

Transethosomes: A Comprehensive Review of Ultradeformable Vesicular System for Enhanced Transdermal Drug Delivery System

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Abstract: Transdermal drug delivery systems (TDDS) have gained significant attention as a non-invasive and effective approach for systemic and localized drug delivery. However, the primary limitation of this route is the barrier function of the stratum corneum, which restricts drug permeation. To overcome this challenge, ultra-deformable vesicular carriers such as transethosomes have been developed. Transethosomes are advanced lipid-based nanocarriers composed of phospholipids, high concentrations of ethanol, and edge activators, integrating the advantages of both ethosomes and transfersomes. These vesicles exhibit superior deformability, enabling them to penetrate through narrow intercellular pathways of the skin and deliver drugs into deeper layers. The presence of ethanol enhances skin permeability by fluidizing the lipid bilayer, while edge activators improve vesicle flexibility and stability. As a result, transethosomes demonstrate enhanced drug entrapment efficiency, improved permeation, and increased bioavailability compared to conventional liposomes and other vesicular systems. Various preparation techniques such as cold method, hot method, thin-film hydration, and reverse-phase evaporation are employed for their formulation. Key characterization parameters including particle size, polydispersity index, zeta potential, and entrapment efficiency are critical for evaluating their performance. Transethosomes have been widely explored for the delivery of diverse therapeutic agents such as antifungal, anti-inflammatory, antihypertensive, and anticancer drugs. Despite their promising advantages, certain limitations such as formulation instability and potential skin irritation due to high ethanol content need to be addressed. Overall, transethosomes represent a novel and efficient ultra-deformable vesicular carrier system with great potential to enhance transdermal drug delivery and improve therapeutic outcome.

Key Words: Transdermal drug delivery systems, Transethosomes, Formulation instability, potential skin irritation, ultra-deformable vesicular carrier.

1. Introduction

Skin Membrane and Its Barriers

Skin is the largest organ in the human body, making up around 16% of the total body weight. On average, an adult skin weighs approximately 8 pounds and has a surface area of about 22 square feet. The skin's thickness is usually about 2 mm,

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though this can change based on where it is on the body, as the skin's texture and makeup can be different in various areas. The skin is made up of several layers, such as the epidermis, which includes the stratum corneum, stratum lucidum, stratum granulosum, stratum mucosum, and stratum germinativum, along with the dermis and subcutaneous tissues. It also contains sweat glands, blood vessels, sebaceous glands, lymph vessels, collagen, elastin, and hair. Blood vessels are found in both the dermis and subcutaneous tissues.

Epidermis

The epidermis is composed mainly of keratinocytes and forms the outermost layer of the skin. It consists of two distinct layers: the viable layer and the non-viable layer. The non-viable layer is known as the stratum corneum. This layer is made up of 10 to 25 layers of dead cells called corneocytes, which are heavily keratinized and held together by a matrix of lipid bilayers. The stratum corneum acts as the primary barrier of the skin, preventing substances from penetrating into the deeper layers.

When a topical product is applied, the active ingredient must pass through the stratum corneum. The slow movement of the substance through this dead, outer layer is what limits how quickly it can be absorbed. As a type of membrane, the stratum corneum is hydrophobic, meaning it repels water. The rate at which substances, such as organic non-electrolytes with both low and high molecular weights, pass through this layer is generally determined by its structure.

Dermis

The dermis provides strength and flexibility to the skin, and its thickness varies depending on the area of the body. These properties are due to its composition, which is mostly made up of 70% collagen and elastin fibers.

Hypodermis

Below the dermis is the hypodermis, also known as the subcutis. This layer contains a large number of fat cells that serve as cushions for nerve endings and blood vessels. The hypodermis is typically between 4 and 9 millimeters thick, although its actual thickness can vary from person to person and also depends on the specific body region.

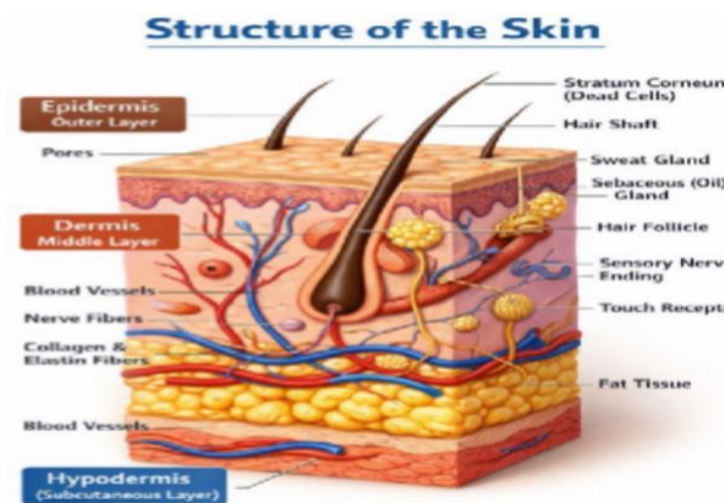


Fig 1: structure of skin

Skin Barriers

The stratum corneum serves as a significant barrier that prevents drugs from penetrating the skin. It is the outermost layer composed of tightly packed keratinocytes arranged in a dense structure, which effectively blocks the passage of most endogenous substances. This barrier plays a crucial role in protecting the body by preventing the entry of most drugs and foreign materials, while also helping to maintain the body's internal balance.

The structural features of the stratum corneum enable drugs to pass through the skin via three distinct pathways: intercellular, transcellular, and appendageal. These pathways are illustrated in Figure 2. In the intercellular pathway, drug molecules move between the tightly packed, lipophilic (fat-soluble) cells of the stratum corneum, traveling through the complex lipid matrix that exists between the keratinocytes. This pathway is generally considered the most important for drug delivery. Typically, small and lipophilic molecules use this route to transport drugs through the skin. In the transcellular pathway, drugs pass directly through the cells of the stratum corneum, specifically the keratinocytes.

The structural features of the stratum corneum enable drugs to pass through the skin via three distinct routes: the intercellular, transcellular, and appendage pathways. These pathways are illustrated in Figure 2. They move through the complex network of lipids that lies between the keratinocytes, and this route is considered the most significant for drug delivery.

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Generally, small molecules that are lipophilic, meaning they dissolve in fat, can use this pathway to transport the drug. In the transcellular pathway, drugs pass directly through the cells, specifically the keratinocytes, of the stratum corneum.

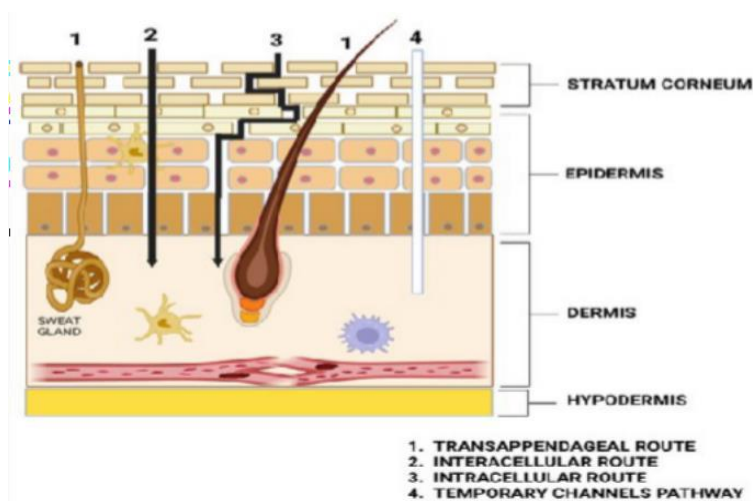


Fig 2: Pathway of transdermal drug delivery

This route involves passing through both the lipid-based membranes and the water-based parts of cells. It is a difficult path for delivering drugs because of the cellular components in between. The appendageal pathway allows drugs to be delivered by using sweat glands and hair follicles.

Hair follicles and sweat glands cover a smaller area of the skin's surface, but they offer an alternative way to avoid the outermost layer of the skin, known as the stratum corneum. Therefore, this route allows the platform to deliver nanoparticles through this pathway.

The dermis, which has different thicknesses in various parts of the body, mainly gives the skin its strength and flexibility because it contains a high amount of collagen (around 70%) and elastin fibers.

Its main role is to eliminate toxins via the lymphatic system. The hypodermis, which is the deepest layer of the skin, acts as a cushion between the skin and the tissues beneath it. It is mainly made up of fat cells. These cells help in insulating the body from heat, protecting against shocks, and serving as paths for nerves and blood vessels to pass through.

Vesicular Drug Delivery System

Oral delivery systems are commonly used to give medicines because they offer many advantages, such as being easy to use, convenient for patients, and generally safe. However, there are some issues, such as the drug not being stable in the digestive system and being affected by first-pass metabolism. Enzymes in the body or the acidic environment of the stomach can cause the drug to break down. Additionally, the drug's ability to pass through the intestines and dissolve in bodily fluids may also limit how much of it is absorbed, as these processes can act as barriers to effective absorption.

Vesicular systems offer an alternative method for drug delivery that reduces side effects compared to traditional physical and chemical approaches. Various transdermal delivery techniques, such as solid lipid nanoparticles (SLNs), lipid nanovesicles, and nanostructured lipid carriers (NLCs), have distinct characteristics that make them suitable for drug delivery through the skin.

SLNs and NLCs are not as stable or efficient in drug loading as lipid nanovesicles like liposomes and niosomes. Solid lipid-based SLNs may have a lower capacity for drug loading and might release the drug when they crystallize. On the other hand, NLCs, which are a type of second-generation lipid nanocarrier, mix liquid and solid lipids to improve stability and reduce drug leakage. Due to their small size and lipid-like structure, lipid nanovesicles can effectively deliver both water-soluble and fat-soluble drugs through the skin. Vesicular drug delivery systems are lipid-based structures with one or more layers in the presence of water, designed to transport drugs through the stratum corneum while minimizing systemic side effects. These systems include ethosomes, liposomes, niosomes, and transferosomes. One type of second-generation vesicular system is deformable liposomes, which incorporate edge activators or penetration enhancers.

These vesicles offer greater flexibility, elasticity, and ability to penetrate the skin compared to conventional liposomes. They are used in various drug delivery applications, such as the administration of proteins, ophthalmic drugs, vaccines, small molecules, and monoclonal antibodies. Ethosomes, a third-generation nano-vesicular system composed of phospholipids and 10–50% ethanol, enhance the transport of both hydrophilic and hydrophobic drugs. Their key feature is the ability to permeate the skin without causing allergic or harmful reactions. Ethosomes can be further grouped based on their composition and method of skin penetration. By utilizing the unique properties of vesicular systems, formulation scientists can address many limitations of traditional transdermal delivery methods and improve patient compliance and treatment effectiveness.

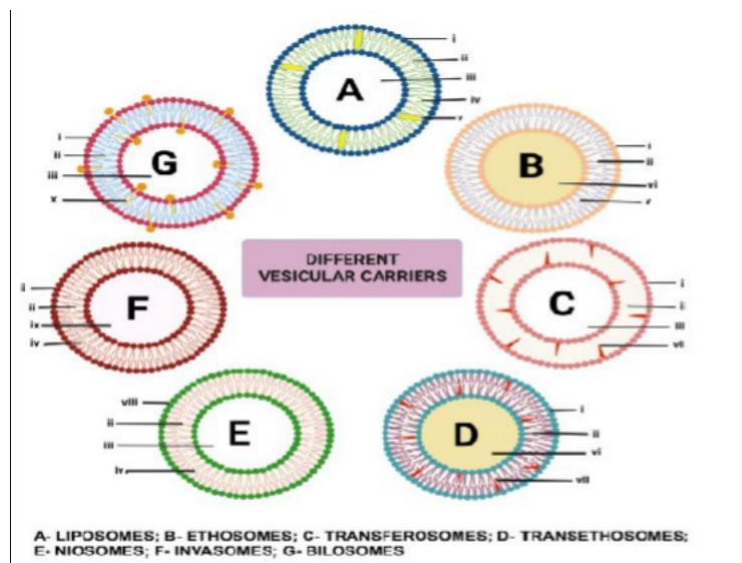


Fig 3: shows the structure and composition of different vesicular carriers

2. Transethosomes

Structure of Transethosomes

To combine the benefits of transferosomes and ethosomes, a new delivery system called transethosomes was developed. Transethosomes, which are part of the third generation of vesicular carriers, are more effective than transferosomes and ethosomes due to their improved drug release properties. Figure 4 illustrates the structure of transethosomes. This advanced system incorporates characteristics from both vesicle types to enhance transdermal drug delivery, and it can also be used for ophthalmic, transvaginal, and pulmonary applications. Transethosomes provide notable advantages in terms of patient compliance, stability, and biocompatibility. They have an irregular spherical shape and greater deformability and flexibility compared to ethosomes and transferosomes, enabling them to return to their original form after passing through cells. Transethosomes are composed of 2–5% phospholipids, edge activators, 30–40% ethyl alcohol (ethanol), and water, which helps improve skin penetration.

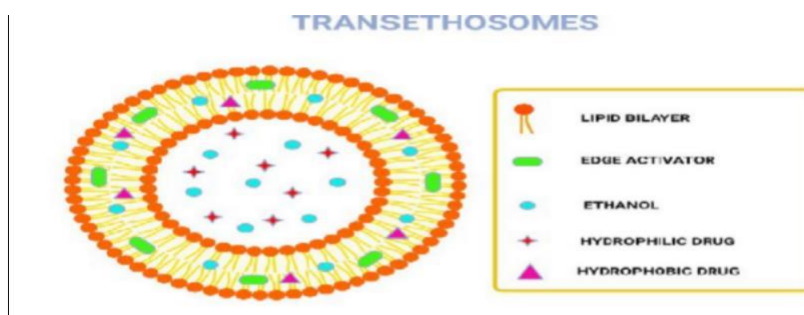


Fig 4: structure of transethosomes

Efficient drug delivery .Because phospholipids are naturally found in eukaryotic cells and are both biocompatible and biodegradable, they are used as carriers that can encapsulate drugs. This helps the drugs to avoid the first-pass metabolism in the liver and also helps them to escape detection by the reticuloendothelial system, making them useful for both topical and systemic use. These carriers also help drugs to enter the body more effectively and work better with the body's natural processes. Ethanol and surfactants, also known as edge activators, help the vesicles to be more flexible and easier to pass through the body's membranes. Surfactants can change the structure of the outer layer of skin, making it more flexible and easier for the drug to penetrate. Cholesterol is added to the formulation to make the vesicles more stable, control how fast the drug is released, reduce the need for taking the drug frequently, and help to minimize side effects in other parts of the body.

Transethosomes can hold both water-soluble and fat-soluble drugs within their inner water layer or outer phospholipid layer.

These transethosomes can be modified by adding chemicals such as polymers and carbohydrates to help them target specific parts of the body more effectively. They can also be tailored to work better in the lungs by improving how well the drug moves through lung tissues, which helps in making the treatment more effective. When used in the vaginal route, transethosomes bypass the gastrointestinal tract and the first-pass metabolism, which increases the amount of drug that reaches the bloodstream

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and reduces possible side effects. However, transethosomes might have some issues, like causing skin irritation due to the high amount of alcohol they contain. Despite these challenges, transethosomes are easy to make on a large scale and are a promising choice for industrial use.

The Composition of Transethosomes

Phospholipids are important in making these vesicles and can be made naturally. Natural phosphoglycerides or phospholipids can be found in sources like soybeans, egg yolks, and sunflower seeds. These natural phospholipids help to make the outer layer of the skin more flexible, which in turn helps drugs to pass through more easily. Saturated (hydrogenated) phospholipids also help to repair the skin's natural barrier, making the drug stay longer in the skin. There are different types of phospholipids, such as phosphatidic acid, phosphatidylcholine, phosphatidylinositol, phosphatidylethanolamine, phosphatidylserine, and phosphatidylglycerol. Natural phospholipids are less stable than synthetic ones because they contain unsaturated chains. Synthetic phospholipids include examples like dimyristoyl phosphatidylcholine, distearoyl phosphatidylcholine, and dipalmitoyl phosphatidylcholine. The type and amount of phospholipids used affects how much drug is carried, the size of the vesicles, their charge, how well they can penetrate the skin, and how stable they are. Typically, phospholipids are used in concentrations from 0.5% to 5% when making transethosomes. Ahmed et al. found that a higher ratio of drug to phospholipid leads to larger vesicles, which can improve how well the drug can pass through the skin. Transethosomes made with unsaturated phospholipids have better skin penetration and drug trapping efficiency compared to those made with saturated phospholipids. Ethanol helps improve the performance of transethosomes by making their membrane softer and more deformable, and it also helps in drug penetration.

The amount of ethanol used has a big impact on the size of the particles, their charge, how well they can penetrate the skin, how efficiently they carry the drug, and their overall stability. Ethanol concentrations in transethosomal formulations usually range from 10 to 50%. However, at higher concentrations, ethanol can make phospholipids dissolve more easily, which may reduce how well the drug is trapped due to its interaction with the lipid layer. Adding cholesterol to the mixture can help improve the stability and flexibility of the transethosomes, even at high ethanol levels. Tamer et al. showed that formulations with 10 to 20% ethanol had better stability and flexibility compared to those with 30 to 50%. Similarly, Abdulbaqi et al. reported that increasing the ethanol concentration from 10 to 30% (w/v) leads to smaller vesicles. However, going beyond this range can cause the layers of the vesicles to break down, slightly increasing their size.

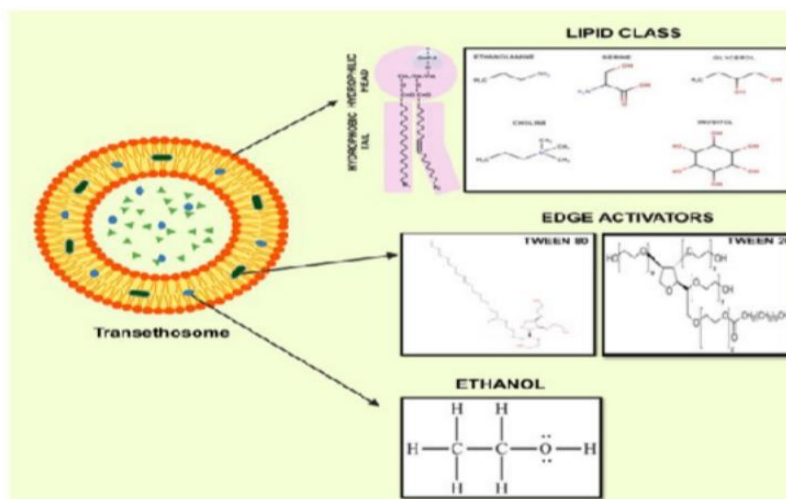


Fig 5: Composition of transethosomes

Cholesterol

Cholesterol, when used in transethosomal formulations, helps improve the stability of the system and enhances the efficiency with which drugs are trapped inside the vesicles.

It is also observed that including cholesterol may lead to an increase in the size of the vesicles. In various studies, a concentration of 3% cholesterol has been used in formulations to stabilize the vesicular system by preventing the particles from clumping together. Stabilizers are important in transethosomal formulations because they help prevent particle aggregation, maintain the vesicle size and cohesion, and extend the shelf life of the formulation. Cholesterol is the most commonly used stabilizer as it offers structural support to the transethosomal system. It was found that adding cholesterol to transethosomal formulations improved their strength and flexibility, especially when the concentration of ethanol was higher. However, increasing the amount of cholesterol can reduce the efficiency of drug encapsulation due to its limited solubility. Cao et al. examined the effects of different ratios of sodium deoxycholate (SDC) on the stability and drug release properties of transethosomes carrying the antiparasitic drug ivermectin. Their results showed that SDC concentrations above 1% reduced the stability of the transethosomes and led to drug leakage, which could affect the effectiveness of the treatment.

Edge Activator

Edge activators in transethosomal formulations improve the deformability and flexibility of phospholipid vesicles. The type and amount of edge activators influence the permeability of the drug. A high amount of edge activators may increase the zeta potential, which suggests better stability and a threefold improvement in encapsulation efficiency. This is probably because the interaction between deoxycholate anions and drug cations reduces the polarity. Common surfactants used as edge activators include Polysorbate 20, sodium cholate, dipotassium glycyrrhizinate, bile salts, Sorbitan laurate 80, 9-Octadecenoic acid, and Polysorbate 80. In a recent study, Fe-chlorophyllin transethosomes were made using surfactants like Sorbitan laurate 20, Polysorbate 20, Polysorbate 80, and Cetyl Stearyl Polyether-25 at low concentrations (0.1% and 0.3% w/v) along with phosphatidylcholine and 20% w/v ethanol. The transethosomes had a typical vesicle size ranging from 456 to 685 nm.

3. Mechanism

Transethosomes Mechanism in Skin

The mechanism of transethosomes in the skin involves how endogenous molecules can pass through the stratum corneum via either transcellular or intercellular pathways. The intercellular pathway is the main route for drug molecules to pass through the skin and reach the systemic circulation. Based on Fick's law of diffusion, drug movement through the skin typically occurs through a diffusion process. There are also other pathways in the skin, such as diffusion shunts and appendages, which help certain large, highly hydrophobic molecules or specific electrolytes to cross the skin membranes.

The lipidal layer within the stratum corneum, which has a high concentration of blood vessels, is essential for maintaining the skin's barrier function. Liposomal formulations cannot pass through the epidermal layers because they contain cholesterol. The compact structure of the stratum corneum causes the liposomes to break down, releasing the drug into the epidermis. Due to their ethanol-based and non-invasive nature, these formulations may allow medicines to penetrate deeper into the skin layers or even enter the systemic circulation without harming other cells.

Transferosomes have special properties that allow them to move through deeper tissue layers by traveling from the dry outer surface of the skin to the moist areas underneath, while maintaining their vesicular structure and adaptability. Through pores or hydrophilic channels between cells, transferosomes pass through the skin and deliver the full drug payload without breaking down. The unique properties of ethanol, such as its ability to deform and be flexible, enable ethosomes to deeply enter the skin, improving the absorption and penetration of drugs. Because of the lipophilic nature of the carbon tail in ethanol, it can easily pass through the lipid-rich areas of the bilayer. Initially, transethosomes attach to the surface of the stratum corneum and then enter it by widening the intercellular junctions.

The widening of these tight junctions allows transethosomes to pass through the skin. After topical application, transethosomes dry and partially dehydrate the skin during the first stage of penetration. The vesicles follow the local hydration gradient, moving fully into the more hydrated skin layers to maintain complete hydration. Two main mechanisms are responsible for the increased permeation of transethosomes into the skin.

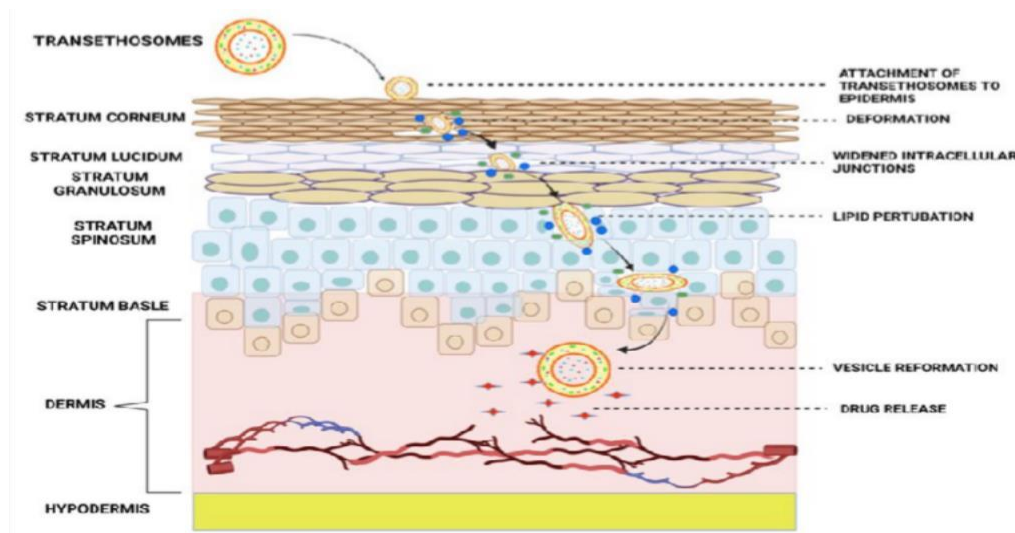


Fig 6: Mechanism of transethosomes -mediated skin permeation

4. Method of Preparation

- Cold method
- Hot method
- Reverse phase evaporation method
- Mechanical dispersion method

1. Cold Method

In this method, ethanol is first taken and phospholipid is added to it. The mixture is then stirred continuously at room temperature until the phospholipid dissolves completely. After that, propylene glycol is added to the solution and stirred thoroughly. The resulting mixture is then heated to a temperature of 30°C. At the same time, water is also heated to the same temperature. Both ethanol and water are used as solvents so that the drug can dissolve in whichever solvent it is more compatible with. Stirring continues for an additional five minutes. The suspension is then cooled to room temperature. In the final step, techniques such as extrusion or sonication are used to adjust the size of the vesicles. The prepared ethosomal vesicles are then stored in the refrigerator. The cold method is illustrated in Figure 7.

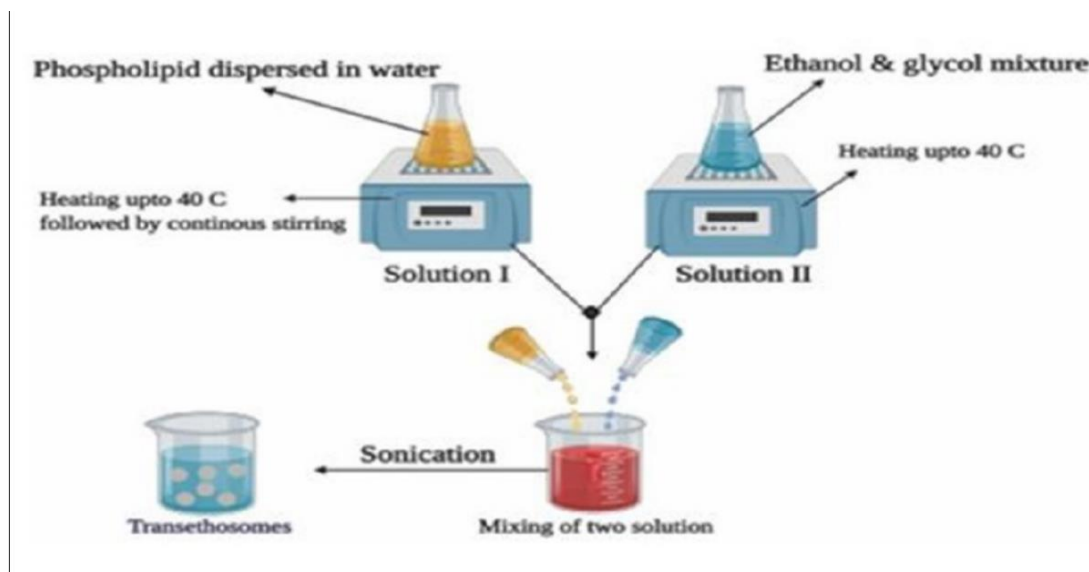


Fig 7: cold method

2. Hot Method

The heating process is continued at the same temperature until a colloidal solution is formed. At the same time, in another container, two additional solvents—glycol and ethanol—are added and heated to 40°C. Once both mixtures have reached 40°C, the organic phase is added to the aqueous phase. Stirring is carried out for the next five minutes, after which the vesicle suspension is cooled to room temperature. Depending on the drug's ability and affinity to bind with hydrophilic and hydrophobic solvents, it either dissolves in water or in ethanol. The desired size of ethosome vesicles can be easily achieved using methods such as extrusion or sonication. The hot method is illustrated in Figure 8.

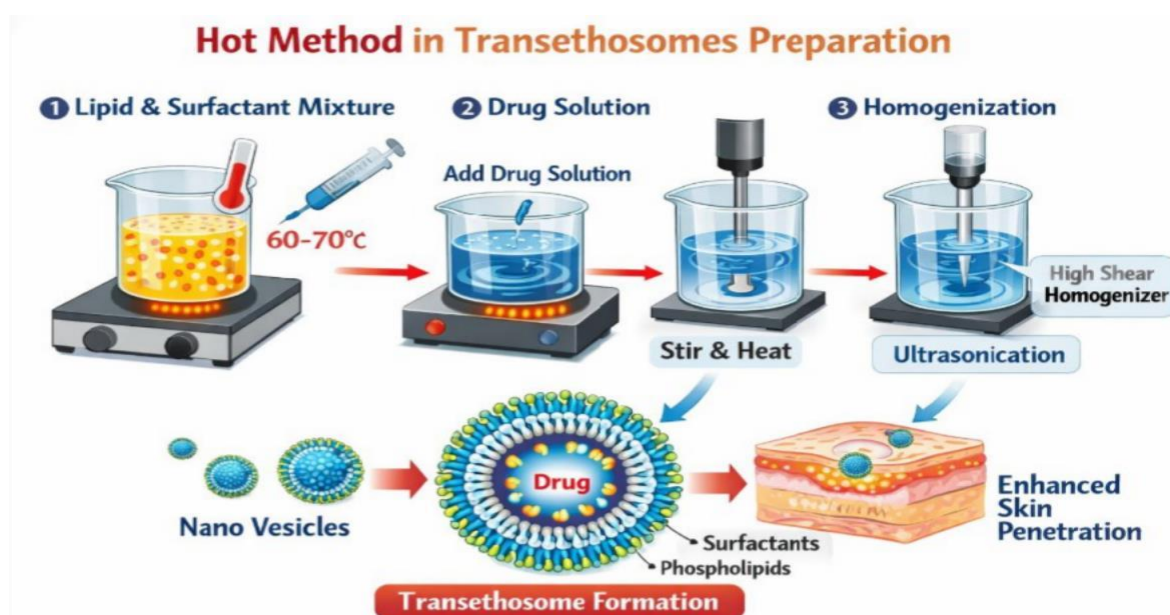


Fig 8: Hot method

3. Reverse Phase Evaporation

This method involves mixing lipids in ethanol, which serves as the organic solvent. The aqueous phase contains edge activators. The organic phase is then combined with the aqueous phase. The resulting mixture is placed in an ultrasonic bath to separate the two phases. Afterward, the gel is formed by removing the organic solvent under low pressure. The lipid layer is then encapsulated, and the liquid is stirred before being filtered. The Reverse Phase Evaporation method is illustrated in Figure 9.

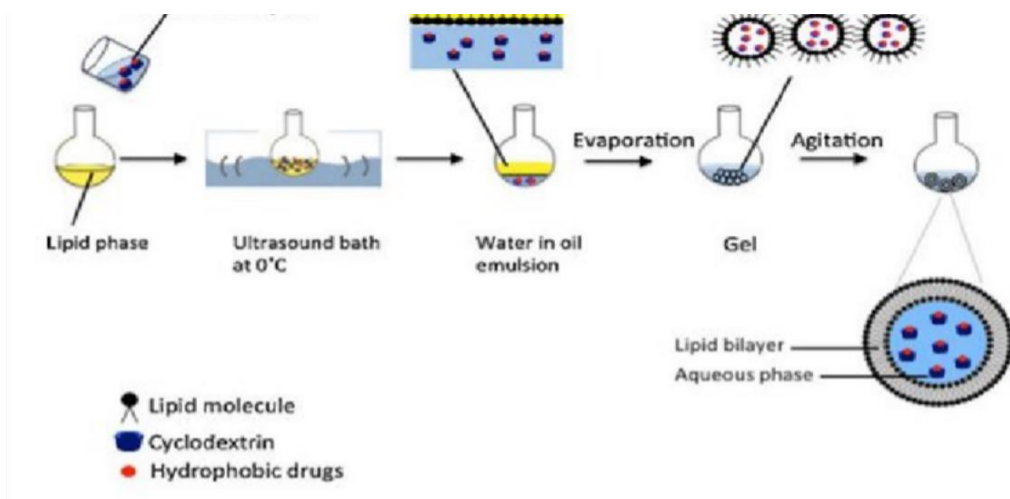


Fig 9: Reverse phase evaporation

4. Mechanical Dispersion

This method involves mixing lipids in ethanol, which acts as an organic solvent. The aqueous phase contains edge activators. The organic phase is combined with the aqueous phase. The mixture is then placed in an ultrasonic bath to separate the two phases. After this, the gel is formed by evaporating the organic solvent under low pressure. The lipid layer is then encapsulated, and the liquid is stirred before being filtered. The Reverse Phase Evaporation method is illustrated in Figure 8.

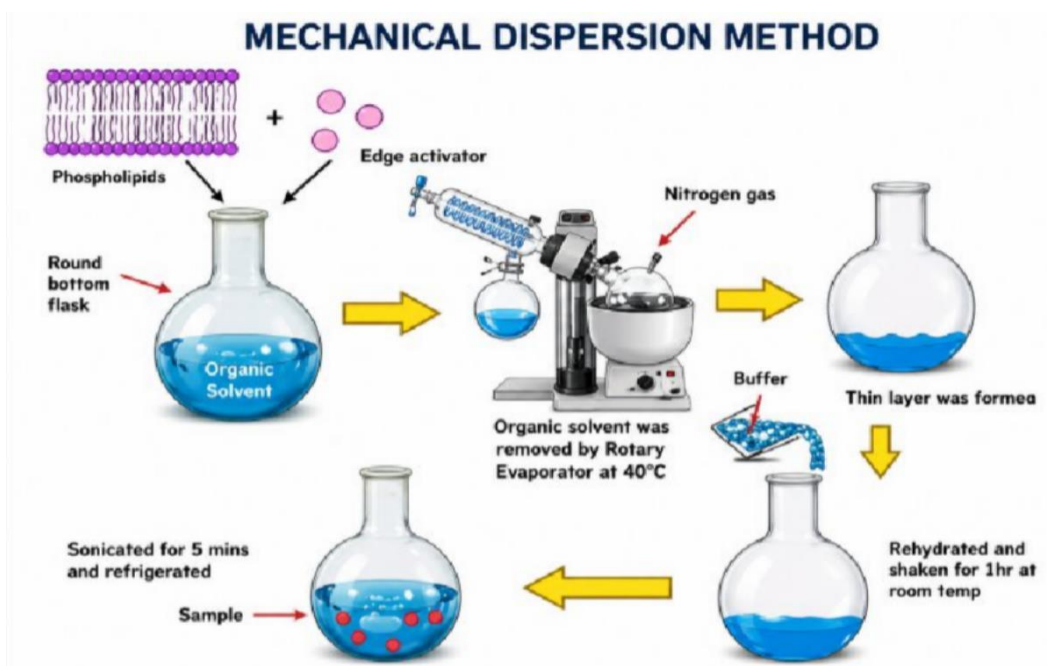


Fig. 8 mechanical dispersion method

5. Ethanol Injection Method

This method involves mixing lipids in ethanol, which acts as an organic solvent. The aqueous phase contains edge activators. The organic phase is combined with the aqueous phase. The mixture is then placed in an ultrasonic bath to separate the two phases. After this, the gel is formed by evaporating the organic solvent under low pressure. The lipid layer is then encapsulated, and the liquid is stirred before being filtered. Phospholipids, such as phosphatidylcholine, and various drugs are dissolved in ethanol. The mixture is continuously stirred at 35°C to form the organic phase. This organic phase is then slowly

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added to an aqueous phase made up of water and an edge activator. Continuous stirring is maintained to ensure a uniform solution. Ethanol stays in the final solution and does not evaporate, as it remains inside the glass container. Formulated Eugenol-Loaded Transethosomal Gel for Treatment of Atopic Dermatitis The study used the ethanol injection method to prepare transethosomes. Phospholipon 90G, Eugenol, and Tween 80 were dissolved in the organic solvent ethanol. The aqueous phase consisted of a phosphate buffer with a pH of 7.4 and was maintained at a temperature of 50°C. Once both the organic and aqueous phases were prepared, the organic phase was slowly added to the aqueous phase while continuous stirring was kept.

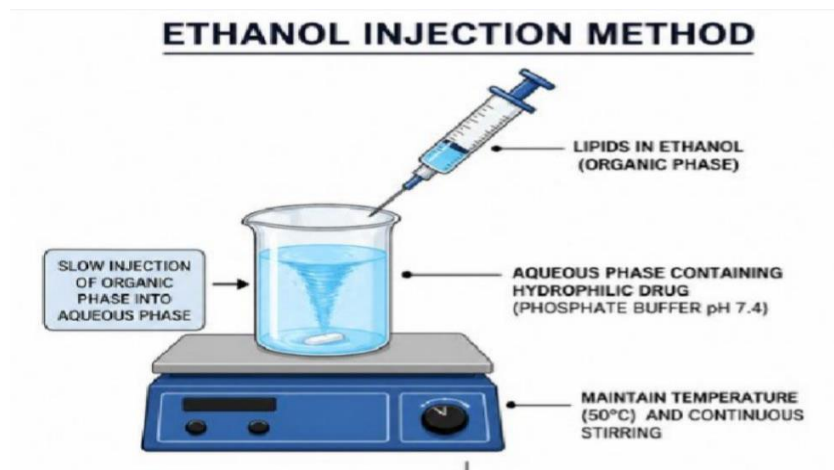


Fig.9 Ethanol injection method

5. Evaluation of Transethosomes

1. Entrapment efficiency

The entrapment efficiency is expressed as the percentage entrapment of the drug added. This can be measured by ultra-centrifugation technique. % EE can be expressed as:

$$\% \text{ Entrapment efficiency} = \frac{\text{Drug loading}}{\text{Theoretical drug loading}} \times 100.$$

2. Transmission electron microscopy

TEM was used for imaging of the transethosomes vesicles. Transethosomal dispersion (selected formulation) was diluted tenfold utilizing distilled water, then a drop was placed on a copper grid with a 300-mesh carbon coating and allowed to sit for a minute to allow some of the vesicles to stick to the carbon substrate. A piece of filter paper was used to remove extra dispersion, and the grid was then rinsed twice in deionized water for 3-5 seconds. The sample was then examined under a microscope utilizing 10–100× magnification and an accelerating voltage of 100 kV.

3. Zeta potential and vesicle size

In colloidal dispersion, electrostatic attraction and repulsion are measured by the zeta potential. Zeta potential can also give details about the surface attraction. Dynamic light scattering (DLS), computerized inspection system, and photon correlation spectroscopy (PCS) can be used to evaluate particle size and zeta potential.

The vesicular diameter can also be determined by DLS as particles having different size are scattered by the light at different wavelengths. TE's formulation having a particle size of less than 300nm can penetrate the stratum corneum layer. A high zeta potential in the dispersion medium suggests highly charged particles, which prevents coagulation, aggregation, and flocculation. When the zeta potential is low, attraction dominates the repulsion and coagulation occurs.

4. In vitro release

The quantity of drug release can be studied using dialysis bag method. In this method, the transethosome formulation is loaded in dialysis membrane. The loaded membrane is taken into conical flask containing buffer solution and is incubated. At given time intervals, aliquots are withdrawn and centrifuged by using mini column centrifugation. The free drug is assessed by suitable method.

5. Interaction studies by using FTIR spectroscopy

The drug-excipients interaction was studied using a Fourier transform infrared spectrophotometer (FTIR). IR spectra for drug and powdered transethosomes were recorded in a Fourier transform infrared spectrophotometer (FTIR 1615, Perkin Elmer, USA) with Potassium Bromide (KBr) pellets. The spectra were scanned over the 3600 to 400 cm⁻¹ range.

6. Drug content

UV spectrophotometer can be used to measure the drug content of transethosomes. This can also be measured quantitatively by a modified high-performance liquid chromatographic method (HPLC).

7. Stability study

The stability testing is done to guarantee the quality, safety, and effectiveness of the active medication ingredient in dosage forms throughout storage. For six months in a stability chamber, transethosomal gel formulations were kept in tightly closed, amber-coloured glass containers sealed with aluminium foil at three different temperatures: room temperature ($25^{\circ}\text{C} \pm 2^{\circ}\text{C}/60\% \text{RH} \pm 5\%$), refrigerator temperature ($4.0^{\circ}\text{C} \pm 1.0^{\circ}\text{C}$), and accelerated temperature ($40^{\circ}\text{C} \pm 2^{\circ}\text{C}/75\% \text{RH} \pm 5\%$). The samples were taken out at the end of the first, second, third, and six months to assess the in-vitro drug release, pH, entrapment efficiency, and drug concentration.

8. Phase transition

Phase transition temperature is studied to understand the release of drug from the vesicles. It can be identified using Differential Scanning Calorimeter (DSC). Each sample is analysed at a temperature range under a constant nitrogen stream. With the help of differential thermal curves, the samples are compared.

9. Skin Permeation Determinations

The ability of the transethosomes to penetrate the layers of the skin can be determined by using confocal laser scanning microscopy (CLSM).

10. Surface Tension

The surface tension activity of a drug in aqueous solution can be measured by the ring method in a Du Nou ring tensiometer.

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