As a skin penetration enhancer*
Phospholipid	*Phosphatidylcholine from soya Egg phosphatidylcholine*	*Vesicles forming component*
Vehicle	*Carbopol 934*	*Act as a Gel forming agent*
Cholesterol	*Cholesterol*	*Provides stability to vesicle membranes*
Dye	*Rhodamine red Fluorescent (FITC)*	*For Identification study*

TABLE 1: Different Components Used in the Formulation of Ethosomes [9]

4. MECHANISM OF ACTION [10]

The real benefit of ethosomes over liposomes is improved drug penetration. The process by which drugs are absorbed from ethosomes is unknown. The following two phases of medication absorption are most likely to occur:

1. ***Ethanol effect***
   - *The mechanism by which ethanol helps to enhance skin penetration is well known. It penetrates lipids in the intercellular spaces, reduces the density of lipid multilayers in cell membranes, and increases the fluidity of lipids in the cell membranes.*
2. ***Ethosomes effect***
   - Due to increased lipid fluidity within the cell membrane caused by ethanol in ethosomes, skin permeability is also increased, so the ethosomes penetrate very easily inside the deep skin layers, fusing with skin lipids and releasing the drugs into that layer of skin.

5. IDEAL CHARACTERISTICS OF ETHOSOMES [11]

1. ***Visualization***
- *Scanning electron microscopy (SEM) and Transmission electron microscopy (TEM) techniques are used to examine ethosomes.*

2. ***Vesicle size and Zeta potential***
- *Using a computer-based inspection system and photon correlation spectroscopy, dynamic light scattering (DLS) can be used to measure particle size and zeta potential (PCS).*

3. ***Surface Tension Activity Measurement***
- *A Du Nouy ring tensiometer can be used to measure the drug's surface tension activity in an aqueous solution using the ring method.*

4. ***Entrapment Efficiency***
- *The ultracentrifugation technique can be used to determine the drug's entrapment efficiency by ethosomes.*

5. ***Penetration and Permeation Studies***
- *Confocal laser scanning can be used to see the depth of penetration from ethosomes.*

6. ***Vesicle Stability***
- *The size and shape of vesicles can be measured over time to determine their stability. TEM detects mean DLS and structure changes, used to assess size.*

7. ***In vitro drug release study and Drug Deposition study***
- *Franz diffusion cell with artificial or biological membrane, Dialysis bag diffusion can be used for in vitro drug release studies and drug deposition of ethosomal preparation.*

6. PREPARATION TECHNIQUES FOR ETHOSOMES [12-15]

• Ethosomes can be made using two simple and convenient methods:

6.1 By using the Cold method
6.2 By using the Hot method

6.1 Cold Method

For the preparation of ethosomal formulation, this is the most usual procedure. Phospholipids, drugs, and other lipid compounds are dissolved in ethanol at room temperature in a covered vessel with vigorous stirring using a mixer in this process. During the stirring process, propylene glycol or another polyol is introduced. In a water bath, this combination is heated to 300°C. In a separate vessel, heat the water to 300°C, add it to the mixture and whisk for 5 minutes in a closed container. The vesicular size of an ethosomal formulation can be decreased to the required extent using a sonication or extrusion procedure. Finally, the mixture is placed in the refrigerator.

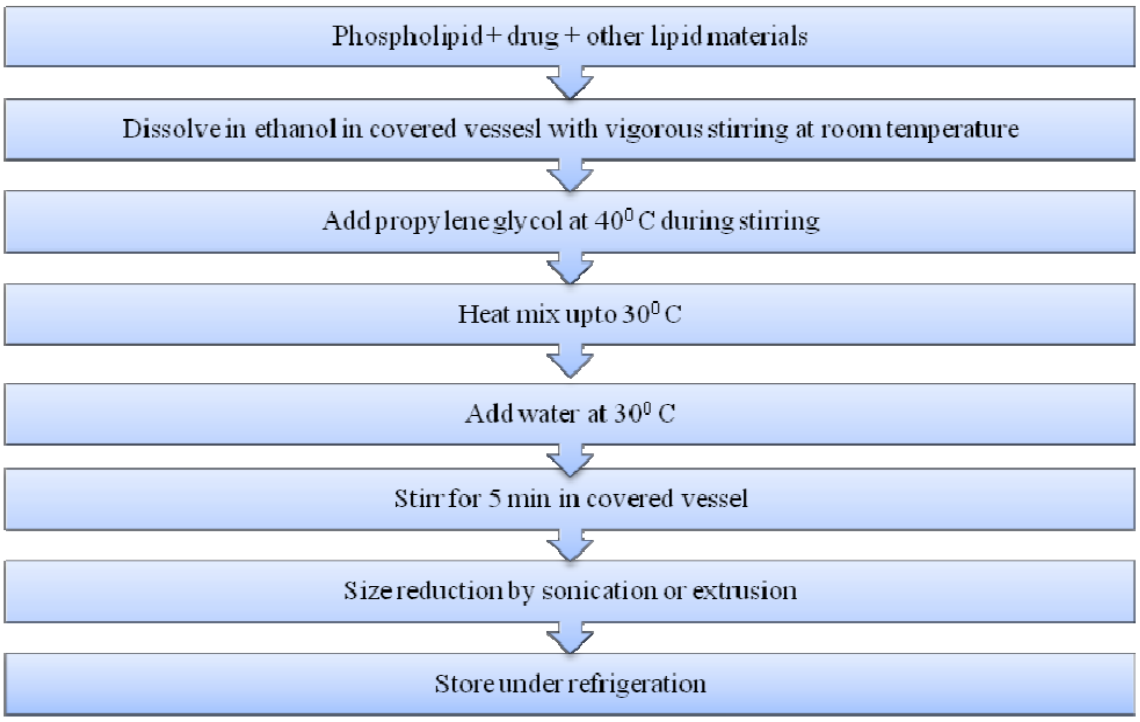

Figure 2: Ethosome Preparation Using a Cold Method [12]

6.2 Hot method

The phospholipid is dispersed in water using this process, which involves heating it in a water bath at 400°C until it becomes a colloidal solution. Ethanol and propylene glycol are combined in a separate vessel and heated to 400 degrees Celsius. The organic phase is introduced to the aqueous phase once both mixtures reach 400°C. Depending on the drug's hydrophilic or hydrophobic characteristics, it is dissolved in water or ethanol. Using probing sonication or the extrusion approach, the vesicle size of an ethosomal formulation can be reduced to the required size.

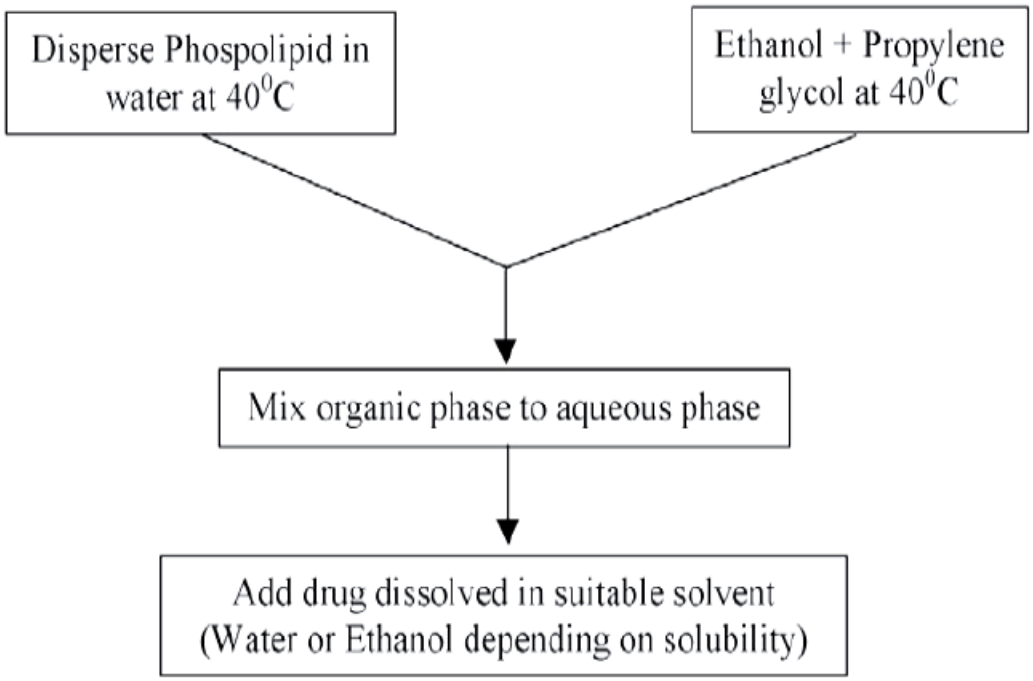

Figure 3: Ethosome Preparation Using a Hot Method [12]

7. EVALUATION OF ETHOSOMES [16,17]

7.1 ***Filter Membrane-Vesicle Interaction Study by Scanning Electron Microscopy*** [18,19]

It entails coating a filter membrane with a pore size of 50 nm with vesicle suspension (0.2 mL) and depositing it in diffusion cells. The upper surface of the filter was exposed to air, while the lower surface was in contact with phosphate buffer saline solution (pH 6.5). After 1 hour, the filters were removed. The samples were prepared for SEM examinations by overnight fixation in Karnovsky's fixative at 4°C, followed by dehydration with graded ethanol solutions like 30, 50, 70, 90, 95, and 100 percent v/v in water. Finally, gold-coated filters were inspected in a scanning electron microscope (Leica, Bensheim, Germany).

7.2 ***Skin permeation studies*** [20]

Hair was carefully trimmed short (2 mm) on test animals (rats), and the abdomen skin was separated from the connective tissue with a scalpel. The removed skin was placed on aluminum foil, and the dermal side was gently teased off to remove any clinging fat and subcutaneous tissue. The diffusion cell volume permeation area was 1.0 cm2, and the receptor cell volume permeation area was 10 ml. The temperature was maintained at 32 degrees Celsius plus or minus 1 degree Celsius. Saline solution with phosphate buffer was kept in the receptor compartment (10 ml pH 6.5. The donor and receptor compartments were partitioned by excised skin. The ethosomal formulation was applied to the skin's epidermal surface (1.0 ml). A high-performance liquid chromatography test was performed to evaluate samples (0.5 ml) obtained within 2-hour, 4-hour, 8-hour, 12-hour, 16-hour, 20-hour, and 24-hour time intervals, respectively, via the diffusion cell's sampling port.

7.3 ***Stability Study*** [21]

By keeping the vesicles stored at 4°C 0.5°C, the stability of the vesicles was assessed. The method mentioned before quantifies vesicle size, zeta potential, and entrapment efficiency after 180 days.

7.4 ***Drug uptake studies*** [22]

The drug was injected into MT-2 cells (1106 cells/mL) in 24-well plates (Corning Inc) with 100 liters of RPMI medium. The cells were cultured with 100 liters of drug solution in phosphate-buffered saline solution (pH 7.4), ethosomal formulation, or marketed formulation. The drug uptake was then measured using an HPLC technique to analyze the drug content.

7.5 HPLC Assay [23,24]

The amount of drug penetrated in the receptor compartment during in vitro skin permeation assays and in the MT-2 cell was evaluated using an HPLC assay using a 70:20:10 vol/vol mixture of methanol, distilled water, and acetonitrile as mobile phase delivered at 1 mL/min by an LC 10-AT VP pump (Shimadzu, Kyoto, Japan). A twenty-microliter injection was eluted in the C-18 column (4.6150 mm, Luna, 54, Shimadzu). The SPDM10A VP diode array UV detector was used to monitor the column eluent at a wavelength of 271 nm. The standard curve's coefficient of variation (CV) ranged from 1.0 to 2.3 percent, with a squared correlation coefficient of 0.9968.

7.6 ***Statistical Analysis*** [25,26]

ANOVA was preferably used to examine the statistical significance of all the data obtained, followed by a studentized range test. The results were interpreted using PRISM with a confidence limit of P.05 (GraphPad, Version 2.01, San Diego, CA).

7.7 ***Vesicle-Skin Interaction Study by TEM and SEM*** [27,28]

Ultra-thin segments were cut (Ultracut, Vienna, Austria), collected on formvar-coated grids, and analyzed using a transmission electron microscope. After dehydration, the skin segments were attached to stubs and covered with gold-palladium alloy using a fine coat ion sputter coater for SEM analysis. A scanning electron microscope was used to look at the cells.

7.8 ***Vesicle-Skin Interaction Study by Fluorescence Microscopy*** [29,30]

The TEM and SEM study methodology were followed when performing fluorescence microscopy. MT-2 cells (Tlymphoid cell lines) were propagated in Dulbecco's modified Eagle medium (HIMEDIA, Mumbai, India), which contains 10% foetal calf serum, 100 U/mL penicillin, 100 mg/mL streptomycin, and 2 mmol/L Lglutamine at 37°C under a 5% CO2 atmosphere. The cytotoxic dosage 50 (CD50) that caused a 50% drop in absorbance at 540nm was used to measure cytotoxicity.

8. APPLICATION OF ETHOSOMES [31,32,33,34]

Ethosomes are mainly used in the replacement of liposomes for transdermal drug delivery. They help transport hydrophilic and impermeable drugs through the skin.

8.1 Pilosebaceous targeting [35,36]

In addition, hair follicles and sebaceous glands are increasingly recognized as potentially significant elements in percutaneous drug delivery. The follicles can also be used as transport shunts for the systemic delivery of drugs. A lipid-soluble drug called minoxidil is topically applied to the scalp as a baldness treatment using pilosebaceous delivery. Pilosebaceous units can be used as depots for localized therapies, especially for follicle-related disorders like acne.

8.2 Hormone delivery [37]

Several issues arise when oral hormone preparations are administered, including high first-pass metabolism, poor oral bioavailability, and various dose-dependent side effects. Furthermore, oral hormone preparations heavily depend on patient compliance with these side effects. For example, Touitou et al. studied transdermal testosterone-loaded ethosomes (Testosome), compared to transdermal testosterone injections, and found that the ethosomes were more efficient for hormone delivery. (Testoderm patch, Alza) rabbit pinna skin showed approximately 30-times higher skin permeation of testosterone from ethosomal formulation. It was found that after treating with ethosomal formulation, the volume of drug deposited after 7 hrs was significantly higher (p50.05) than that after treating with Testoderm or Testosome. There was a significant improvement in the area under the curve (AUC) and Cmax of testosterone after applying Testoderm.

8.3 Transcellular Delivery [38,39]

Clinical trials are currently investigating ethosomes as penetration enhancers and carriers to deliver therapeutic agents transcellular. The fluorescence was almost nonexistent when liposomes were embedded in hydroethanolic solutions. During the incubation period of 3 minutes, the presence of each probe in the cytoplasm was evident.

8.4 Delivery of problematic drug molecules [40]

Large biogenic molecules, such as peptides and proteins, are difficult to transmit orally since they are destroyed in the GI tract. When it comes to oral delivery issues, non-invasive protein administration is a better option. Researchers looked at how ethosomal insulin administration reduced blood glucose levels in diabetic and non-diabetic SDI rats in vivo. The results showed that insulin delivered by this patch caused a significant reduction in BGL in both diabetic and non-diabetic rats (up to 60%). An insulin injection from a control formulation, on the other hand, does not affect BGL.

8.5 Delivery of anti-arthritis drug [41,42]

Anti-arthritis medication delivered topically is a better option for site-specific administration and avoids the complications associated with standard oral therapy. Cannabidiol (CBD) is a therapeutic candidate for treating rheumatoid arthritis that was only recently found. Low bioavailability, first-pass metabolism, and GIT degradation are all problems related to his oral administration. A carrageenan-mediated rat paw edema model investigated the CBD-ethosomal formulation's biological anti-inflammatory efficacy. As a result, it was determined that encapsulating CBD in ethosomes improved its skin penetration, accumulation, and hence physical activities.

8.6 Delivery of Antibiotics [43,44,45]

Antibiotics used topically are a better option for boosting their therapeutic efficacy. Oral therapy used in the past has resulted in several allergic responses and several adverse effects. External preparations with a poor permeability to deep skin layers and subdermal tissues are common.

Ethosomes can solve this problem by distributing enough antibiotics into the deeper layers of the skin. Ethosomes penetrate the epidermis quickly, delivering a significant amount of medications to the deeper layers of skin and suppressing infection at its source.

8.7 ***Cosmeceutical application of ethosomes*** [46,47,48]

The use of ethosomes in cosmeceuticals improves the stability of cosmetic chemicals, reduces skin irritation from irritating cosmetic chemicals, and improves transdermal penetration, especially in inelastic varieties. Furthermore, the compositions and sizes of the vesicles are critical concerns that must be addressed to attain the cosmeceutical benefits of elastic vesicles.

8.8 ***Topical delivery of DNA*** [49,50]

Many infections from the environment attempt to enter the body via the skin. The skin has evolved into an excellent defense barrier that is both immunologically active and capable of gene expression. Based on the preceding, the most critical application of ethosomes is for topical transport of DNA molecules to skin cells for gene expression. Ethosomes may be employed as carriers for gene therapy applications that need transient gene expression, according to one theory. The findings also revealed that ethosomes might be used to administer vaccines via transdermal delivery successfully. As a result of the increased ethosomal skin penetration capacity, these dosage forms can now help immunize drugs.

9. Conclusion:

It's easy to see how ethosomes outperform liposomes when it comes to skin permeation. When compared to transdermal and dermal administration, ethosomes offer more benefits. Ethosomes are distinguished by their ease of preparation, safety, and potency. They can be upgraded to allow more active drugs to penetrate the skin. Ethosomes can help penetrate the epidermis barrier is the critical limiting factor in transdermal medication delivery systems. Ethosomes provide more effective delivery of small, medium, and large therapeutic molecules while minimizing adverse effects. When it comes to delivering medications to the skin, ethosomes outperform liposomes and hydroalcoholic solutions. Ethosomes are non-invasive drug delivery vehicles that allow medicines to reach deep layers of the skin before being transferred to the systemic circulation. Large molecules, such as peptides and protein molecules, are transported. The development of ethosomal carriers creates new problems and potential for developing new, better medicines. Additionally, a study in this field will allow for improved regulation of medication release in vivo and long-term safety analysis, providing more effective treatment. Hydrophilic pharmaceuticals, cationic medications, proteins, and peptides have all been encapsulated using ethosomes. In conclusion, ethosomal preparations have a promising future in delivering bioactive substances via transdermal distribution.

COMPETING INTERESTS DISCLAIMER:

Authors have declared that no competing interests exist. The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

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