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Review Article

Evolution of Ethosomes in Pharmaceutical Sciences: From Basic Vesicles To Advanced Nanocarrier Systems

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A B S T R A C T

Transdermal drug delivery systems (TDDS) have emerged as an effective alternative to conventional drug administration by overcoming limitations such as poor bioavailability, gastrointestinal degradation, and hepatic first-pass metabolism. Among various vesicular carriers, ethosomes have gained significant attention due to their superior ability to enhance drug permeation through the skin. Ethosomes are soft, flexible phospholipid vesicles containing high concentrations of ethanol, which increase membrane fluidity and disrupt the lipid organization of the stratum corneum, facilitating deeper skin penetration. They can efficiently encapsulate hydrophilic, lipophilic, and amphiphilic drugs, enabling controlled and sustained drug release with improved therapeutic efficacy and patient compliance. Since their introduction by Touitou and co-workers in the late 1990s, ethosomes have been extensively investigated for the delivery of anti-inflammatory, antifungal, antiviral, hormonal, anticancer, and peptide-based therapeutics. This review highlights the history, composition, mechanism of skin penetration, methods of preparation, characterization techniques, advantages, limitations, and diverse pharmaceutical applications of ethosomes. Recent advancements, including Nanoethosomes, ligand-modified systems, and integration with hydrogels and microneedles, have further expanded their potential in targeted and personalized drug delivery. Overall, ethosomes represent a promising and versatile carrier system for enhanced transdermal and topical therapy.

Keywords: Ethosomes, Transdermal Drug Delivery System, Nanocarriers, Controlled Drug Release, Nanotechnology in Drug Delivery,etc.

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INTRODUCTION

The skin is the biggest organ in the human body and acts as a strong protective barrier against external environmental elements, viruses, and chemicals. (1,2) Although the oral route is still the most popular method of drug administration due to its convenience and patient acceptance, it has several limitations, including enzymatic degradation in the gastrointestinal tract, poor absorption, variable bioavailability, and extensive hepatic first-pass metabolism.(3,4) These limitations can drastically impair therapeutic efficacy and necessitate frequent dosage, thereby decreasing patient compliance.Transdermal drug delivery systems (TDDS) are becoming more popular in pharmaceutical research to address these problems.(6) Transdermal administration allows medications to penetrate the skin and reach systemic circulation without gastric degradation or hepatic first-pass metabolism.(6,7)

Transdermal administration has several advantages, including sustained drug release, higher bioavailability, reduced dose frequency, and increased patient compliance.(7,8) However, the stratum corneum, the skin's outermost layer, acts as a formidable barrier, limiting the entry of most therapeutic agents, particularly hydrophilic compounds and macromolecules.(9) Several methods have been developed to promote transdermal drug transport, such as chemical penetration enhancers, iontophoresis, sonophoresis, microneedles, and vesicular carrier systems.(10,11) Among these techniques, lipid vesicular carriers such as liposomes, niosomes, transferosomes, ethosomes, and transethosomes have demonstrated great potential.(12,13) Ethosomes are one

of the most effective vesicular carriers designed specifically for increased skin delivery.(14)

Ethosomes are soft, flexible phospholipid vesicles carrying a relatively high concentration of ethanol, which typically ranges from 20% to 45%, together with water andPropylene glycol is an example of a polyol.(14,15) Ethosomes differ from normal liposomes in that they have a high ethanol concentration, which gives them unique physicochemical qualities such as increased membrane fluidity, deformability, and skin permeability. (15,16)Ethanol alters the stratum corneum's highly ordered lipid structure, increasing permeability and allowing vesicles to penetrate deeper into the epidermal layers.(17) Touitou and colleagues established the concept of ethosomes in the late 1990s.(14,18) Since then, substantial study has shown that they can successfully deliver a wide range of therapeutic agents, such as anti-inflammatory medications, antifungal agents, antivirals, hormones, antibiotics, anticancer treatments, peptides, and cosmetic actives.(19,21) Ethosomes can hold both hydrophilic and lipophilic molecules, resulting in regulated medication release, longer therapeutic activity, and increased drug accumulation at target areas.(20,21)

Recent breakthroughs in nanotechnology have increased the potential applications of ethosomes. Nanoethosomes, ligand-modified ethosomes, and stimuli-responsive ethosomal systems are now being studied for targeted drug delivery and personalized treatment.(22,23) The coupling of ethosomes with microneedles, hydrogels, and wearable delivery systems has also yielded promising results in terms of therapeutic outcomes.(23,24)

This article gives a thorough overview of ethosomes, including their history, composition, mechanism of skin penetration, manufacturing methods, characterisation techniques, benefits, limits, and therapeutic uses in modern drug delivery systems.

ADVANTAGES OF ETHOSOMES

Ethosomes provide various benefits that make them appealing carriers for transdermal and topical medication delivery.

Advantages of the system include improved penetration across the skin barrier, enhanced bioavailability of poorly absorbed drugs, avoidance of hepatic first-pass metabolism, and the ability to encapsulate hydrophilic, lipophilic, and amphiphilic drugs. Additionally, the system provides controlled and sustained drug release, enhanced therapeutic efficacy, reduced systemic toxicity, improved patient compliance, and non-invasive administration. Other benefits include high drug loading capacity, greater stability than conventional liposomes, ease of preparation and scale-up, biocompatibility, biodegradability, suitability for cosmetic formulations, and potential for delivery of proteins, peptides, and nucleic acids.[12–18]

Disadvantages of Ethosomes

Despite several advantages, the system also presents certain limitations, including possible skin irritation due to high ethanol concentration, vesicle aggregation during long-term storage, drug leakage, and instability caused by temperature and humidity variations. In addition, higher manufacturing costs compared with conventional formulations, phospholipid oxidation leading to reduced shelf life, limited loading of some hydrophilic macromolecules, and restricted regulatory approval for many formulations remain important challenges.[2,11–16].

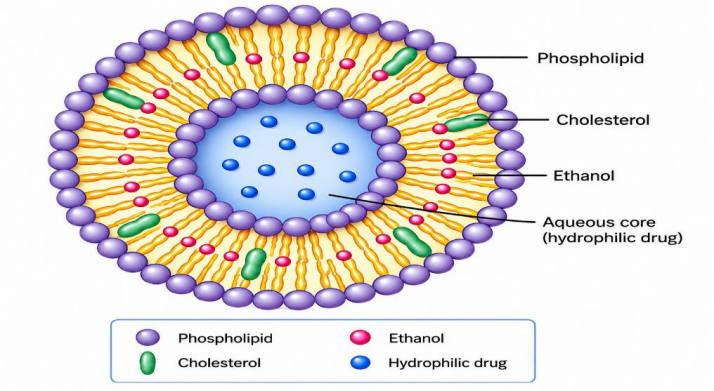

Figure 1: Structure of Ethosome

History of Ethosomes

Ethosomes represented a big step forward in the evolution of vesicular drug delivery systems.(14) Before ethosomes, ordinary liposomes were widely studied for transdermal medication delivery. However, its clinical applicability was limited due to insufficient penetration of the stratum corneum.(12,13) Researchers noticed that changing vesicular makeup could improve skin permeability and medication delivery.(14) Touitou and colleagues introduced the notion of ethosomes in 1996-1998.(14,18) They developed a new phospholipid vesicular carrier with high ethanol concentrations and tested its potential to carry medicines into deep epidermal layers and systemic circulation. (18) Their revolutionary research demonstrated dramatically enhanced transdermal distribution compared to ordinary liposomes.(14)

In the early 2000s, various studies were conducted to better understand the mechanism of action of ethosomes.(16,17) Researchers discovered that ethanol fluidizes both the phospholipid bilayer and the interstitial lipids of the stratum corneum, resulting in transient routes that allow vesicle

penetration.(17) Studies using testosterone, acyclovir, and trihexyphenidyl hydrochloride demonstrated the advantage of ethosomes for transdermal drug delivery.(19,20)

Between 2005 and 2015, ethosomal technology was widely accepted for the delivery of anti-inflammatory, antifungal, antiviral, and cosmetic drugs.(20,22) Researchers also created nanoethosomes with lower vesicle sizes and increased stability.(22) Advanced characterisation techniques such as dynamic light scattering (DLS), transmission electron microscopy (TEM), and confocal laser scanning microscopy (CLSM) have improved understanding of vesicle behavior and skin penetration mechanisms.(23)

Over the last decade, major attempts have been made to integrate ethosomes with nanotechnology and customized drug delivery systems.(22,24) Gel-based ethosomes, ethosomal patches, ligand-conjugated ethosomes, and stimuli-responsive ethosomes are examples of novel formulations under investigation for cancer therapy, hormone replacement therapy, and chronic inflammatory disease treatment.(24,26) Current research remains focused on enhancing the stability, scalability, and clinical translation of ethosomal drug delivery systems.

Table 1: History of Ethosome

Year	Milestone	Details
1996–1998	Introduction of Ethosomes	Touitou et al. developed ethosomes as ethanol-containing phospholipid vesicles for transdermal drug delivery.
Early 2000s	Expansion of Research	Studies demonstrated improved skin permeation of hormones, antivirals, and anti-inflammatory drugs.
2003–2010	Mechanistic Understanding	Ethanol–lipid interaction and enhanced penetration mechanisms were investigated extensively.
2010–2018	Pharmaceutical Applications	Ethosomes applied for topical and systemic delivery of various therapeutic agents.
2018–2023	Nanotechnology Integration	Development of nanoethosomes with improved stability and targeting capabilities.
2024–2025	Advanced Drug Delivery Systems	Combination with hydrogels, microneedles, and nanotechnology platforms for enhanced therapeutic efficacy.

Mechanism of Skin Penetration of Ethosomes

The exceptional transdermal distribution capability of ethosomes is due to a synergistic combination of ethanol, phospholipids, and skin lipids.(16,17) Unlike other vesicles, ethosomes have a unique mechanism that allows them to penetrate deep into the skin.(14)

The penetration mechanism includes two complementary processes:

Ethanol Effect

Ethanol enhances penetration by reacting with the stratum corneum's lipid molecules.(16,17) It inserts itself into lipid bilayers, disrupting their highly organized structure. This interaction lowers the lipid transition temperature (17), increases membrane fluidity, and causes transient skin barrier deficiencies. As a result, skin permeability increases dramatically.(16)

Ethosomal Effect

The high ethanol level makes the phospholipid vesicles more flexible and deformable. (14,15)These adaptable vesicles can pass through limited intercellular passageways in the stratum corneum and travel to deeper skin layers.(17,21) Once the vesicles reach the viable epidermis and dermis, they release the encapsulated medication, allowing for local or systemic therapeutic activity.(20,21)

Ethosomes have significantly stronger drug penetration than ordinary liposomes due to the combined action of ethanol induced skin permeabilization and vesicle-mediated transport.

Components of Ethosomes

The composition of ethosomes influences their physicochemical properties, stability, drug-loading capacity, deformability, and skin penetration ability. Ethosomes are largely made up of phospholipids, ethanol, and water, with optional additions including cholesterol, glycols, penetration enhancers, antioxidants, preservatives, and buffering agents. Each component makes a distinct contribution to the creation and performance of the vesicular system.

Ethanol

Ethanol is the main component that distinguishes ethosomes from traditional liposomes and accounts for their enhanced skin penetrating properties.(14,17) It performs several activities, including penetration enhancement, membrane fluidization, stabilization, and co-solvent for medicinal molecules.(15,16) The ethanol percentage in ethosomal formulations typically ranges from 20% to 45% (v/v), while the exact concentration varies depending on the nature of the medicine and desired formulation properties.(15)

Ethanol improves transdermal medication distribution through interacting with the stratum corneum's lipid domains. Ethanol alters the highly organized structure of skin lipids, increasing membrane fluidity and permeability. This forms temporary channels that aid in the passage of vesicles and medicament molecules into deeper skin layers.(16) Ethanol

also provides flexibility to the phospholipid bilayer, lowering vesicle hardness and allowing the vesicles to bend and travel through small intercellular spaces within the skin.(17) Ethanol also influences vesicle properties such particle size, zeta potential, entrapment efficiency, and stability. Moderate ethanol concentrations often reduce vesicle size due to increased membrane fluidization and electrostatic repulsion between vesicles.(15)Furthermore, ethanol frequently contributes a negative surface charge to ethosomal vesicles, reducing aggregation and increasing colloidal stability. However, high ethanol concentrations can disrupt the vesicular membrane, leading to drug leakage and lower trapping efficiency.(17)

According to studies, formulations with ethanol concentrations ranging from 20% to 40% showed excellent vesicle flexibility, stability, and drug absorption. Concentrations that exceed the optimum range can result in severe membrane rupture and vesicle instability. Thus, careful ethanol content optimization is required for successful ethosomal formulation creation.(20,21)

Phospholipids

Phospholipids are the primary structural component of ethosomes, forming a bilayer membrane around the aqueous center. They are amphiphilic molecules with both hydrophilic and hydrophobic areas, allowing spontaneous vesicle formation when hydrated.(12,14) The type and concentration of phospholipids have a substantial effect on vesicle size, entrapment efficiency, membrane flexibility, stability, and drug release behaviour.

- Common phospholipids utilized in ethosomal formulations are:
- Soy lecithin
- Egg phosphatidylcholine
- Hydrogenated phosphatidylcholine
- Lipoid S100
- Lipoid E80
- Phosphatidylserine
- Phosphatidylethanolamine (15,21)

Natural phospholipids are widely selected for their high biocompatibility, biodegradability, and low toxicity. Unsaturated phospholipids have more membrane fluidity and allow for better skin penetration, but saturated phospholipids have increased membrane rigidity and storage stability.(15)

The content of phospholipids normally ranges between 0.5% and 5% (w/v). Increasing phospholipid concentration improves drug entrapment efficiency because more bilayer material is accessible for drug inclusion. However, high phospholipid concentrations may increase vesicle size and viscosity, reducing skin permeability.(21) Phospholipids also help to maintain medication release by regulating diffusion across the vesicular membrane. Furthermore, they interact with skin lipids, allowing the vesicles to fuse with biological membranes, so improving medication transport across the skin barrier.(21)

Cholesterol

Cholesterol is widely used in ethosomal formulations as a membrane stabilizer. It intercalates between phospholipid molecules in the bilayer, affecting membrane fluidity and permeability. The addition of cholesterol improves vesicle integrity, minimizes medication leakage, and increases storage stability.(20)The usual cholesterol concentration in ethosomal preparations is between 0.5% and 3% (w/v). At sufficient doses, cholesterol strengthens the phospholipid bilayer by reducing ethanol-induced membrane fluidity. This stabilization inhibits vesicle fusion and aggregation during storage.(21)

Cholesterol increases vesicle size because it takes more space within the bilayer membrane. Moderate concentrations increase entrapment efficiency by enhancing membrane organization. However, high cholesterol concentrations may compete with drug molecules for integration into the bilayer, limiting drug-loading capacity and entrapment efficiency.(20)

In addition to enhancing stability, cholesterol aids in the maintenance of vesicle shape and mechanical strength under changing environmental conditions, making it a crucial ingredient in many ethosomal compositions.(21)

Water

Water is the hydration medium and continuous phase of ethosomal systems. It promotes the self-assembly of phospholipid molecules into vesicular structures and helps to generate the aqueous core, which can be used to encapsulate hydrophilic medicines.(14)

Purified water, distilled water, or Milli-Q water are frequently used in formulation preparation. The quality of water has a considerable impact on vesicle stability and repeatability. The water content is quantitatively adjusted to achieve the final volume and concentration of formulation components. The aqueous phase also influences viscosity, vesicle hydration, and formulation stability. Proper phospholipid hydration is required to produce ethosomal vesicles of consistent size and stability.(15)

Polyols and Co-solvents

Polyols such as propylene glycol, polyethylene glycol, glycerol, and Transcutol® are commonly included in ethosomal formulations to increase medication solubility, stability, and skin permeation.

Propylene glycol is a popular co-solvent because of its high compatibility with phospholipids and skin tissues. (20)It functions as both a humectant and a penetration enhancer, enhancing stratum corneum moisture and promoting medication diffusion.

- Polyols serve various functions, including:
- Increasing skin hydration.
- Improves permeation efficiency.
- Stabilizing vesicles
- Improved medication solubility.
- Prevents vesicle aggregation.

Polyol concentrations typically range from 5% to 20%, depending on the formulation requirements.(22)

Penetration Enhancers

Although ethanol is a significant penetration enhancer, other permeation enhancers are occasionally added to promote medication transport through the skin.(20)

Examples include:

- Oleic acid.
- Isopropyl myristate.
- Dimethyl Sulfoxide (DMSO)
- Menthol.
- Terpenes.
- Lauric acid.

These chemicals interact with stratum corneum lipids to improve membrane fluidity, allowing for deeper penetration of ethosomal vesicles and encapsulated medicines.(22)

Antioxidants

Phospholipids are subject to oxidative breakdown during storage, especially when unsaturated phospholipids are present. Antioxidants are thus added to preserve the vesicle components from oxidation.(21)

Common antioxidants include:

- α-Tocopherol (vitamin E).
- Butylated hydroxytoluene (BHT).
- Ascorbic acid.
- Sodium metabisulfite.

These compounds enhance formulation stability and increase shelf life.(22)

Preservatives

Preservatives are applied to protect against microbial contamination during storage and use.

Examples include:

- Methylparaben
- Propylparaben.
- Benzalkonium chloride.
- Phenoxyethanol.

Their concentration must be carefully adjusted to achieve antibacterial efficacy while maintaining vesicle stability.(21)

Buffering Agents

Buffer systems are used to keep the pH stable and make the product skin friendly.

Typical buffering agents include:

- Phosphate buffer.
- Citrate buffer
- Acetate buffer.

The pH of ethosomal formulations is typically kept between 5.0 and 7.0 to reduce skin irritation and increase formulation durability.(20,21)

Method of preparation of Ethosome

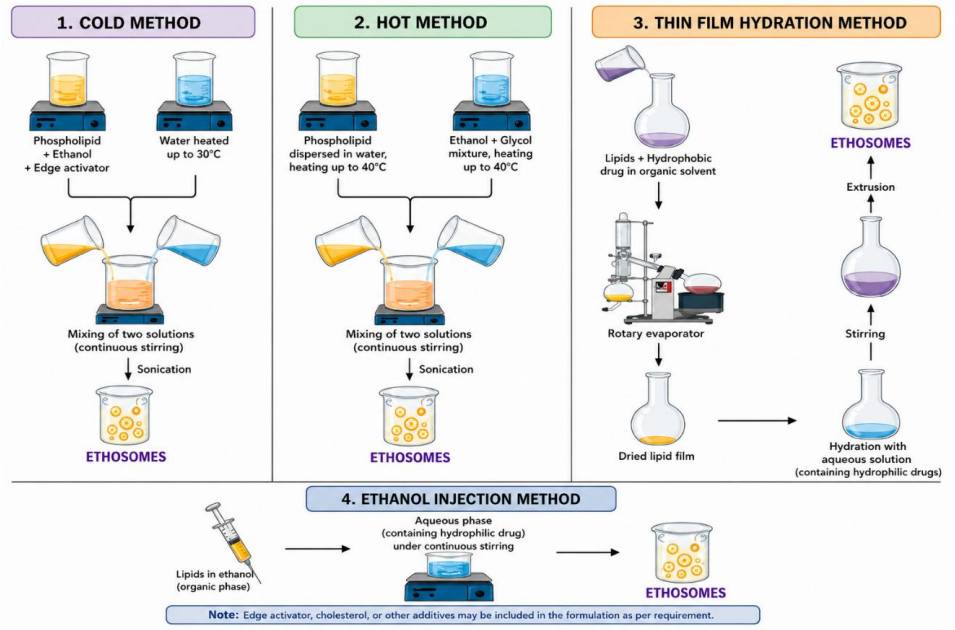

Figure 2: Method of Preparation of Ethosome

Cold Method

The cold approach is the most popular and easiest way for creating ethosomal vesicles. It was invented by Touitou and colleagues and is chosen because it does not require high temperatures, making it appropriate for thermolabile and heat-sensitive medicines.(14)

Principle

In this process, phospholipids are dissolved in ethanol while continuously stirring to generate an alcoholic phase. Water is then progressively added while being constantly mixed. The interaction of ethanol, phospholipids, and water causes spontaneous production of ethosomal vesicles. Ethanol serves as both a solvent and a membrane-fluidizing agent, encouraging vesicle formation and increasing deformability.(20)

Procedure

Initially, phospholipids such as soy lecithin or phosphatidylcholine are thoroughly dissolved in ethanol with vigorous magnetic swirling. If the medication is lipophilic, it dissolves with phospholipids in the ethanolic phase. Hydrophilic medicines can be dissolved independently in the aqueous phase.

The ethanolic solution is agitated at room temperature (25-30°C) until a clear, homogenous solution forms. Purified water, previously kept at the same temperature, is gradually added to the alcoholic phase in a fine stream while continuously stirring. The addition of water leads phospholipid hydration and spontaneous vesicle formation. Continuous stirring is required for about 30-60 minutes to achieve complete vesicle production. The resultant dispersion is then probe sonicated or extruded through polycarbonate membranes to minimize vesicle size and obtain a more uniform size distribution.(20,21)

Advantages

- Easy and affordable approach.
- No need for sophisticated equipment.
- Ideal for heat-sensitive medications.
- High entrapment efficiency.
- Easy to scale up for industrial manufacturing.

Disadvantages

- Vesicles may aggregate during storage.
- Ethanol concentration needs to be carefully optimized.
- Large vesicles can develop without size reduction procedures.

Applications

The cold approach has been widely used in the development of ethosomal formulations including acyclovir, testosterone, diclofenac sodium, ketoconazole, and a variety of other medicinal drugs.

Hot Method

The hot method is an alternate method for preparing ethosomal formulations that requires higher temperatures to enable phospholipid hydration and vesicle formation. This approach is very effective for phospholipids with high phase transition temperatures.(20)

Principle

The procedure entails heating the aqueous and alcoholic phases separately before mixing them under controlled conditions. Elevated temperature stimulates phospholipid dispersion and increases vesicle production.

Procedure

In the first step, phospholipids are dispersed in filtered water and heated to 40-45°C while continuously stirring. Ethanol and any cosolvents, such as propylene glycol, are heated separately to the same temperature. The medicine is dissolved in the phase based on its solubility profile. Hydrophilic medications are added to the aqueous phase, while lipophilic pharmaceuticals are dissolved in the ethanolic phase. The ethanolic solution is progressively added to the aqueous phase while stirring continuously and keeping the temperature constant. Mixing is continued for around 30 minutes to guarantee complete vesicle production. The resulting vesicular suspension is cooled to room temperature before being exposed to sonication or membrane extrusion to produce smaller vesicles with a limited size distribution.(22)

Advantages

- Suitable for phospholipids with high hydration temperatures.
- Improved dispersion of lipid components.
- Excellent repeatability. • Uniform vesicle formation.

Disadvantages

- Unsuitable for heat-sensitive medications.
- Active compounds may degrade under heat conditions.
- Additional temperature control needed.

Applications

The heated process has been used to create ethosomal formulations including anti-inflammatory medicines, hormones, and antibiotics.

Ethanol Injection Method

The ethanol injection method is a simple and repeatable procedure for creating tiny ethosomal vesicles. This approach relies on the rapid diffusion of ethanol into an aqueous solution, which results in the spontaneous production of phospholipid vesicles.(20)

Principle

When a phospholipid solution in ethanol is introduced into an aqueous phase, the ethanol quickly diffuses into the water, limiting phospholipid solubility and inducing self-assembly into vesicular structures.

Procedure

Phospholipids and lipophilic medicines are entirely dissolved in ethanol with steady stirring. The resulting ethanolic solution is put into a syringe fitted with a tiny needle.The aqueous phase, which contains purified water and,

if necessary, hydrophilic medication, is stirred continuously at room temperature.Anethanolic solution is slowly injected into the aqueous phase at a controlled rate. During injection, ethanol disperses into the aqueous media, resulting in the spontaneous creation of vesicles.The dispersion is further agitated for around 30 minutes to ensure that the vesicles are completely stabilized. Probe sonication or high-pressure homogenization may be used to minimize vesicle size and increase homogeneity.(23)

Advantages

Simple and quick process.

- Creates tiny vesicles.
- Excellent repeatability.
- Ideal for laboratory-scale preparation.

Disadvantages

Hydrophilic medicines exhibit lower trapping efficiency.

- Injection rate must be precisely controlled.
- Limited scalability without specialised equipment.

Applications

This technology is commonly utilized to create nanoethosomes for improved skin penetration and regulated medication delivery.

Thin-Film Hydration Method (Rotary Evaporation Method)

Thin-film hydration is one of the most used ways for creating vesicular systems, including ethosomes. It is ideal for encapsulating lipophilic medicines and obtaining excellent drug loading efficiency.(21)

Principle

Phospholipids dissolved in organic solvents evaporate, leaving a thin lipid coating. Multilamellar vesicles are formed after being hydrated with an aqueous solution containing ethanol.

Procedure

In a round-bottom flask, phospholipids, cholesterol, and lipophilic medicines are dissolved in an organic solvent combination consisting of chloroform and methanol.The organic solvents are evaporated under reduced pressure with a rotary evaporator that is kept above the phospholipids' phase transition temperature. This technique creates a thin, consistent lipid layer on the flask's inner wall.After the solvent has been completely removed, the lipid film is hydrated with an aqueous solution containing ethanol and gently rotated. Hydration is commonly done at temperatures ranging from 40 to 60°C.To achieve thorough hydration and vesicle maturation, the hydrated suspension is continually spun for about an hour before being allowed to stand overnight.Sonication, extrusion, or high-pressure homogenization diminish the size of the resulting multilamellar vesicles.(22)

Advantages

- High entrapment efficiency.
- Ideal for lipophilic medicines.
- Excellent repeatability.
- Well-established approach.

Disadvantages

- Time-consuming process.
- Requires organic solvents.
- Additional size reduction processes are required.

Applications

This approach is commonly used to encapsulate poorly water-soluble medicines like curcumin, ketoconazole, and several anticancer medications.

Reverse-Phase Evaporation Method

The reverse-phase evaporation approach is especially useful for achieving high encapsulation efficiency of hydrophilic medicines in ethosomal vesicles.

Principle

A water-in-oil emulsion is initially formed, followed by the removal of the organic solvent at reduced pressure. This process promotes the production of vesicles with extensive aqueous compartments that can entrap hydrophilic medicines.(20)

Procedure

Phospholipids and cholesterol are dissolved in organic solvents like diethyl ether, chloroform, or isopropyl ether.The aqueous phase containing the medication is progressively introduced into the organic phase. The resulting mixture is sonicated to produce a stable water-in-oil emulsion.To extract organic solvents, the emulsion is exposed to reduced pressure with a rotary evaporator. As the solvent evaporates, the solution produces a gel-like structure and eventually vesicles.The resulting ethosomal dispersion is then hydrated with an ethanol-containing aqueous solution and constantly agitated to form a homogenous suspension.Finally, the formulation is sonicated or extruded to ensure uniform vesicle size.(21)

Advantages

Improved encapsulation efficiency for hydrophilic pharmaceuticals.

- High internal aqueous volume.
- Ideal for macromolecules and peptides.
- Effective medication retention.

Disadvantages

- Use organic solvents.
- Complex procedure.
- Extended preparation time.
- Additional purification processes may be needed.

Applications

The reverse-phase evaporation method is widely used to deliver peptides, proteins, nucleic acids, and water-soluble medicinal compounds.

Evaluation of Ethosomes

Comprehensive characterization and evaluation of ethosomal formulations is required to verify their quality, stability, safety, and therapeutic efficacy. Several physicochemical, morphological, and biological factors are analyzed.

Morphological Examination

Morphological study reveals details on vesicle shape, surface properties, aggregation behavior, and structural integrity.

a. Scanning Electron Microscopy (SEM)

SEM can produce precise images of the exterior surface morphology of ethosomal vesicles. Samples are coated with a conductive substance and then examined under high vacuum conditions. SEM detects aggregation, fusion, and surface imperfections.(20)

b. Transmission Electron Microscopy (TEM)

TEM is commonly used to examine interior structure and vesicle morphology. Ethosomes resemble spherical or nearly spherical unilamellar vesicles with smooth surfaces. TEM allows for direct assessment of vesicle diameter and membrane thickness.(21)

c. Atomic Force Microscopy (AFM)

AFM provides three-dimensional surface imaging and high-resolution analysis of vesicle topology without the need for extensive sample preparation.(20)

Vesicle Size and Size Distribution

Vesicle size is one of the most important factors influencing skin penetration, drug release, stability, and bioavailability.

Dynamic Light Scattering (DLS)

Dynamic light scattering (DLS) is one of the most commonly used techniques for determining the particle size and size distribution of ethosomal vesicles. This method measures the fluctuations in scattered light caused by the Brownian motion of particles suspended in a medium. The important parameters obtained from DLS analysis include mean vesicle size and polydispersity index (PDI). The mean vesicle size is generally expressed in nanometers (nm), while the PDI indicates the uniformity of vesicle distribution within the formulation. A PDI value below 0.3 represents a uniform and narrow particle siz distribution, whereas a value around 0.5 indicates a broad and heterogeneous distribution. The size of ethosomal vesicles usually ranges between 50 and 500 nm depending on the composition of phospholipids, ethanol concentration, and other formulation variables.(20,21)

Entrapment Efficiency (EE%)

Entrapment efficiency is an important parameter used to determine the percentage of drug successfully incorporated within ethosomal vesicles compared to the total amount of drug added during formulation preparation. It provides information regarding the loading capacity and effectiveness of the vesicular system. Common methods employed for determining entrapment efficiency include ultracentrifugation, dialysis, and gel filtration techniques. High entrapment efficiency indicates effective drug incorporation and optimized formulation characteristics. Several formulation factors influence entrapment efficiency, including drug solubility, ethanol concentration, phospholipid concentration, cholesterol content, and vesicle size.(20,21)

The entrapment efficiency is calculated using the following formula:

%Drug Entrapment=DrugAmount of Entrapped Drug /Total Amount of ×100

Zeta Potential

Zeta potential is used to evaluate the electrical charge present on the surface of ethosomal vesicles and serves as an important indicator of colloidal stability. The measurements are generally carried out using electrophoretic light scattering techniques. Vesicles with zeta potential values above +30 mV or below –30 mV are considered stable due to sufficient electrostatic repulsion between particles, whereas values near ±10 mV indicate poor stability and a higher tendency for aggregation. In ethosomal systems, the presence of ethanol generally imparts a negative surface charge to the vesicles, which helps reduce vesicle aggregation and improves formulation stability through electrostatic repulsion.(20,21)

Drug Content Determination

Drug content determination is performed to estimate the actual amount of drug present within the ethosomal formulation. This evaluation ensures uniformity and accuracy of drug incorporation in the prepared vesicles. Various analytical techniques such as UV-visible spectrophotometry, high-performance liquid chromatography (HPLC), and LC-MS/MS are commonly employed for drug content analysis. The drug content is usually expressed either as percentage drug content or concentration per unit volume of formulation.(20,22)

Elasticity and Deformability Index

The elasticity and deformability of ethosomal vesicles play a major role in enhancing skin penetration and transdermal drug delivery. Ethosomes possess flexible lipid bilayers that allow them to deform and pass through narrow pores of the skin. The deformability index is commonly determined using the extrusion method, in which vesicles are passed through membranes of defined pore size. Higher deformability values indicate greater vesicle flexibility and improved penetration capability through the skin barrier.(21,23)

pH Determination

The pH of ethosomal formulations is an important parameter affecting formulation stability, skin compatibility, and drug release behavior. The pH is measured using a calibrated digital pH meter under controlled conditions. For topical and transdermal applications, the optimal pH range of ethosomal preparations is generally maintained between 5.0 and 6.5, which is close to the normal physiological pH of human skin. Maintaining the formulation within this range minimizes skin irritation and enhances patient compatibility.(20,21)

In Vitro Drug Release Studies

In vitro drug release studies are conducted to evaluate the release kinetics of the encapsulated drug from ethosomal vesicles over a specified period of time. These studies help in understanding the drug release profile and predicting formulation performance. Commonly employed methods include the dialysis membrane diffusion technique and Franz diffusion cell method. During the study, samples are withdrawn at predetermined time intervals and analyzed using spectrophotometric or chromatographic methods to determine the amount of drug released.(21,22)

Ex Vivo Skin Permeation Studies

Ex vivo skin permeation studies are carried out to investigate the penetration and permeation of drugs through excised animal or human skin. The Franz diffusion cell method is most commonly used for this purpose. In this method, the receptor compartment contains phosphate buffer maintained at a temperature of 37 ± 0.5°C to simulate physiological conditions. Important parameters evaluated during the study include cumulative drug permeation, flux, permeability coefficient, and drug retention within the skin. These studies provide valuable information regarding the transdermal delivery potential of ethosomal formulations.(17,21)

Stability Studies

Stability studies are performed to evaluate the integrity and performance of ethosomal formulations during storage under different environmental conditions. These studies help determine the shelf life and physical stability of the formulation. Parameters commonly evaluated include vesicle size, polydispersity index (PDI), zeta potential, entrapment efficiency, drug content, physical appearance, and pH. Monitoring these characteristics over time ensures that the formulation maintains its stability, efficacy, and safety throughout the storage period.

Storage conditions typically include:

- Refrigerated at 4 ± 2°C.
- Room temperature: 25 ± 2°C.
- Accelerated conditions (40 ± 2°C/75% RH).
- Assessment periods typically range from one to six months. (20,22)

Applications of Ethosomes

Table 2: Applications of Ethosomes

Application Area	Examples / Uses	Key Benefits
Oncology (Cancer Therapy)	Transdermal delivery of anticancer drugs like 5-fluorouracil, tamoxifen	Non-invasive administration, reduced systemic toxicity, controlled release
Neurological Disorders	Delivery of drugs for Parkinson's disease, Alzheimer's (e.g., dopamine agonists)	Bypasses blood–brain barrier via transdermal route, sustained drug levels
Cardiovascular Therapy	Nitroglycerin, propranolol, carvedilol	Avoids hepatic first-pass metabolism, steady plasma concentration
Diabetes Management	Transdermal insulin delivery	Non-invasive alternative to injections, improved patient compliance
Peptide & Protein Drugs	Delivery of biomolecules like interferons, growth hormones	Protects fragile molecules, enhances absorption through skin
Gene Therapy	Experimental delivery of DNA/RNA fragments	Potential for non-invasive genetic treatment, localized targeting
Wound Healing	Incorporation of growth factors, antimicrobial peptides	Accelerated healing, reduced infection risk
Veterinary Medicine	Transdermal delivery of antiparasitic and anti-inflammatory drugs in animals	Easy administration, reduced stress for animals

Future Prospects

Future research on ethosomes is likely to concentrate on increasing vesicle stability, targeted distribution, and controlled release properties. Integration with microneedles, hydrogels, nanoparticles, and smart wearable devices may enhance therapeutic efficacy. The development of ligand-targeted and stimulus-responsive ethosomes could lead to more precise drug delivery for cancer, inflammatory illnesses, and dermatological problems. Furthermore, ethosomes have potential for the delivery of biologics, peptides, proteins, nucleic acids, and vaccines, opening up new avenues in customized therapy.

CONCLUSION

Ethosomes are advanced vesicular drug delivery systems widely used for topical and transdermal applications due to their enhanced skin penetration ability. They are mainly composed of phospholipids, ethanol, and water, where ethanol plays a crucial role in increasing membrane flexibility and improving drug permeation through the stratum corneum. Compared to conventional liposomes, ethosomes provide better drug entrapment, improved bioavailability, and deeper skin penetration. They can effectively deliver both hydrophilic and lipophilic drugs, making them suitable for pharmaceutical, cosmetic, and biomedical applications. Ethosomes are extensively studied for the delivery of anti-inflammatory, antifungal, antiviral, and anticancer agents. However, challenges such as stability issues and large-scale production limitations still exist. Continuous advancements in nanotechnology and formulation techniques are expected to enhance their clinical applicability and establish ethosomes as promising carriers for future transdermal drug delivery systems.

REFERENCES

1. Touitou E, Dayan N, Bergelson L, Godin B, Eliaz M. Ethosomes novel vesicular carriers for enhanced delivery: characterization and skin penetration properties. *J Control Release*. 2000;65(3):403–418. doi:10.1016/S0168-3659(99)00222-9.
2. Touitou E, Godin B, Weiss C. Enhanced delivery of drugs into and across the skin by ethosomal carriers. *Drug Dev Res*. 2000;50(3–4):406–415.
3. Godin B, Touitou E. Ethosomes: new prospects in transdermal delivery. *Crit Rev Ther Drug Carrier Syst*. 2003;20(1):63–102.
4. Elsayed MMA, Abdallah OY, Naggar VF, Khalafallah NM. Deformable liposomes and ethosomes: mechanism of enhanced skin delivery. *Int J Pharm*. 2006;322(1–2):60–66. doi:10.1016/j.ijpharm.2006.05.027.
5. Verma P, Pathak K. Therapeutic and cosmetic applications of ethosomes: an overview. *J Adv Pharm Technol Res*. 2010;1(3):274–282.
6. Benson HAE. Transfersomes for transdermal drug delivery. *Expert Opin Drug Deliv*. 2006;3(6):727–737.
7. Paolino D, Lucania G, Mardente D, Alhaique F, Fresta M. Ethosomes for skin delivery of ammonium glycyrrhizinate. *Int J Pharm*. 2005;295(1–2):235–244.
8. Dubey V, Mishra D, Asthana A, Jain NK. Transdermal delivery of anti-inflammatory agents using ethosomal carriers. *Drug Dev Ind Pharm*. 2007;33(11):1193–1198.
9. Jain S, Umamaheshwari RB, Bhadra D, Jain NK. Ethosomes: a novel vesicular carrier for enhanced transdermal delivery of an anti-HIV agent. *Indian J Pharm Sci*. 2004;66(1):72–81.
10. Fang JY, Hwang TL, Huang YB, Tsai YH. Transdermal iontophoretic delivery of sodium nonivamide acetate using lipid vesicles. *Int J Pharm*. 2003;255(1–2):153–160.
11. Jain NK. Advances in controlled and novel drug delivery. New Delhi: CBS Publishers; 2012.
12. Abdulbaqi IM, Darwis Y, Khan NAK, Assi RA, Khan AA. Ethosomal nanocarriers: the impact of constituents and formulation techniques on ethosomal properties, in vivo studies, and clinical trials. *Int J Nanomedicine*. 2016;11:2279–2304. doi:10.2147/IJN.S105016.
13. Ascenso A, Raposo S, Batista C, Cardoso P, Mendes T, Praça FG. Development, characterization and skin delivery studies of related ultra-deformable vesicular systems. *Int J Cosmet Sci*. 2015;37(4):351–359.
14. Mbah CC, Builders PF. Nanovesicular carriers as alternative drug delivery systems. *J Pharm Investig*. 2014;44(6):343–356.
15. Sharma G, Kumar A, Sharma AR. Ethosomes for transdermal and topical drug delivery: current advances and future perspectives. *J Drug DelivSci Technol*. 2021;61:102164.
16. Niu M, Lu Y, Hovgaard L, Guan P, Tan Y, Lian R, Qi J, Wu W. Ethosome-based topical delivery systems. *Int J Pharm*. 2017;521(1–2):191–198.
17. Song CK, Balakrishnan P, Shim CK, Chung SJ, Chong S, Kim DD. A novel vesicular carrier, transethosome, for enhanced skin delivery. *Colloids Surf B Biointerfaces*. 2012;92:299–304.
18. Mishra V, Bansal KK, Verma A, Yadav N, Thakur S. Ethosomal drug delivery systems: a review of composition, preparation and characterization. *J Pharm Innov*. 2022;17:1048–1063.
19. Kaur LP, Guleri TK. Topical and transdermal drug delivery systems based on vesicular carriers. *Drug Deliv*. 2013;20(5):213–220.
20. Singh RP, Sharma PK, Malviya R. Ethosomal systems: preparation, characterization and therapeutic applications. *J Liposome Res*. 2023;33(4):345–360.
21. Cevc G, Blume G. Biophysical and skin penetration properties of ultradeformable vesicles and ethosomes. *Int J Pharm*. 2015;489(1–2):23–36. doi:10.1016/j.ijpharm.2015.04.003.
22. Sharma N, Gupta R, Singh S, Kumar P. Recent advances and future perspectives of ethosomal drug delivery systems. *Pharmaceutics*. 2024;16(5):612. doi:10.3390/pharmaceutics16050612.