Review Article

ETHOSOMES: A Novel Approach in Vesicular Drug Delivery Systems

Abstract:

Transdermal drug delivery is a dosage form that is applied topically to the skin layer (epidermis) which helps to deliver the drug into the skin layer before entering the systemic circulation. Ethosomes are soft, and flexible vesicles which helps in rapid drug absorption. Ethosomes have better pharmaceutical properties than the conventional liposomes such as room temperature stability, and improved compatibility with the Stratum Corneum barrier. Ethosomes are non-toxic in nature and can be used in the preparation of cosmeceutical and it has better drug absorption to the skin. The most common disadvantage of ethosome is that it might not be cost-effective. The ethosomal patches might not stick to all skin types. The mechanism of ethosomes is mainly occurred due to increased lipid fluidity within the cell membrane caused by the ethanol in ethosome. As a result, skin permeability is increased. Ethosomes can be prepared by using cold method and hot method. Ethosome consists of various evaluation parameters which includes permeability studies, drug content studies, interaction study between the vesicle and filter membrane. Ethosomes can deliver various highly lipophilic drugs like minoxidil, testosome, CBD, and other antibiotic drugs. The most widely used application for ethosomal formulation is to transport the DNA topically into the skin layer for gene expression and hence ethosomes are used in the delivering the vaccines by transdermal route. Additionally, a study in this field allows for improved regulation of medication release in vivo and long-term safety analysis, providing effective treatment. Ethosomal preparations have promising future in delivering bioactive substances via transdermal distribution. The discovery of ethosomes and vesicle derivatives was a crucial breakthrough in vesicle research. Ethosomes are preferred because they are non-irritant to the GIT tract and avoid first-pass metabolism.

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

1. INTRODUCTION

Ethosomes are preferred because they don't cause any irritation to the GIT tract and first-pass metabolism; transdermal drug delivery systems (TDDS) have shown promising outcomes compared to oral drug delivery systems. Main disadvantage of TDDS is that the drug might interact with the Stratum Corneum barrier, meaning that only lipophilic drugs with molecular weight less than 500 Da can cross the Stratum Corneum barrier. Chemical and physical enhancers, including iontophoresis, sonophoresis, and other similar methods, have all been studied to promote drug permeation through the skin layer (epidermis). Drug permeation across the Stratum Corneum barrier has also been observed to be increased by liposomes, niosomes, transferosomes, and ethosomes. Permeation enhancers like alcohols (Ethanol, Isopropyl Alcohol) increases the rate of permeation in the skin, allows the drugs to pass through the skin 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), Differential Scanning Calorimetry (DSC), and other microscopic techniques like TEM, Raman, Scanning Electronic Microscope (SEM) and various surface analysis techniques like XPS, and Electron Spin Resonance (ESR). Vesicles is recognized by the researchers due to their particle transportation in nature in the systemic circulation. 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 [1-3]

2. ADVANTAGES AND DISADVANTAGES OF ETHOSOMES

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. *Its composition can be used for preparation of pharmaceuticals and cosmetics.*
5. *It has a low-risk profile.*
6. *Patient compliance is high.*
7. *Ethosomes can be directly marketed due to its non-invasive properties.*
8. *Ethosome systems is used in various industries including pharmaceuticals, biotechnologies, cosmetics, nutraceuticals and veterinary medicines.*
9. *Compared to iontophoresis, phosphophoresis, and other complex processes, this is a simple drug delivery approach* [4,5].

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. *Loss of product is observed during the phase transition from organic to water medium.*
10. *It's only for potent chemicals that require a daily dose of long or less.*
11. *The excipients and penetration enhancers that are used in formulation of ethosomes can cause skin irritation (dermatitis)* [6,7].

3. COMPOSITION OF ETHOSOMES

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

- *Ethosomes mainly comprises of ethanol (10-50%), water and phospholipids (such as phosphatidylcholine which helps in the formation of bilayer lipid vesicles).*
- *It consists of non-aqueous phase (22-70%). The alcohol that is used in the composition can be ethyl or isopropyl alcohol.*
- *Ethosomes is hydroalcoholic in nature due to the high alcohol content caused by the mixture of alcohols.*
- *Ethanol and isopropyl alcohol are examples of alcohols that can be employed. Propylene glycol (05-20%) and transcutol are the most often utilized glycols.*
- *Non-ionic surfactants like polyethylene glycol can be combined with the phospholipids (1-5%) for the formulation of ethosomes.* [8,9]

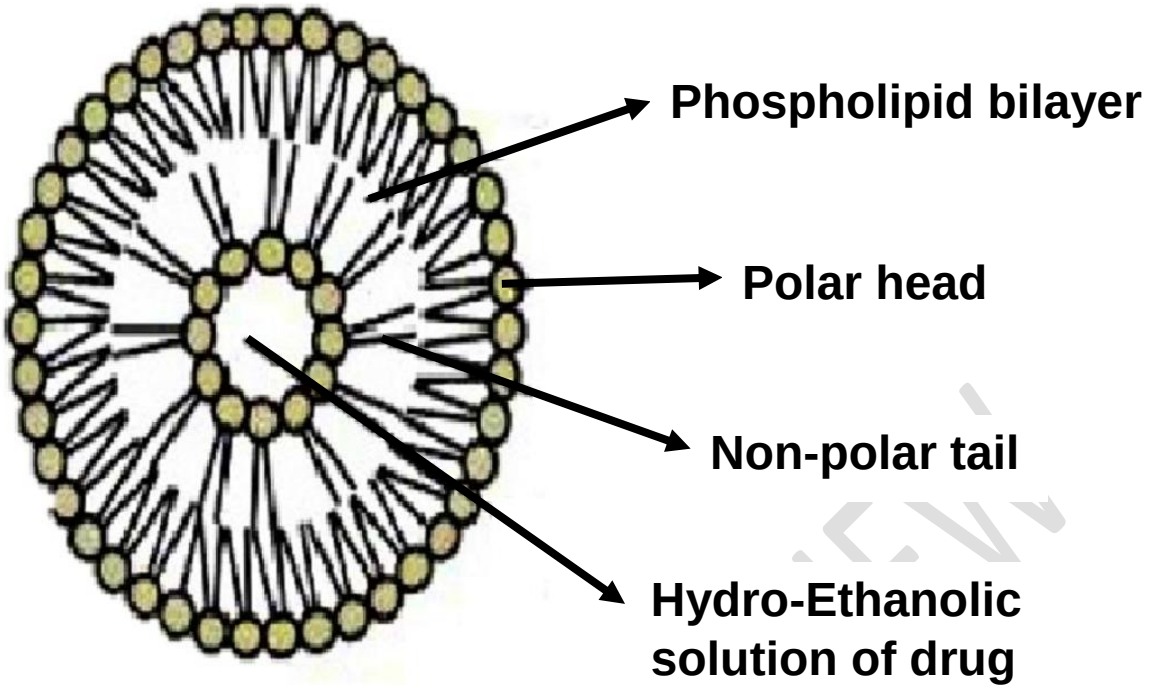

Figure 1: Diagrammatic representation of Ethosomes [8]

Table 1: Different Ingredients Used in the Preparation of Ethosomes [9]

Ingredients	***Examples***	***Uses***
Alcohols (Solvents)	*Ethanol (10-50%)* *Isopropyl Alcohol (15%)*	*Penetration Enhancer*
Phospholipids	*Phosphatidylcholine from Soya* *Egg Phosphatidylcholine*	*Vesicles forming component*
Vehicles	*Carbopol 934*	*Act as a gel forming agent in vesicle formation.*
Cholesterol	*Cholesterol*	*Stability provided to the vesicle membrane.*
Dye	*Rhodamine red*	*Identification purpose*
Polyglycol	*Transcutol (05-20%)*	*Skin Permeation*

4. MECHANISM OF ACTION

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 observed in ethosomes:

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 viscosity of the lipid bilayer.*

2. Ethosomes effect

- Due to increased lipid fluidity within the cell membrane caused by ethanol in ethosomes, skin permeability is also increased. As a result, rapid skin permeation is observed in the skin layers *[10]*

5. IDENTIFICATION OF ETHOSOMES

1. Visualization

Ethosomes can be identified/visualised by using various microscopic techniques like SEM and TEM.

2. Vesicular size and Zeta potential

Using a computer-based approach and photo emission spectroscopy, zeta potential and vesicle size of the ethosome can be determined.

3. Surface Tension Activity Measurement

Surface tension of a particular ethosome can be estimated by an instrument called Dunuoy ring tensiometer which measures the presence of drug concentration in the aqueous solution.

4. Entrapment Efficiency

Entrapment efficiency of an ethosome can be determined by using ultra-centrifugation techniques.

5. Vesicle Stability

Ethosomes shapes and sizes are measured to determine stability by using TEM and DLS technique.

6. Drug Deposition study

Drug deposition studies of ethosomes can be performed by using an instrument called Franz diffusion cell. *[11]*

6. PREPARATION TECHNIQUES FOR ETHOSOMES- *Preparation of ethosomes is done by using two methods.*
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6.1 By using Cold Method

Cold method is the most widely used procedure for the preparation of ethosomes. In this method, the phospholipids, drugs and other lipid compounds are dissolved in ethanol medium at a room temperature in a container. All the ingredients are mixed vigorously with the help of stirrer. Then add propylene glycol at 40 C during stirring process. In a separate container, heat the water at 30 C, add it to the mixture and stir for 5 minutes in a closed container. Size reduction of the ethosomes is done by using sonicator. Then the mixture is stored under refrigeration. [12,13]

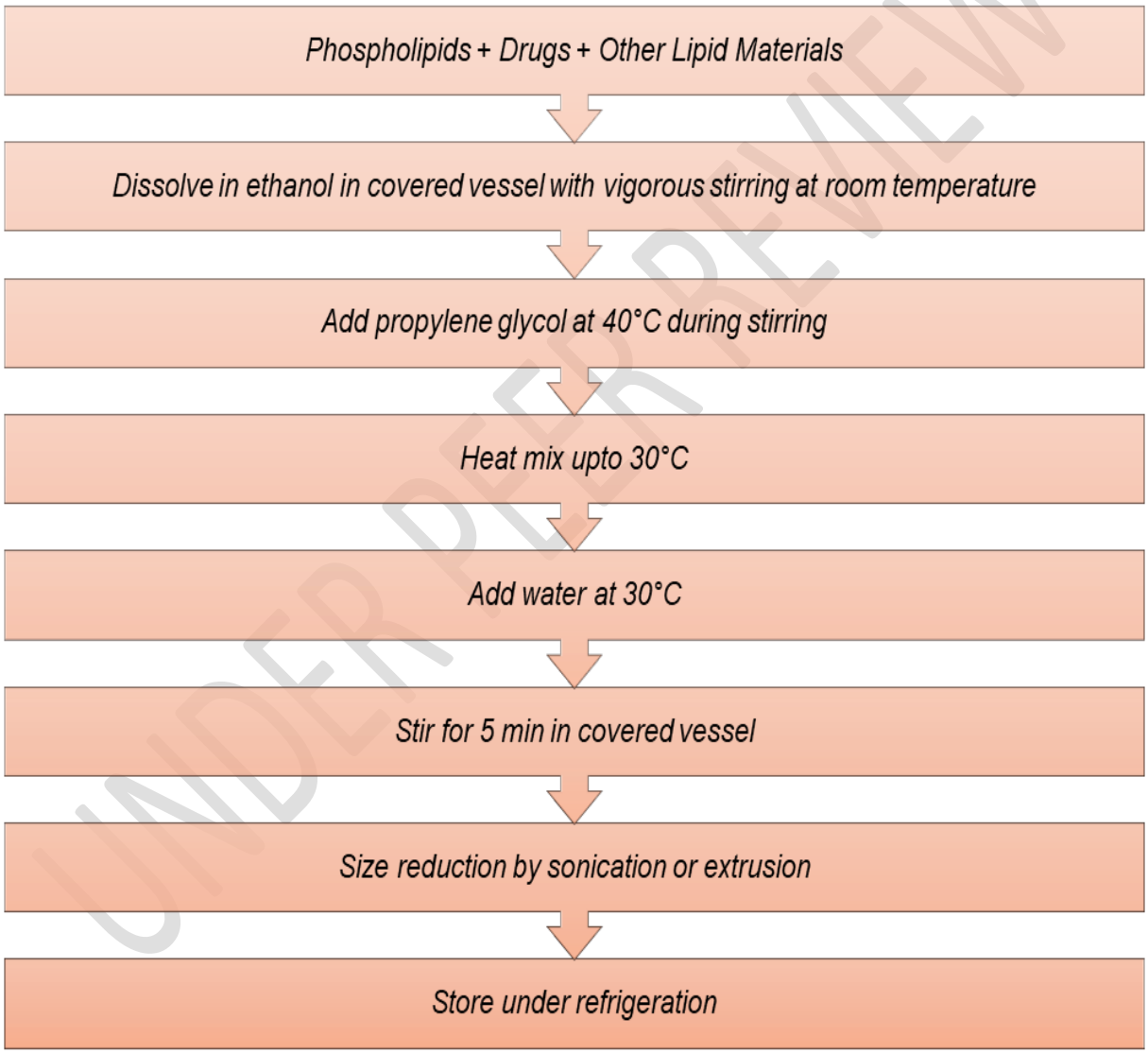

Figure 2: Ethosome Preparation Using a Cold Method [12]

6.2 By using Hot method

Hot method is the process which involves heating of the phospholipid which is dispersed in the water at 40°C. Heating is continued until the phospholipid is completely dispersed in water. In another container, mixture of ethanol and propylene glycol is heated at 40°C.When both the containers temperature reaches 40°C, then mix organic phase into the aqueous phase. Based on the drugs hydrophilic or lipophilic in nature, add suitable solvent accordingly and dissolve it (water or ethanol). Size reduction of ethosomes can be done by using probe sonication (direct sonication) techniques. [14,15]

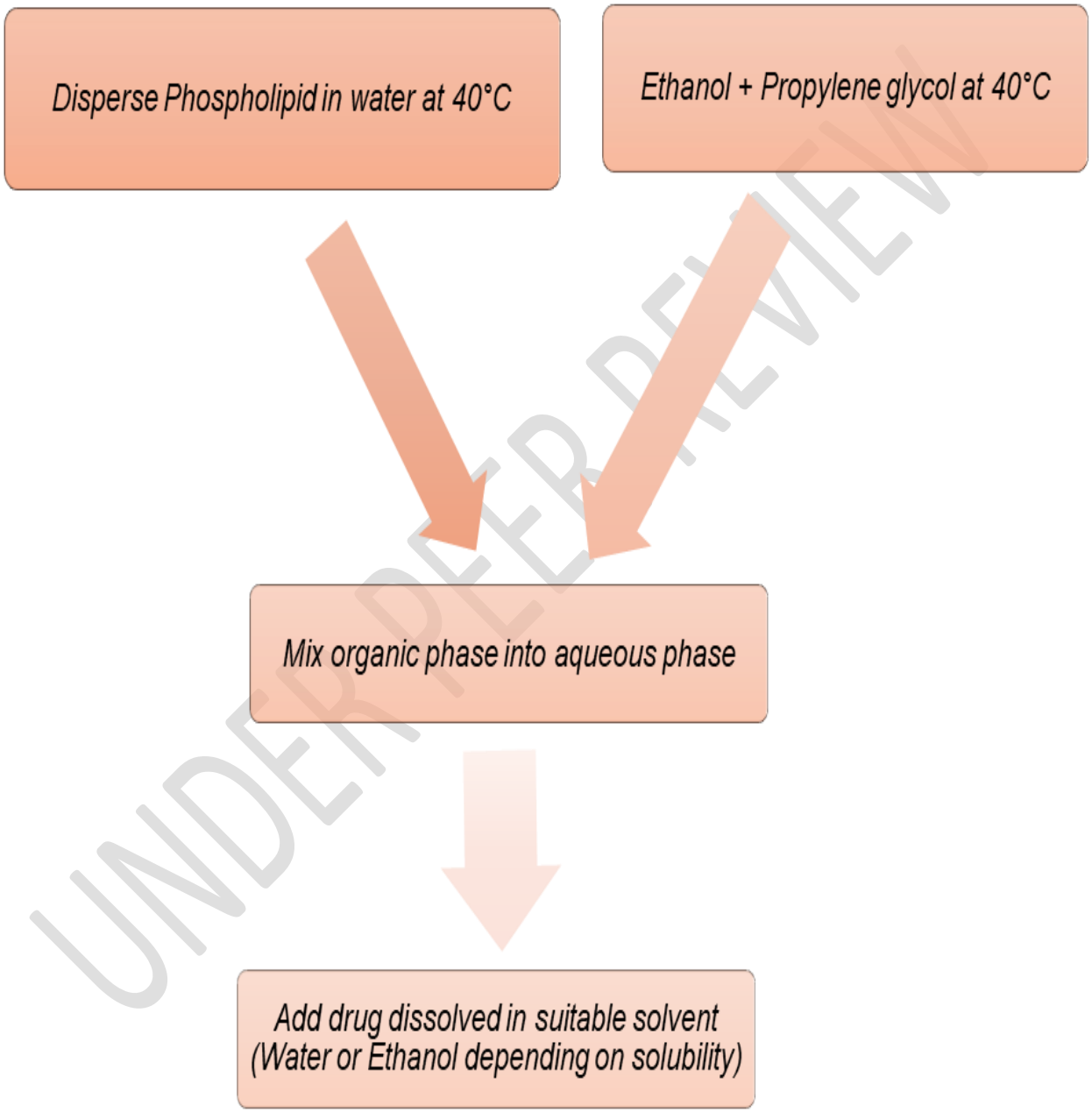

Figure 3: Ethosome Preparation Using a Hot Method [12]

7. EVALUATION OF ETHOSOMES

7.1 Study of Interactions between Vesicle and Filter Membrane by Using SEM

- *In this method, coating of filter membrane is done by using a suitable vesicular suspension (0.5 mL). Then the coated filter membrane is placed in Franz diffusion cell apparatus.*
- *After placing filter membrane in the Franz Diffusion cell apparatus, top portion of the filter membrane was open and exposed to air and bottom portion of the filter membrane was suspended in phosphate buffer (pH 6.5) and keep it for 1 hour.*
- *Later, the filters were removed and sample preparations is done for SEM analysis.*
- *The sample preparation for SEM analysis is done by a reagent called Karnovsky's fixative reagent at 04 C and also ethanol graded solutions is used for sample preparation. Later, the filters were analysed by SEM.* **[16,17,18,19]]**

7.2 Permeability studies

- *In skin permeability studies, rodent animals are used like rats or guinea pigs. In this method, the hair follicles of the rodent animals are carefully trimmed (2mm).*
- *Now with the help of scalpel, the abdomen epidermis was separated and from that the connective tissue was extracted.*
- *The removed skin was kept on a sheet of aluminium foil. Slowly remove the dermal side.*
- *The temperature is maintained at 32 degrees Celsius plus or minus 1 degrees Celsius. 10 mL saline solution was kept in the receptor compartment. The donor and receptor compartments were partitioned by excise skin. The ethosomal formulation was applied to the skin's epidermal surface (1.0 mL). High-performance chromatography test was performed to evaluate samples (0.5 mL).*
- *Collect the samples respectively with time-intervals: 2,4,6,8,12,16,20,24 hours accordingly.* **[20]**

7.3 Stability Study

Storing the vesicles at cool temperature (04°C). The various stability studies of ethosomes includes, entrapment efficiency, vesicular size and zeta potential. **[21]**

7.4 Drug content studies

Drug content studies can be conducted by using HPLC method to determine the amount of drug uptake. **[22]**

7.5 HPLC Assay

- *It is an invitro permeation assay which measures the amount of drug permeated in the receptor compartment and assay is conducted by using HPLC method.*
- *In this method methanol: distilled water: acetonitrile is used as mobile phase and ratio of mobile phase is 70:20:10 vol/vol.*
- *Flow rate of mobile phase pass through the column is at 1ml/min.*
- *Column used in this method is octadecylsilane(C-18).* **[23,24]**

7.6 **Statistics data analysis**

Ethosomes can also be Evaluated by various statistical method one of the methods that is widely used is the ANOVA technique which preferably used to examine the statistical data. Obtained, followed by a studentized ranged test. the results were interpreted using PRISM statistical software. [25,26]

8. APPLICATION OF ETHOSOMES

Ethosomes are mainly used in the replacement of liposomes and they help to transport both hydrophilic and lipophilic drug which are impermeable in nature through transdermal route. [27,28,29,30]

8.1 **Pilosebaceous targeting**

In addition, sebaceous gland and follicle have significant potential to deliver the drug percutaneously. Hair follicles act as a transport carrier and delivers the drug sytemically. For example, a lipophilic drug like minoxidil used in the treatment for baldness and minoxidil is applied topically into the scalp through pilosebaceous delivery. Pilosebaceous units can be used as depots, for localised therapies, especially for follicle-related disorders like acne. [31,32]

8.2 **Hormone delivery**

Hormone delivery can also be done in ethosomal formulation but several issues are observed during oral hormone administration like low drug absorption, and various side effects based on dose. Furthermore, oral hormone preparations heavily depend on patient compliance with these side effects. For example, Testosome is a testosterone-based ethosome. Compared to normal transdermal patch containing testosterone (Testoderm), it has higher rate of skin penetration of testosterone into the skin layer and later found out that the ethosomal formulations are more efficient for hormone delivery. [33]

8.3 **Transcellular Delivery**

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. [34,35]

8.4 **Delivery of Biomolecules**

Delivery of large biomolecules like proteins and peptides are difficult to administer orally and can cause degradation of the drug molecule in the GIT. When it comes to oral delivery issues, non-invasive protein administration is a better option. Researchers found out that insulin invasive protein administration reduces BGL and later results showed that the insulin which was delivered through transdermal route reduces BGL in both diabetic and non-diabetic conditions in the rats (up to 60%). An insulin injection from a control formulation, on the other hand, does not affect BGL. [36]

8.5 **Delivery of anti-arthritis drug**

Anti-arthritic drugs can also be delivered through topical route of administration at specific site and also the delivery of anti-arthritic drug through topical route is better alternative than oral drug delivery. Certain complications are observed in oral drug delivery. For example, Cannabidiol is a drug used for the treatment of rheumatoid arthritis. It is recently found out that poor drug absorption and first-pass metabolism and degradation are all problems related to oral drug 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 Cannabidiol which is present in the ethosomal formulation improves skin permeation. [37,38]

8.6 ***Delivery of Antibiotic Drugs***

Antibiotic drugs can also be delivered topically and has better option for enhancing therapeutic efficacy. Oral drug therapy used in the past has resulted in several allergic responses and several adverse effects. External preparations have several issues like poor drug permeation into the skin layer. As a result, ethosomal formulations are preferred to distribute enough antibiotic drugs into the skin layer. It can easily penetrate the epidermis layer of the skin, delivering significant amount of medication to the skin layer. [39,40,41]

8.7 ***Cosmeceutical application of ethosomes***

Ethosomes can be used in the preparation of cosmeceutical due to its stability, non-irritant (chemicals) nature to the skin, and improves better skin permeation. [42,43,44]

8.8 ***Topical delivery of DNA***

Many infections from the environment enters the body through the skin. As a result, skin shows defense mechanism that is effective immunologically and capable of gene expression. The most commonly used application for ethosomal formulation is to transport the DNA topically into the skin layer for gene expression. Ethosomes can also be used as carriers for gene expression. Hence ethosomes can be used to administer vaccine via transdermal route of administration. As a result of the increased ethosomal skin penetration capacity, these dosage forms can now help immunize drugs. [45,46]

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. 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 minimum adverse effects. When it comes to delivering medications to the skin, ethosomes outperform liposomes and hydroalcoholic solutions. Ethosomes has better advantage over liposomes and hydroalcoholic solutions. It has low risk profile, ethosomes can also be used for preparation of cosmeceuticals. It can also be directly marketed due to its non-invasive properties. Ethosomal formulations allows the drug to reach into the skin layer before entering the systemic circulation. Large biomolecules can also be delivered like proteins and peptide molecules. 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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