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INNOVATIVE DRUG DELIVERY SYSTEMS: EXPLORING ETHOSOMES AND TRANSETHOSOMES

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ABSTRACT

Ethosomes and transethosomes are advanced lipid-based vesicles designed to enhance drug delivery through the skin. This review explores their types, preparation methods, advantages, and challenges. Ethosomes use ethanol to increase skin permeability, while transethosomes incorporate additional penetration enhancers for improved transdermal delivery. Key preparation techniques include lipid film hydration and solvent injection. Advantages of these systems include controlled drug release, reduced systemic toxicity, and enhanced therapeutic efficacy, though they face issues like stability and environmental sensitivity. Characterization involves TEM, SEM, DLS, and drug entrapment efficiency assessments. Applications range from dermatology and cosmeceuticals to systemic drug delivery. Pharmacological evaluations highlight their effectiveness in anti-inflammatory, analgesic, and anti-gout treatments. Overall, ethosomes and transethosomes represent promising platforms for innovative drug delivery solutions.

KEYWORDS: Ethosomes, Transethosomes, Drug Delivery, Skin Permeation Cosmeceuticals, Characterization Techniques, Transdermal Systems.

INTRODUCTION

The continuous advancement in pharmaceutical sciences has been largely driven by the quest to enhance the efficacy, safety, and patient compliance of therapeutic agents. A significant aspect of this progression is the development of innovative drug delivery systems that can effectively address the challenges posed by conventional methods. Among the cutting-edge approaches, vesicular systems have garnered considerable attention as a promising strategy for drug delivery, particularly in overcoming biological barriers. Ethosomes and transethosomes, two advanced vesicular systems, have emerged as potent carriers, particularly in transdermal drug delivery, due to their unique composition and enhanced ability to transport drugs through the skin.[1]

The imperative for novel drug delivery systems

Traditional drug delivery methods, such as oral administration and injections, are often hampered by several limitations, including low bioavailability, rapid metabolic degradation, and systemic adverse effects. These challenges necessitate the exploration of alternative routes and novel delivery systems that can improve therapeutic outcomes. Transdermal drug delivery has become an appealing option due to the skin's vast surface area and accessibility. However, the stratum corneum, the skin's outermost layer, presents a formidable barrier to drug penetration, limiting the effectiveness of many therapeutic agents. Therefore, developing novel delivery systems that can breach this barrier and facilitate efficient drug transport is crucial for advancing treatment modalities.[2]

Vesicular systems as pioneers in drug delivery

Vesicular systems, including liposomes, niosomes, and ethosomes, represent a significant leap forward in drug delivery technology. These systems are constructed from lipid bilayers that encapsulate therapeutic agents, enhancing their stability and facilitating controlled release. The inherent advantages of vesicular systems—such as improved bioavailability, protection of drugs from degradation, and minimized systemic side effects—make them ideal candidates for a wide range of pharmaceutical applications. Among these, ethosomes and transethosomes stand out due to their superior capabilities in transdermal drug delivery, particularly for both hydrophilic and lipophilic drugs.[3,4]

Ethosomes and Transethosomes: Revolutionizing transdermal drug delivery

Ethosomes and transethosomes are specialized lipid-based vesicles designed to transcend the limitations of conventional vesicular systems. Ethosomes are characterized by their unique composition, which includes phospholipids, a high concentration of ethanol, and water. The ethanol content disrupts the tightly packed lipids in the stratum corneum, enhancing the permeability of the skin and facilitating deeper drug penetration. Transethosomes, an advanced variant of ethosomes, incorporate additional components such as surfactants or penetration enhancers. These modifications further boost their ability to traverse the skin barrier and deliver drugs more effectively. The versatility of ethosomes and transethosomes in encapsulating a wide range of drugs positions them as powerful tools in the ongoing effort to optimize drug delivery and improve patient outcomes.[5,7]

Transdermal drug delivery systems (TDDS) have gained considerable attention due to their ability to deliver therapeutic agents effectively while minimizing systemic side effects. Among various carriers, ethosomes have emerged as a promising alternative for enhancing skin permeation of drugs. Ethosomes are lipid-based vesicular systems composed of phospholipids and a high concentration of ethanol (20-45%), which significantly enhances the permeability of the stratum corneum, the outermost layer of the skin. The unique composition of ethosomes allows them to solubilize lipophilic drugs within a double-layer phospholipid matrix, facilitating their passage through biological membranes. Ethanol plays a crucial role by disrupting the lipid structure of the stratum corneum, thus increasing the fluidity of skin lipids and enhancing drug permeation. This characteristic makes ethosomes particularly effective for delivering a wide range of therapeutic agents, including hydrophilic and lipophilic compounds, proteins, and peptides.Recent advancements have led to the development of transethosomes, a novel class of ethosomes that incorporate surfactants as edge activators. This modification enhances the flexibility and stability of the vesicles, further improving their transdermal delivery capabilities. The incorporation of surfactants not only alters the packing characteristics of the vesicular bilayer but also enhances the overall drug delivery efficiency, making transethosomes a superior option for targeted and controlled release of drugs through the skin.The versatility of ethosomes and transethosomes allows for their formulation into various dosage forms, including gels, creams, and patches, which can be tailored to specific therapeutic needs. As research continues to explore the potential applications of these novel carriers, ethosomes and transethosomes are poised to revolutionize the field of transdermal drug delivery, offering improved bioavailability and patient compliance.In summary, the innovative design and functional properties of ethosomes and transethosomes present significant advantages over traditional drug delivery systems, making them a focal point for ongoing research and development in pharmaceutical sciences.[9-10]

How do ethosomes and transethosomes work[11-13]

Ethosomes

Ethosomes are advanced lipid-based vesicles composed primarily of phospholipids, water, and a significant amount of ethanol. The presence of ethanol is pivotal in their function, as it enhances the ability of ethosomes to penetrate the skin. Ethanol disrupts the tightly packed lipid molecules in the stratum corneum, the skin's outermost layer, thus improving the permeability of drugs through the skin. The formation of ethosomes begins with the mixing of phospholipids, such as phosphatidylcholine, with ethanol and water, resulting in a flexible lipid bilayer structure. This flexibility is crucial for adapting to the contours of the skin's surface. The drug intended for delivery is then encapsulated within the ethosome, either through passive loading or by active methods like sonication or extrusion. Upon application to the skin, the ethosomal formulation interacts with the stratum corneum. The ethanol in the ethosomes facilitates the disorganization of the skin's lipid structure, creating a temporary pathway for the ethosomes to penetrate. The ethosomes, being highly adaptable and flexible, then pass through the skin, carrying the encapsulated drug. Once inside, the drug is gradually released from the vesicle, allowing it to diffuse into the surrounding tissue, thereby achieving its therapeutic effect.

Transethosomes

Transethosomes are an evolved version of ethosomes, distinguished by the inclusion of an additional component, such as a penetration enhancer or a surfactant, which further amplifies their drug delivery capabilities. The fundamental mechanism of action of transethosomes is similar to that of ethosomes, with the added component providing a more robust enhancement of skin penetration. The formation of transethosomes involves the combination of phospholipids, ethanol, and the extra component, resulting in a lipid bilayer structure that is even more flexible and adaptable than that of ethosomes. The drug is encapsulated within this vesicle, using techniques similar to those used in ethosome preparation, such as sonication or extrusion. When the transethosomal formulation is applied to the skin, it encounters the stratum corneum, where both the ethanol and the additional component collaborate to enhance penetration. This dual action disrupts the stratum corneum barrier more effectively, creating a more pronounced pathway for the transethosomes to pass through. As with ethosomes, the adaptable structure of transethosomes allows them to penetrate the skin, carrying the encapsulated drug. Once inside the skin, the drug is released from the vesicle and diffuses into the surrounding tissue, achieving the desired therapeutic effect.

Key differences between Ethosomes and Transethosomes

While ethosomes and transethosomes both serve as effective vehicles for delivering drugs through the skin, there are several key distinctions between them. Transethosomes include an additional component that enhances their penetration capabilities beyond what is possible with ethosomes alone, making them more effective at delivering drugs across the skin. This additional component also contributes to the greater flexibility and adaptability of transethosomes, allowing them to penetrate deeper into the skin and deliver drugs more efficiently. Furthermore, transethosomes tend to be more stable than ethosomes, which can be prone to aggregation and fusion, thereby making transethosomes a more reliable option for drug delivery.

Types of ethosomal systems[12,13]

Ethosomal systems can be categorized into three main types based on their composition and formulation characteristics:

Classical ethosomes

Classical ethosomes are lipid-based vesicles that consist of phospholipids, a high concentration of ethanol (up to 45% w/w), and water. They are a modification of traditional liposomes and are known for their smaller size, negative ζ-potential, and higher entrapment efficiency. These properties contribute to better skin permeation and stability compared to classical liposomes, making them effective for transdermal drug delivery. The molecular weights of drugs encapsulated within classical ethosomes typically range from 130.077 Da to 24 kDa.

Binary ethosomes

Binary ethosomes are an extension of classical ethosomes, developed by incorporating an additional type of alcohol, such as propylene glycol (PG) or isopropyl alcohol (IPA), into the formulation. This modification aims to enhance the flexibility and permeability of the ethosomal system, potentially improving drug delivery efficiency. The addition of these alcohols can alter the vesicular characteristics, thereby affecting the overall performance of the ethosomal system in transdermal applications.

Transethosomes

Transethosomes represent the latest generation of ethosomal systems, first reported in 2012. They retain the basic components of classical ethosomes but include penetration enhancers or edge activators (such as surfactants) in their formulation. This combination aims to merge the advantages of classical ethosomes with those of deformable liposomes (transfersomes), resulting in enhanced drug delivery capabilities. Transethosomes have shown superior properties over classical ethosomes in terms of drug entrapment and skin permeation, accommodating drugs with molecular weights ranging from 130.077 Da to 200-325 kDa. Each type of ethosomal system is designed to optimize drug delivery through the skin, leveraging unique formulations to enhance permeability, stability, and entrapment efficiency.

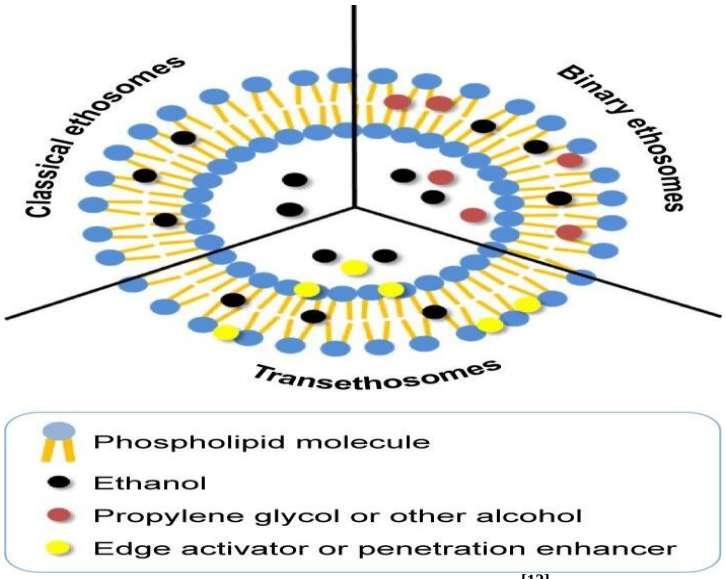

Figure 1: Ethosomal subtypes.[12]

Advantages of ethosomes

1. Ethosomes significantly enhance the permeation of drugs through the skin, enabling more effective transdermal delivery.
2. Ethosomes are suitable for dermal, transdermal, and intracellular drug delivery, making them a versatile system for various therapeutic needs.
3. Ethosomes can efficiently deliver a wide range of molecules with varying physicochemical properties, including hydrophilic and lipophilic molecules, peptides, proteins, and other macromolecules.
4. The components of ethosomes are generally recognized as safe (GRAS), non-toxic, and approved

for pharmaceutical and cosmetic use, ensuring a low risk of adverse effects.
5. The ethosomal structure presents a low risk in drug development, as its toxicology profiles are well-established and documented in scientific literature.
6. The ethosomal system is passive and non-invasive, making it suitable for immediate marketing without the need for complex regulatory approvals.
7. Ethosomal drug delivery systems can be widely applied in pharmaceutical, biotechnology, veterinary, cosmetic, and nutraceutical fields.
8. The ethosomal drug is typically delivered in a semi-solid form, such as a gel or cream, which enhances patient compliance due to ease of application and comfort.
9. Compared to methods like iontophoresis and sonophoresis, ethosomes offer a simpler and less complicated approach to drug delivery.
10. Ethosomes are relatively simple to manufacture, requiring no complicated technical investments, and can be easily scaled up to produce multiliter amounts.
11. Ethosomes enhance drug permeation across the skin efficiently, ensuring that the drug reaches the desired site within the skin or enters the bloodstream effectively.
12. Ethosomes typically show higher drug entrapment efficiencies compared to liposomes, resulting in more effective drug delivery.
13. Ethosomes exhibit excellent stability over extended periods, making them reliable for long-term storage and use.
14. The alcohol content in ethosomes acts as a natural preservative, eliminating the need for additional preservatives.
15. The cost of manufacturing ethosomes is low, making them an economically viable option for large-scale production.
16. The transport of drugs across the skin using ethosomes is not dependent on concentration, providing consistent delivery outcomes.[14,15]

Disadvantages of ethosomes

1. Ethosomes can cause allergic reactions in patients sensitive to ethanol or other components.
2. They are primarily limited to transdermal drug delivery and are not suitable for multiple administration routes.
3. Ethanol's flammability necessitates careful handling during all stages of ethosomal use.
4. Ethosomal formulations often yield poorly, making them less economical.
5. There is a risk of product loss when transferring ethosomes from organic to aqueous media, reducing efficiency.
6. Ethosomes are generally limited to delivering potent molecules requiring small or infrequent doses.
7. They are designed for steady, continuous drug release, not for rapid drug input or bolus administration.
8. Adequate drug solubility in both lipophilic and aqueous conditions is required to ensure proper microcirculation and access to systemic circulation.
9. The molecular size of the drug must be suitable for percutaneous absorption, limiting the range of drugs that can be effectively delivered.
10. Ethosomal adhesives may not adhere well to all skin types, potentially affecting drug delivery.
11. Excipients and penetration enhancers in ethosomes may cause skin irritation or dermatitis.
12. If the vesicle structure is not well stabilized, ethosomes can coalesce or disintegrate when transferred to water, compromising the formulation's integrity.[16,17]

Applications

1. Ethosomes have demonstrated effectiveness in treating viral and microbial skin infections. Trials involving animal models with deep skin infections have shown promising results with ethosomal systems containing bacitracin and erythromycin.
2. Ethosomes containing ammonium glycyrrhizinate have been manufactured and tested, revealing their anti-inflammatory effects on human skin. These trials were conducted with volunteer subjects to evaluate the therapeutic impact.
3. In vivo studies on rabbits have shown that ethosomal patches are effective for managing androgen insufficiency in men and alleviating menopausal symptoms in women, demonstrating significant improvements in these conditions.
4. Research suggests that ethosomes may provide analgesic and antipyretic benefits, as well as effective treatment for erectile dysfunction, indicating their potential in pain management and sexual health.
5. Ethosomes have also been investigated for their ability to deliver DNA molecules topically, enabling skin cells to express specific genes, which opens up possibilities for gene therapy applications.

Composition of ethosomes[18-22]

Ethanol

Ethanol is a highly effective penetration enhancer in ethosomal systems. It significantly influences the vesicles' dimensional characteristics, including size, ζ-Potential, stability, and skin permeability. Ethanol concentrations in ethosomal formulations typically range from approximately 10% to 50%. Research has shown that increasing ethanol concentration generally leads to a reduction in ethosome size, which can enhance skin permeability.

However, exceeding the optimal ethanol concentration can have detrimental effects. High ethanol levels may cause the vesicle bilayers to become leaky, resulting in an increase in vesicle size and a notable decrease in drug entrapment efficiency. Excessive ethanol concentrations can also solubilize the vesicles, compromising their structural integrity.

Additionally, ethanol affects the vesicular charge. At higher concentrations, ethanol shifts the vesicular load from positive to negative, providing a negative charge to the ethosomal surfaces. This change in charge helps to prevent vesicle aggregation through electrostatic repulsion, thus contributing to the overall stability of the ethosomal system. Ethanol also enhances the efficiency of drug entrapment within ethosomes, with increased concentrations generally improving trapping efficacy.

Phospholipids

Phospholipids are essential components of ethosomal systems, as they form the structural basis of the vesicles. They determine the vesicle's size, entrapment efficiency, stability, and skin penetration properties. Different types of phospholipids can be used, including those derived from soy, egg, or synthetic sources. The choice of phospholipid type and concentration is crucial for optimizing the ethosomal system. For instance, phosphatidylcholine is commonly used due to its ability to form stable bilayers. The specific phospholipid selected affects how the ethosomes interact with the skin and how well they encapsulate and release the drug. The various phospholipids used in ethosomal formulations are summarized in Table 1.

Alcohols

Ethanol: Ethanol is a key ingredient in ethosomal formulations due to its role as a penetration enhancer. It significantly affects the ethosome's size, charge, stability, entrapment efficiency, and skin permeability. Ethanol concentrations in ethosomal systems typically range from 10% to 50%. Increasing the ethanol concentration often leads to a reduction in ethosome size, which enhances skin penetration. Ethanol disrupts the stratum corneum, allowing ethosomes to penetrate more effectively.

Propylene Glycol (PG): PG is another common penetration enhancer used in ethosomal systems. It affects ethosome characteristics such as size, entrapment efficiency, permeability, and stability. PG is typically used at concentrations between 5% and 20%. It significantly reduces particle size compared to formulations without PG and is believed to enhance viscosity and offer antihydrolysis properties, improving ethosome stability.

Isopropyl Alcohol (IPA): IPA has been studied for its effects on ethosome entrapment efficiency and skin permeation. Research by Dave et al. found that IPA significantly affects entrapment efficiency but has less impact on drug release. Further studies are needed to understand IPA's effects on other ethosomal characteristics.

Cholesterol

Cholesterol is a rigid steroid molecule added to ethosomal formulations to improve stability and drug entrapment efficiency. It helps to reduce vesicle leakage, fusion, and permeability. Cholesterol is commonly included at concentrations up to 70% of the total phospholipid content, though it is typically used at around 3% of the total formulation. While cholesterol enhances the stability of ethosomes, it can also increase the vesicle size, which must be considered when optimizing the formulation.

Edge Activators and Penetration enhancers

Edge activators and penetration enhancers are crucial in enhancing the properties of ethosomal systems. These agents improve the vesicles' ability to penetrate the skin and deliver drugs effectively. Common edge activators and penetration enhancers include:

N-Decylmethyl Sulfoxide (NDMS) and Dimethyl Sulfoxide (DMSO): These compounds are known for their ability to disrupt lipid bilayers, facilitating drug penetration.

Tweens and Spans: These surfactants help stabilize the vesicles and improve drug delivery by altering the ethosome's surface properties.

Oleic acid: Oleic acid is a fatty acid that enhances skin penetration by fluidizing the lipid bilayer.

L-Menthol: L-Menthol can enhance skin permeability and improve the drug delivery efficiency of ethosomes.

Sodium stearate: This surfactant helps in the stabilization of ethosomal vesicles and enhances drug penetration.

Bile Acids and Salts: These compounds act as penetration enhancers by interacting with the skin's lipid matrix.

Polyethylene Glycol 4000 (PEG 4000): PEG 4000 helps in improving the stability and penetration of ethosomes.

Hexadecyltrimethylammonium Bromide (HTAB): HTAB is a surfactant that can enhance the penetration and stability of ethosomal formulations.

Cremophor: Cremophor acts as a solubilizer and penetration enhancer, improving drug delivery.

Skin-Penetrating and Cell-Entering (SPACE) Peptide: This peptide enhances drug penetration into the skin and cellular uptake.

Sodium Dodecyl Sulfate (SDS): SDS is a surfactant that helps to enhance skin permeability and ethosome stability.

Methods of preparation for ethosomes[23-29]

1. Cold method

The cold method is one of the most common and straightforward techniques for preparing ethosomes. In this method, phospholipids, ethanol, and the drug are mixed together at a low temperature (usually 4°C) in a suitable container. The mixture is then stirred continuously until the phospholipids are completely dissolved, forming a uniform ethosomal suspension. After the phospholipids are fully dissolved, the aqueous phase, which may contain additional components like water or a buffer solution, is added slowly with continuous stirring. This gradual addition helps in the formation of ethosomal vesicles. The final ethosomal suspension can be sonicated or homogenized to reduce

the vesicle size and ensure a uniform distribution of the drug within the vesicles. The cold method is advantageous due to its simplicity and ability to incorporate both lipophilic and hydrophilic drugs.

2. Hot method

The hot method involves the preparation of ethosomes at an elevated temperature, typically between 30°C to 40°C. In this method, the phospholipids are dissolved in ethanol, and the solution is heated to the desired temperature. The drug is then dissolved in the aqueous phase, which is also heated to the same temperature as the ethanol-phospholipid mixture. The heated aqueous phase is added to the ethanol-phospholipid mixture with continuous stirring, leading to the formation of ethosomal vesicles. After the addition of the aqueous phase, the mixture is stirred for a specific period to allow the ethosomal vesicles to form and stabilize. This method is particularly useful for drugs that are more soluble at higher temperatures, but it requires careful temperature control to prevent degradation of temperature-sensitive components.

3. Mechanical dispersion method

The mechanical dispersion method is a technique that uses mechanical forces to prepare ethosomes. In this method, phospholipids are dispersed in water under high shear conditions, such as those created by a homogenizer or a high-speed stirrer. Once the phospholipids are well-dispersed in the aqueous phase, ethanol is gradually added to the dispersion while continuing to apply mechanical forces. The combination of mechanical forces and the ethanol's penetration-enhancing effect leads to the formation of ethosomal vesicles. This method is particularly useful for scaling up ethosomal production and can be adapted for large-scale manufacturing processes.

4. Classic lipid film hydration method

The classic lipid film hydration method, also known as the thin-film hydration method, is adapted from liposome preparation techniques. In this method, phospholipids and other lipid components are dissolved in an organic solvent, typically chloroform or methanol. The organic solvent is then evaporated under reduced pressure using a rotary evaporator, leaving behind a thin film of lipids on the walls of the container. The drug, dissolved in the aqueous phase, is then added to the lipid film, and the mixture is subjected to hydration with continuous stirring. The hydration process leads to the formation of ethosomal vesicles as the lipid film absorbs the aqueous phase. This method is particularly suitable for encapsulating lipophilic drugs and allows for the preparation of ethosomes with a controlled size and high encapsulation efficiency.

5. Sonication method

The sonication method is often used as a complementary step in ethosome preparation to reduce vesicle size and achieve a more uniform distribution of drug-loaded vesicles. After preparing ethosomes using any of the above methods, the ethosomal suspension is subjected to ultrasonic waves in a sonicator. The ultrasonic energy breaks down larger vesicles into smaller ones, resulting in a more uniform ethosomal suspension with reduced vesicle size. This method is especially useful when a smaller vesicle size is desired for enhanced drug penetration through the skin.

6. Extrusion method

The extrusion method is another technique used to control the size of ethosomal vesicles and ensure uniformity. In this method, the ethosomal suspension is passed through a membrane filter with a specific pore size using an extruder. The process is repeated several times, with the ethosomal suspension being forced through the filter under pressure. This mechanical process reduces the size of the vesicles and produces a more uniform ethosomal suspension. The extrusion method is particularly useful for preparing ethosomes with a narrow size distribution, which can be important for certain drug delivery applications.

Ethosomal dosage forms[12,30,31]

Ethosomal systems are frequently studied in their original suspension forms, which typically contain a high concentration of alcohol. To optimize their use for dermal and transdermal administration, ethosomal suspensions are often incorporated into various vehicles. This approach offers several benefits, including preventing ethanol evaporation, extending skin contact time, enhancing the therapeutic effect of the encapsulated drug, improving the stability and storage of the final dosage form, and increasing patient compliance. Recent advancements have led to the development of ethosomal formulations in different dosage forms such as gels, patches, and creams.

Ethosomal gels

Ethosomal gels are assessed for several critical parameters, including pH, viscosity, spreadability, and extrudability. The most commonly used gel-forming agents for ethosomal systems are Carbopol and hydroxypropyl methylcellulose (HPMC) in their various grades. These polymers provide the necessary viscosity and bioadhesive properties, and research has demonstrated their compatibility with ethosomal systems.

Ethosomal patches

The preparation of ethosomal patches involves forming a mold, making the process more complex compared to the preparation of ethosomal gels. Ethosomal patches require careful formulation and evaluation to ensure their efficacy and consistency.

Ethosomal creams

Ethosomal creams have been explored in a limited number of studies, primarily focusing on incorporating Curcuma longa extract-loaded ethosomal systems into a

cream base. These studies have investigated the use of ethosomal creams for photoprotection and anti-wrinkle effects. The application of these ethosomal creams to human volunteers has shown promising results, highlighting their potential as effective photoprotective and anti-wrinkle agents.

Characterization of Ethosomes and Transethosomes[32,33]

1. **Vesicle morphology:** Vesicle morphology is a key parameter in the characterization of ethosomes and transethosomes. This involves examining the shape and structural integrity of the vesicles. Techniques such as Transmission Electron Microscopy (TEM) and Scanning Electron Microscopy (SEM) are used for this purpose. TEM provides high-resolution images of the vesicles' internal structure by negative staining with agents like phosphotungstic acid, while SEM offers surface views, helping to assess the overall morphology and uniformity of the vesicles.

2. **Vesicle Size and Size distribution:** The size and size distribution of ethosomes and transethosomes are critical for their performance and stability. Dynamic Light Scattering (DLS) is commonly used to measure these parameters. DLS determines the size range of vesicles, which typically falls between nanometers and microns depending on the formulation. The size distribution affects the drug release profile and skin penetration capabilities, with smaller vesicles generally offering better performance.

3. **Configuration of the vesicular bilayer:** The vesicular bilayer configuration is crucial for the ethosomal system's efficacy. Nuclear Magnetic Resonance (NMR) spectroscopy is employed to investigate the bilayer's formation and integrity. This analysis helps ensure that the bilayer is optimized for effective drug entrapment and release, which is essential for the stability and performance of ethosomal formulations.

4. **Drug entrapment efficiency:** Drug entrapment efficiency measures how effectively the drug is encapsulated within the ethosomes. Two primary methods are used for this characterization:

- **Ultracentrifugation:** This method involves centrifuging the ethosomal suspension to separate the drug-loaded vesicles from free drug.
- **Dialysis:** Drug-loaded vesicles are placed in dialysis bags and immersed in a phosphate buffer saline (PBS) solution. The amount of drug released into the buffer over time is measured using High-Performance Liquid Chromatography (HPLC), allowing the calculation of entrapment efficiency based on the drug content in the dialysis medium.

5. **Permeation distinctiveness:** The permeation enhancement of ethosomes is evaluated to understand their effectiveness in drug delivery. Ethanol, a key component in ethosomes, enhances skin permeability through mechanisms such as increased thermodynamic activity and reduced barrier properties of the skin. The synergistic effect between ethanol, the vesicles, and skin lipids contributes to improved drug penetration.

6. **Physical stability:** Physical stability of ethosomes is assessed through freeze-drying techniques, which ensure long-term storage stability. Freeze-dried ethosomes typically form lightweight, glassy cakes that rehydrate quickly. Stability studies monitor drug encapsulation and leakage over time. The presence of antioxidants like α-tocopherol can help prevent oxidative degradation of phospholipids, thereby maintaining vesicular stability.

7. **Transition temperature:** The transition temperature of vesicular lipids, determined using Differential Scanning Calorimetry (DSC), indicates the temperature at which the lipid bilayer changes phase. This measurement provides insights into the fluidity and stability of the vesicles, which affects their drug delivery performance.

8. **Confocal Laser Scanning Microscopy (CLSM):** CLSM is used to examine the extent and functionality of ethosomal preparations in skin penetration. By scanning the skin in the z-axis, CLSM provides detailed images of how well ethosomes penetrate and distribute within the skin layers, which is crucial for assessing their effectiveness in drug delivery.

9. **Drug content:** The drug content in ethosomes can be quantified using UV spectrophotometry or HPLC. These methods determine the amount of drug encapsulated in the ethosomal system, providing insights into the formulation's quality and effectiveness.

10. **Surface tension measurement:** Surface tension of the ethosomal formulation is measured using a Du Noüy ring tensiometer. This parameter helps in understanding the interactions between the ethosomal formulation and the skin, influencing the overall performance of the drug delivery system.

11. **Phospholipid-Ethanol interaction:** The interaction between phospholipids and ethanol is studied using techniques like 31P-NMR and differential scanning calorimetry. These studies reveal how ethanol affects the phospholipid bilayer and contributes to the ethosomes' stability and drug entrapment efficiency.

12. **Degree of Deformability and Turbidity:** The deformability of ethosomes is assessed using extrusion methods, while turbidity is measured with

a Nephalometer. These parameters provide insights into the vesicles' flexibility and the clarity of the formulation, which affect drug delivery performance.

13. **Drug entrapment efficiency (Additional analysis):** Differential scanning calorimetry (DSC) and anisotropy analysis using fluorescent probes like AVPC help determine the entrapment efficiency and fluidity of the ethosomal bilayers. These methods confirm the ability of ethosomes to trap both hydrophobic and hydrophilic drugs effectively. Research has shown that ethosomes have higher entrapment efficiency compared to conventional liposomes due to their unique composition and structure.

Evaluation of Ethosomes and Transethosomes[34,35]

1. Vesicle skin interaction study

Various visualization techniques are utilized to assess how ethosomes interact with the skin. Transmission Electron Microscopy (TEM) provides high-resolution images of vesicle morphology and their penetration pathways. Eosin-Hematoxylin staining highlights the distribution and localization of ethosomes within skin layers. Fluorescence microscopy uses fluorescent markers to visualize ethosome distribution, while Confocal Scanning Laser Microscopy (CSLM) offers a 3D view of ethosome penetration into deeper skin layers. Compared to traditional liposomes, ethosomes show enhanced distribution and deeper penetration into the dermis.

2. Filter Membrane-Vesicle interaction study by scanning electron microscopy

This study examines how ethosomes interact with filter membranes to evaluate their size and permeability. Vesicle suspension (0.2 ml) is added to filter membranes with a 50 nm pore size in diffusion cells. The upper side of the filter is exposed to air, while the lower side is in contact with phosphate buffer saline solution (pH 6.5). After one hour, the filters are fixed with Karnovsky's fixative at 4°C overnight and then dehydrated with graded ethanol solutions. Scanning Electron Microscopy (SEM) is used to analyze the membranes for vesicle passage and size distribution.

3. Skin permeation studies

These studies assess the penetration of ethosomes into the skin using an in vitro diffusion cell setup. The hair is shaved from the test animals (rats), and the abdominal skin is separated from the underlying tissue. The skin is placed between the donor and receptor compartments of a diffusion cell, with the ethosomal formulation (1.0 ml) applied to the donor side and a saline solution with phosphate buffer (pH 6.5) in the receptor compartment. Samples (0.5 ml) are collected at various intervals (1, 2, 4, 8, 12, 16, 20, and 24 hours) and analyzed using High-Performance Liquid Chromatography (HPLC) to determine drug permeation.

4. Stability study

The stability of ethosomal formulations is evaluated to ensure long-term effectiveness. Vesicles are stored at 4°C ± 0.5°C, and after 180 days, the vesicle size, zeta potential, and drug trapping efficiency are measured. This assessment helps determine any changes in formulation stability over time.

5. Drug uptake studies

These studies measure drug absorption into cells to evaluate the effectiveness of ethosomes. MT-2 cells (1,1106 cells/ml) are cultured in 24-well plates and exposed to ethosomal formulations or control formulations in RPMI medium. After incubation with the drug solution, drug absorption is measured using HPLC to quantify the amount of drug taken up by the cells.

6. HPLC assay

High-Performance Liquid Chromatography (HPLC) is used to quantify drug content in various studies. During in vitro skin permeation and cell uptake experiments, the drug concentration in the receptor compartment is determined using HPLC with a mobile phase consisting of methanol, distilled water, and acetonitrile (70:20:10 v/v).

7. Statistical analysis

Statistical analysis is performed to validate the results of the studies. Data are analyzed using ANOVA (Analysis of Variance) followed by Studentized Range Testing. The PRISM software is used to interpret the results with a confidence limit set at $P<0.05$, ensuring the statistical significance of the observed differences and effects.

Pharmacological screening models for Ethosomes and Transethosomes[36-63]

Pharmacological screening models using experimental animals are essential for evaluating the efficacy and safety of ethosomes and transethosomes as drug delivery systems. These models help in understanding the pharmacokinetics, pharmacodynamics, and overall therapeutic potential of these vesicular systems. Below are detailed explanations of the pharmacological screening models typically employed for ethosomes and transethosomes.

Toxicological studies

Toxicological studies are essential to assess the safety profile of ethosomes and transethosomes. These studies involve administering varying doses of the formulations to experimental animals and monitoring for any adverse effects. Parameters such as behavioral changes, organ function, histopathological examinations, and biochemical markers are evaluated to determine the safety and biocompatibility of the vesicular systems. This model is crucial for ensuring that the ethosomal formulations do not produce harmful effects when used in clinical settings.

Skin irritation studies
To evaluate the safety and irritation potential of ethosomal and transethosomal formulations, skin irritation studies are performed. This involves applying the formulations to the skin of experimental animals, often rabbits, and monitoring for signs of irritation such as redness, swelling, or ulceration. The formulations are applied under occlusive conditions to mimic real-world usage. The results help to assess the potential of the formulations to cause irritation or allergic reactions when used topically.

Drug efficacy studies
Drug efficacy studies involve assessing the therapeutic effectiveness of ethosomal and transethosomal formulations. For example, in the case of ethosomal formulations designed for topical drug delivery, the effectiveness in treating skin conditions or localized infections is evaluated. Experimental animals are treated with the ethosomal formulation and monitored for improvements in the condition being studied. Parameters such as lesion size, severity, or clinical symptoms are measured to determine the therapeutic efficacy of the formulation.

Pharmacokinetic studies
Pharmacokinetic studies are performed to understand the absorption, distribution, metabolism, and excretion (ADME) of drugs delivered via ethosomes and transethosomes. After administration of the formulations to experimental animals, blood and tissue samples are collected at different time points. These samples are analyzed to determine drug concentrations and to study how the formulation affects the pharmacokinetic profile of the drug. This information is crucial for optimizing dosage regimens and understanding the systemic behavior of the drug.

Anti-inflammatory and Anti-arthritic activity
For ethosomal and transethosomal formulations targeting inflammatory conditions, anti-inflammatory and anti-arthritic activity studies are conducted. Experimental animals with induced inflammation or arthritis are treated with the ethosomal formulations. The reduction in inflammatory markers, pain, and swelling is assessed using various methods, such as paw edema measurements or histopathological examinations. These studies help to evaluate the potential of the formulations in managing inflammatory diseases.

Wound healing studies
In wound healing studies, ethosomal and transethosomal formulations are tested for their efficacy in promoting wound repair. Experimental animals with induced wounds are treated with the formulations, and the rate of wound closure and tissue regeneration are monitored. Parameters such as wound area, epithelialization, and collagen deposition are assessed to determine the effectiveness of the formulations in enhancing wound healing processes.

Behavioral and Neuropharmacological studies
For ethosomal formulations intended for central nervous system (CNS) disorders, behavioral and neuropharmacological studies are performed. Experimental animals are assessed for changes in behavior, cognitive functions, and neurological symptoms following treatment with ethosomal formulations. These studies help to evaluate the potential of the formulations in treating CNS-related conditions and to understand their impact on neurological health.

Each of these pharmacological screening models plays a critical role in evaluating the safety, efficacy, and potential therapeutic benefits of ethosomal and transethosomal formulations. By employing these models, researchers can comprehensively assess the performance of these delivery systems and ensure their suitability for clinical use.

Skin permeation studies
Skin permeation studies are crucial for assessing the transdermal delivery capability of ethosomes and transethosomes. In these studies, animal models, such as rats or rabbits, are used to evaluate how effectively the vesicles can penetrate the skin barrier. The skin is typically isolated and mounted in diffusion cells (e.g., Franz diffusion cells) to simulate the transdermal absorption process. The amount of drug permeated through the skin over time is measured, allowing researchers to compare the permeation efficiency of ethosomes and transethosomes against conventional formulations. This model provides insights into the enhancement of drug delivery due to the unique structural properties of the vesicles, such as their size, elasticity, and the presence of penetration enhancers like ethanol and surfactants.

***In-Vivo* efficacy studies**
In vivo efficacy studies involve administering ethosomes or transethosomes containing therapeutic agents to experimental animals to evaluate their pharmacological effects. For instance, animal models can be used to assess the anti-inflammatory, analgesic, or anti-cancer effects of drugs delivered via these vesicular systems. Observations may include measuring pain response, inflammation markers, or tumor size reduction, depending on the therapeutic target. The results from these studies help determine the effectiveness of ethosomal formulations in achieving the desired therapeutic outcomes compared to traditional delivery methods.

Serum uric acid level measurement
Monitoring serum uric acid levels is a critical aspect of evaluating antigout activity. Elevated uric acid levels are a hallmark of gout. Experimental animals treated with ethosomal or transethosomal formulations are subjected to blood tests to measure serum uric acid concentrations. A reduction in serum uric acid levels indicates that the formulation is effective in addressing one of the primary

causes of gout, providing a direct measure of its therapeutic potential.

Comparative studies

Comparative studies are often performed to evaluate the performance of ethosomes and transethosomes against other drug delivery systems, such as conventional liposomes or emulsions. In these studies, the same drug is formulated in different delivery systems and administered to experimental animals. Parameters such as skin permeation, therapeutic efficacy, and safety profiles are compared to determine the advantages of using ethosomes or transethosomes. This model aids in establishing the superiority of these novel carriers in enhancing drug delivery.

Ethosomes in cosmeceuticals

In the realm of cosmeceuticals, ethosomes offer a promising solution to overcoming the limitations of conventional cosmetic formulations. Many cosmetic products aim to deliver active ingredients to the stratum corneum (SC) of the skin, but the efficacy of these products is often hindered by the SC's natural resistance to penetration. This resistance reduces the effectiveness of topical applications. To address this issue, modifications in formulation are necessary to enhance the permeability of the drug or active ingredient through the skin barrier.

Ethosomes, with their unique composition, serve as an effective carrier system for delivering a diverse range of ingredients via topical routes. Their ability to improve penetration through the skin is particularly advantageous in cosmetic applications. Ethosomes enhance the stability of cosmetic formulations, reduce potential skin irritation caused by certain chemicals, and promote better transdermal permeation, especially in formulations designed for elasticity and adaptability on the skin.

One of the notable benefits of ethosomes in cosmeceuticals is their ability to transport antioxidants efficiently. Antioxidants are crucial for mitigating oxidative damage to the skin, and ethosomal delivery systems can improve their effectiveness by ensuring deeper penetration and sustained release. This approach is increasingly employed to address oxidative stress and promote skin health in cosmetic and cosmeceutical applications.

As the cosmetic and pharmaceutical industries continue to evolve, the application of ethosomes in cosmeceuticals represents a significant advancement. Their potential to enhance the stability and efficacy of cosmetic products while reducing skin irritation and improving ingredient penetration marks a new era in both fields.

Marketed ethosome formulations[64,65]

Table 1: Marketed ethosome formulations.

Product Name	Uses	Manufacturer	Key Ingredients	Region	Formulation Type
Cellutight EF	Topical cellulite cream, accelerates metabolism and fat burning	Hampden Health, USA	Caffeine, L-carnitine	USA	Cream
Decorin Cream	Anti-aging cream addressing wrinkles, sagging, age spots, elasticity, and hyperpigmentation	Genome Cosmetics, Pennsylvania, US	Retinol, Hyaluronic Acid	USA	Cream
Nanominox	First minoxidil-containing ethosome product with 4% Minoxidil for hair growth	Sinere, Germany	Minoxidil, Ethanol	Germany	Solution
Noicellex	Topical anti-cellulite cream	Novel Therapeutic Technologies, Israel	Caffeine, Retinol	Israel	Cream
Skin Genuity	Powerful cellulite buster that reduces orange peel	Physonics, Nottingham, UK	Caffeine, Carnitine	UK	Cream
Supravir Cream	Treatment for herpes virus	Trima, Israel	Acyclovir, Ethanol	Israel	Cream
Body Shape	Gel for skin solidification and stretching	Maccabi CARE	Aloe Vera, Caffeine	Israel	Gel
Osmotics Lipoduction Cellulite Cream	Ethosomal cream for reducing cellulite and fat burning	Osmotics, Israel	Retinol, Caffeine	Israel	Cream

CONCLUSION

Ethosomes and transethosomes have emerged as innovative non-invasive carrier systems for delivering a broad spectrum of drugs with varying physicochemical properties, making them highly effective for both local and systemic applications. These vesicular systems are distinguished by their ability to provide controlled or sustained drug release, enhancing therapeutic efficacy

while minimizing the need for frequent dosing. Their excellent biocompatibility and reduced toxicity further underscore their potential as advanced drug delivery platforms. The fundamental advantage of ethosomes lies in their simplicity of composition and preparation methods. The incorporation of ethanol into ethosomal vesicles not only improves their permeability but also differentiates them from other lipid-based vesicles, especially for topical and transdermal applications. Ethanol facilitates deeper skin penetration, thereby optimizing drug delivery and therapeutic outcomes. Transethosomes, an advanced variant of ethosomes, further enhance this capability by incorporating additional penetration enhancers or edge activators, improving drug penetration through the skin barrier even more effectively. Both ethosomes and transethosomes can be integrated into a variety of dosage forms, including gels, patches, and creams. This adaptability makes them versatile tools in pharmaceutical development, allowing for tailored delivery solutions based on specific therapeutic needs. Their ability to enhance drug delivery, combined with their favorable safety profile and ease of formulation, positions them as promising candidates for future therapeutic applications.

Ethosomes and transethosomes are set to play a significant role in the future of drug delivery systems. Their advanced permeability features and versatile formulation options are likely to improve patient compliance and expand the range of treatable conditions. As research and technological advancements continue, these vesicular systems are expected to contribute substantially to the evolution of effective and patient-friendly therapeutic solutions.

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