AMERICAN JOURNAL OF PHARMTECH RESEARCH

Journal home page: http://www.ajptr.com/

A Systemic Overview On Ethosomes: Advances In Liposomal Technology To Enhance Transdermal Drug Delivery

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ABSTRACT

Ethosomes are advanced and modified form of liposomes, which are applied as carriers for transdermal drug delivery. These are developed by Professor Elka Touitou from the Hebrew University of Jerusalem, to enhance the drug administration of amphiphilic drugs. These are soft and flexible lipid vesicles having phospholipids, water, and alcohol in high concentrations about to 20-45% approximately. Ethosomes are easy to prepare through different common methods named as thin film hydration method, cold method, hot method and injection method. These provide enhanced permeation of drugs, highly drug loading capacity, better control over drug release, non-parenteral drug delivery having ability to encapsulate diverse molecular spectrum range. Another key advantage of ethosomes is that they provide excellent patient compliance since these are able to apply as semi-solid formulations, such as gel or cream. The evaluation criteria, such as vesicle size, zeta potential, entrapment efficiency, *In-vitro* permeation studies and *In-vivo* permeation studies, support their effectiveness in targeted drug delivery. Ethosomes demonstrated positive results and the capability to enhance the distribution of amphiphilic drug molecules. This article provides a comprehensive review of ethosomes including their distinctive properties, composition, methods of preparation, physicochemical properties, methods of penetration and the areas of their application in pharmaceutical and cosmeceutical industries. The article examines objectives of ethosomal research as one way of achieving the goal of developing non-invasive drug delivery systems and reports on recent advancements, issues and future prospects. They also put sufficient evidence of the effectiveness of ethosomal therapy in being viable nanocarriers for drug delivery.

Keywords: Ethosomes, Topical formulations, Transdermal drug delivery, Nano Vesicles, Novel drug delivery system, Liposomes.

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Received 22 January 2025, Accepted 05 April 2025

Please cite this article as: Kumar Y *et al.*, A Systemic Overview On Ethosomes: Advances In Liposomal Technology To Enhance Transdermal Drug Delivery . American Journal of PharmTech Research 2025.

INTRODUCTION

Topical therapies that are anointed, bandaged, massaged, or used on the skin have probably been utilised from the beginning of human history. Written records, such those found on Sumerian clay tablets, provide evidence of these practices. Indeed, a liquid ochre-rich composition found in the Blombos Cave in South Africa around a hundred thousand years in the past has been hypothesised to have functioned as both skin protection and ornamentation. About 80 years ago, Zondek's study appears to have been the first quantitative report of using topical therapy to treat a systemic illness(1). In 1979, first transdermal patch was approved by the United States, which is used to deliver scopolamine in the treatment of motion sickness and then approximately 10 years later, Nicotine patches were introduced in the market which became blockbuster(2). Following this scopolamine patch, a variety of patches and formulations that could administer the medication via the skin were introduced to the market. Transdermal drug delivery is an attractive way to administer the dosage. However, skin function as a barrier to the transdermal drug administration and affects the drug permeation which makes the process of drug administration critical. To overcome these skin barriers and to enhance drug delivery, numerous drug delivery systems are designed by researchers. The main focus of modern era is nanoparticles and nanovesicles based drug delivery systems, which provides numerous advantages such as increased bioavailability, targeted drug delivery, controlled and sustained release, ability to cross biological membranes, reduction in drug degradation, versatility in design and many more(3). Drugs and other active substances can be modified at the nanoscale to greatly increase their therapeutic efficacy and lower the possibility of adverse consequences. Using vesicular systems like liposomes or ethosomes is one way to improve the penetration of medications and cosmetics(4). Nanocarriers can be broadly categorized as lipid based, polymeric based or surfactant-based systems leaving aside polymeric based carriers for now, among the lipid-based nanocarriers, transferosomes, ethosomes, cubosomes, vesicular systems including liposomes, niosomes. These vesicular systems in particular are well known to facilitate drug delivery across skin. Freely permeable structures formed when two lipophilic membranes are separated by an aqueous phase or a polar group or moiety and having amphiphilic properties. These structural arrangements improved both cellular and tissue penetration of the drug on surface of skin and deeper systemic effects(5). Liposomal formulations have been investigated in detail significantly because they are, in essence, biodegradable, easy to manufacture, and economical to produce. But it is a well-known fact that liposomes are limited to use on uppermost surface of the skin, predominantly the stratum corneum, due to the non-penetrating nature of liposomes to deeper skin layers. This property sets them apart

from ethosomes having a high percentage of ethanol which improves skin penetration given the fact that ethanol enhances the elasticity of the formulation. Ethosomes are touted to have a penetrative advantage over liposomes however which makes sense because ethosomes do have an elite, structurally resistant, and flexible sulfur-based coating in the form of the phospholipid bilayer, with ethanol strengthening their structure(6)(7). Ethosomes also have a weaker, but notable covering strength hence their core cannot have significant pressure which gives them a degenerating strength. Ethosomes can range from 20-45% of ethanol which makes them contain nanometric cores. Based on the advantages of ethosomes, it can easily be surmised that ethosomes is recommended over using solvents for diluting the ethanol as they make ethosomal application much more effective in terms of skin penetration (5)(8). This article aims to provide a systematic overview of ethosomes including the justification that these are advanced form of liposomes, their distinctive properties, composition, methods of preparation, physicochemical properties, methods of penetration and the areas of their application in pharmaceutical and cosmeceutical industries. The article aims to examine the objectives of ethosomal research as one way of achieving the goal of developing non-invasive drug delivery systems and reports on recent advancements, issues and future prospects. They also put sufficient evidence of the effectiveness of ethosomal therapy in being viable nanocarriers for drug delivery.

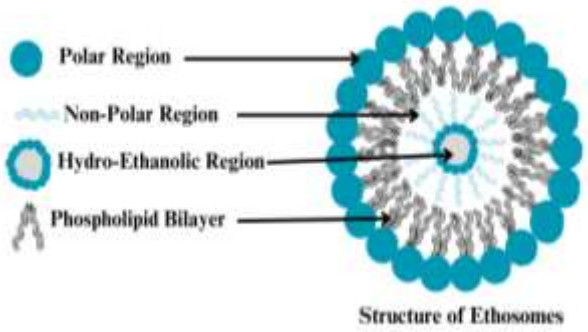

Figure 1: Structure of Ethosomes

Types of Ethosomes:

Ethosomes can be classified based on their internal components into various types which include mainly classical ethosomes, binary ethosomes, and transethosomes. Each of these classifications has significant qualities and advantages of its own.

Classical ethosomes: Classical ethosomes were firstly prepared and presented by Elka Touitou from the Hebrew University of Jerusalem(7). These are the modification form of liposomes containing multilamellar vesicles able to entrap both types of drugs; Hydrophilic and lipophilic. Classical ethosomes are composed of water, phospholipids and ethanol concentration up to 45 %

w/v. These have negative zeta potential and are small in size(9). The negative zeta potential values observed in the vesicles can be associates to the properties of the edge activators or permeation enhancers(10). Classical ethosomes have small size particle distribution. A common range of particle size is 153±4 nm. Donatella Paolino et al. 2005 reported that the size of ethosomes reduced with increasing ethanol concentration, while a decrease in phospholipid concentration led to an increase in ethosomes size(11). Touitou et al. 2000 performed dynamic light scattering and transmission electron microscopy visualization for the examination of the stability of ethosomes via keeping them at room temperature and observed, the average size and size dispersion stayed constant for at least two years. They also observed that the vesicles of ethosomes might have disintegrated or interacted with skin lipids, leading to the formation of new structures. Additionally, the tiny, pliable ethosomal vesicles might have penetrated through the layers of skin(7)(5).

Binary ethosomes:

Binary ethosomes were firstly presented Zhou et al. in 2010(12). A different kind of alcohol was added to classical ethosomes to create binary ethosomes. Binary ethosomes are the modification of conventional ethosomes. Different kind of alcohol such as propylene glycol or isopropyl alcohol are the common alcohol types to form binary ethosomes. When added propylene glycol, it can efficiently enhance the stability of ethosomes. These maintained an intact spherical structure and a lipid bilayer. The average size of binary ethosomes is approximately 142±15.5 nm. Binary ethosomes are especially promising carriers for topical administration as these are smooth, flexible carriers created to enhance the transport of active substances. Zhou et al. 2010 found that the classic passive loading method is not suitable well to form binary ethosomes because the hydrosoluble drug molecules leak from the ethosomal vesicles. They were preferred classic passive loading method to prepare total alkaloids extracted from Sophora alopecuroides (TASA) loaded binary ethosomes(12)(5).

Transethosomes:

Transethosomes were firstly presented by song et al. in 2012(10). This kind of vesicle was created to compose the supremacy of transferosomes and ethosomes into a single composition. Transethosomes are created by combining conventional ethosomes with a permeation enhancer or surfactant. By increasing the space between phospholipids molecules, breaking down of bilayer structure, and improving the ethosomes fluidity, these surfactants become integrated into the phospholipid bilayer. The ethosomes distort and pierce the stratum corneum when applied to hydrated skin, facilitating the drug's transdermal absorption(3).

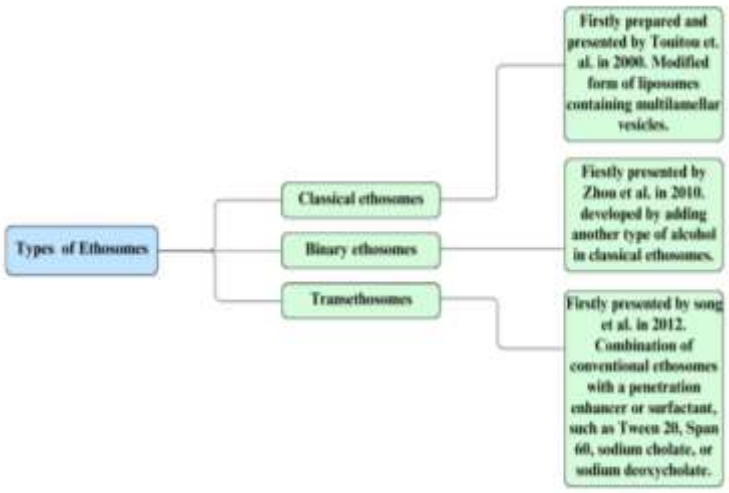

Figure 2: Types of Ethosomes

Materials used in the preparations of Ethosomes:

Phospholipids: Ethosomes or ethosomal systems (i.e. ethosomal gel, patches, creams) have been developed using various phospholipids from several sources(13). Phospholipids have amphiphilic nature as these contain hydrophilic head and hydrophobic tail. This amphiphilic nature of the phospholipids provides the ability to form bilayer structure in the aqueous conditions and helps to be more flexible after the addition of ethanol. Phospholipids create the vesicular structure, encapsulate different kinds of drugs, improve skin penetration, and ensure stability and biocompatibility. These are the essential components in the manufacturing of ethosomes. In ethosomal formulations, phospholipid concentrations are usually found between 0.5% to 5%(14). Phosphatidylcholine is the sole basis for several drug delivery systems, including liposomes and ethosomes(8).

Cholesterol: It is used many times by researchers in preparing ethosomes but 'cholesterol' is not an essential part for preparing ethosomes. Cholesterol is a form of steroid which contains an additional hydrocarbon chain and a sterol component. It is also known as a fluidity buffer, important for the regulation of flow and permeation of phospholipid bilayers in the association of thermal changes. This characteristic reduces the chance of drug leakage, improves the barrier effect of phospholipid bilayer to external effects, and preserves membrane flexibility. In addition, this modification improves encapsulation effectiveness of the drug. Moderate cholesterol levels have been shown to support the integrity of the phospholipid bilayer(15)(16). Drugs such as antivirals, antimalarials, anticancer, and others have been effectively delivered by cholesterol. In comparison to other lipid-based carriers, carriers based on cholesterol are easily controlled, biodegradable, and biocompatible, and they may be generated in huge quantities at a reasonable cost. Additional characteristics of cholesterol is the ability to compose the amphiphilic drugs with

high drug loading efficiency. It is simple to alter the surface of carriers depending on cholesterol. Cholesterol-containing formulations have been shown to inhibit the process of aggregation in the presence of water due to their increased hydrophilic, notable transfection efficiency, and serum compatibility, (17).

Ethanol: One of the most widely used permeation enhancers that can increase drug absorption through the skin and promote percutaneous permeation is the ethanol. It is a key component of ethosomes, distinguishing them from conventional liposomes. The presence of ethanol enhances drug penetration, vesicle flexibility, and stability. Proper ethanol concentration is critical for optimizing ethosomal efficiency in transdermal drug delivery (18). A common concentration of the ethanol used in the development of ethosomes is approximately 20 to 40%. Less than 20% amount of ethanol can lead to the larger size having less permeability of the vesicles of ethosomes and more than 40% amount of ethanol can lead to disrupt vesicle integrity which results in drug leakage, so the ideal range can be 30–40% amount of the ethanol(19)(20).

Propylene glycol: It is used as a permeation enhancer and a common co-solvent in the ethosomes having colourless, transparent characteristics. It helps in the entrapment by its bulge flexibility of the membrane. It can lower the skin barrier's diffusional resistances, potentially as a result of the drug diffusing via the skin while dissolved in the glycol. An increased amount of propylene glycol shows better zeta potential but addition of a large amount of propylene glycol can lead to vesicles leakage (21). Overall, Propylene glycol is significant in ethosomal formulations as it enhances drug solubility, improves vesicle stability, serves as a penetration enhancer, and modifies vesicle properties. Its concentration must be optimized to maintain a balance between drug delivery efficiency and vesicle stability (22).

METHODS OF PREPARATION OF ETHOSOMES:

As we know that ethosomes are modified and advanced form of liposomes with the help of ethanol, so there are various methods to prepare ethosomes such as the liposomes preparation methods like thin film hydration method and Injection method. A variety of ethosomes preparation methods are available, majorly ethosomes preparation methods have been modified by researchers like film rehydration, ultrasonic method, and reverse phase evaporation method. In all of these, four methods are commonly and broadly used to develop ethosomes and these methods are described below.

Thin-film hydration method:

This method is an expansion of standard liposome development method, except that film of lipid is hydrated by the addition of ethanolic-aqueous solution. The phospholipid, first add in chloroform

individually or in a 3:1 or 2:1 mixture of chloroform and methanol. The solvent residue is then extracted from the lipid film by vacuum for a whole night after the organic solvents have been evaporated by a rotating vacuum evaporator operating at a temperature higher than the phospholipid-phase conversion temperature. After that, the lipid film is hydrated with either a phosphate buffer saline-ethanol solution or ethanolic-aqueous solution(14). Sartaj Akhtar Ansari et al. 2021 prepared ethosomes to develop an ethosomal gel of karanjin to treat acne vulgaris using thin film hydration technique. They used 30mg w/w Phospholipid 90G and 3% w/v Karanjin as a drug to dissolve in the menthol and chloroform at a ratio of 1:2 v/v. They performed various methods and techniques to evaluate the prepared ethosomes such as dynamic light scattering for the examination of vesicle shape and size distribution, Differential scanning calorimetry for the examination of physical drug characteristics and excipients, when composed in vesicles (23).

Hot method:

This method involved heating phospholipid in water-based phase at 40°C till the result was a colloidal solution. In another vessel, ethanolic solution and medication were mixed and heated at 40°C. After slowly addition of organic phase, the water-based phase was stirred for five minutes. The Sonication process can be used in the reduction of particle size of ethosomes for a specific size. Ultimately, the preparation should be stored appropriately(24). Ahdaq Ali Faisal Al-Ameri et al. 2024 prepared Meloxicam loaded binary ethosomes to develop ethosomal hydrogel for topical drug delivery using hot method. They prepared organic phase containing meloxicam, propylene glycol and ethanol and aqueous phase contained soya lecithin in deionised water. A syringe pump was used to drop the ethanolic solution phase to the water-based phase at the rate of 200 µL/min via 23-gauge needle while being continuously stirred till one hour at 500 rpm by the help of magnetic stirrer/hotplate. After That, they reduced the vesicle size using a probe sonicator and stored the prepared ethosomes at 4°C. They performed various methods and techniques to evaluate the prepared ethosomes such as dynamic light scattering (DLS) for vesicle size, ultra-centrifugal filter technique and UV spectroscopy for entrapment efficiency(25).

Cold method:

It is known as the classical method. The procedure of this method is simple and broadly used by researchers to develop ethosomes. The ethanolic phase and water-based phase should be individually required to develop ethosomes. The ethanolic phase contained dissolved phospholipids in ethanol or propylene glycol at room temperature. The water-based phase contains water, buffer solution, or regular saline solution, which should be added to the ethanol-based phase. After that, the mixture should be stirred by the help of magnetic stirrer about to 500-700

RPM till 5-10 minutes. Then sonicate the resulting mixture using a probe sonicator to reduce the vesicle size(13). Maha M. Marzouk et al. 2024 developed Carvedilol Ethosomes through the help of Box-Behnken design. They dissolved phospholipid and Carvedilol in ethanol. Stirred the ethanolic phase by the help of magnetic stirrer at 700 RPM and added water drop by drop. They stirred the ethanolic solution for an additional 5 minutes to ensure the homogeneity. The generated ethosomal dispersions were allowed to swell overnight at 4°C in order to develop large multilamellar vesicles. Then they sonicated large multilamellar vesicles on probe sonicator to maintain the small size of the vesicles at 4°C. They performed various methods and techniques to evaluate the prepared Carvedilol ethosomes such as Fourier Transform Infrared Spectroscopy for the Drug excipients interaction studies, dynamic light scattering for the measurement of vesicle shape and size distribution, cooling ultracentrifuge to determine entrapment efficiency(26).

Injection method:

It is the modified process of the conventional method (cold method) having a simple and effective approach to prepare ethosomes with a uniform particle size and improved stability. It involves injecting the lipid phase containing phospholipids and ethanol into an aqueous phase under continuous stirring, leading to the spontaneous formation of ethosomes. Dongmei Qin et al. 2025 prepared Vernonia anthelmintica's total flavonoids loaded ethosomes to treat Vitiligo. They dissolved lecithin and cholesterol in a mixture of the ratio 7:3 of 1,2-propanediol and ethanol. They loaded the resulting solution in a syringe of 5 ml. Approximately 7ml distilled water was added progressively in the period of one hour while magnetic stirring was maintained about to 600 RPM. Then, the mixture was placed in an ice bath and subjected to ultrasonic disrupting for half an hour. Filtered the resulting suspension to produce ethosomes. They used nanoparticle size analyser to examine the vesicle shape and polydispersity index(15).

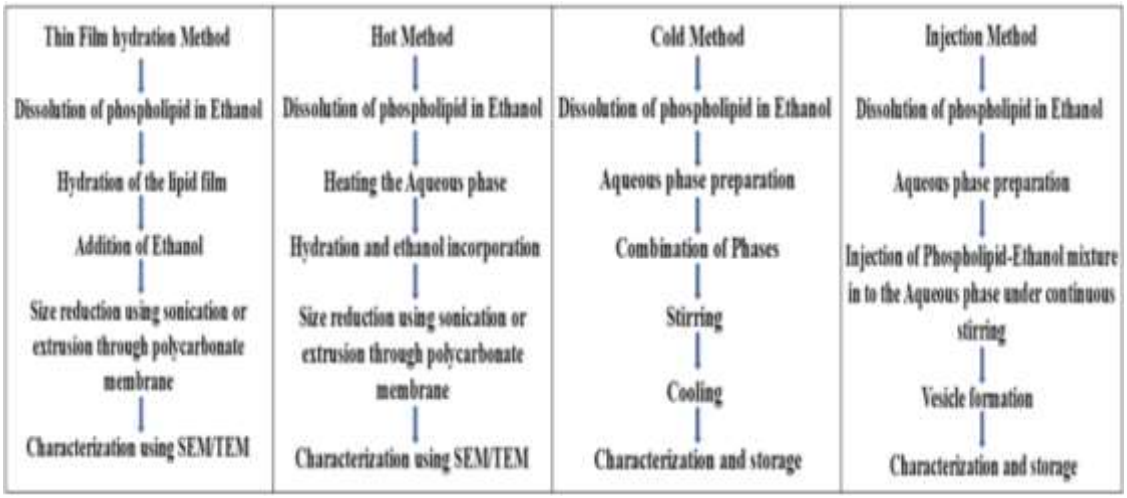

Figure 3: Methods to prepare Ethosomes

Permeation mechanism & studies:

Ethosomes enables drug permeation through the skin via a synergistic mechanism involving lipid disruption, vesicular flexibility, and enhanced partitioning. The ethanol in ethosomal formulations have a significant role to fluidizing stratum corneum lipids, thereby increasing membrane permeability(27). Ethosomal vesicles have great deformability ability, which enables them to pass through tiny intercellular gaps of the skin barriers, enhances this effect even more(28). Additionally, ethosomes interact with skin lipids through fusion and lipid exchange, which further promotes drug deposition in inner parts of the skin. The dual-pathway ethosomal permeation, encompassing both paracellular and transcellular routes, facilitates efficient transdermal drug transport(3). Furthermore, ethosomal composition enhances drug solubility within skin lipids, thereby improving partitioning and retention. Collectively, these mechanisms enable ethosomes for the function of highly efficient vesicles for amphiphilic drugs, offering valuable advantages to the transdermal and topical drug applications.

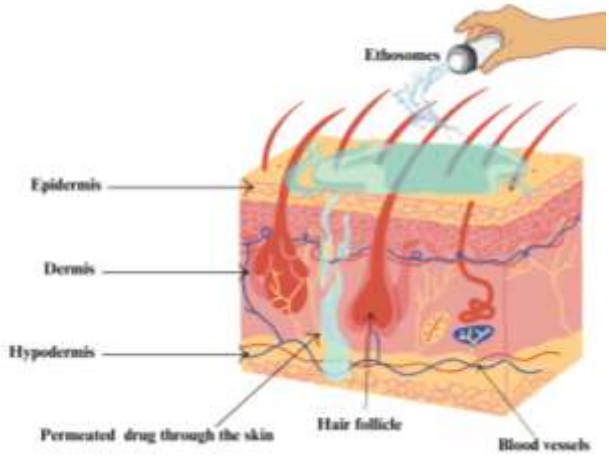

Figure 4: Permeation mechanism & studies

Various ethosomal preparations: Ethosomes are stable and have smaller particles having special benefits of being nontoxic and non-irritating to the skin, which makes them suitable candidates to formulate the transdermal or topical preparations(6). Due to this, researchers are focused on developing various ethosomal preparations such as gels, patches. Some of the common ethosomal preparations are described below.

Ethosomal Gels: These are the semi-solid preparations having ethosomes with a gel base. Ethosomes enhances the topical drug delivery and improves permeation of drug via skin through utilizing ethanol content. Ethosomal gels properties include pH, viscosity, extrudability, and spread ability. The narrow particle size of ethosomes makes them ideal for topical medication administration. Ethosomes and transfersomes are significantly more preferred than liposomes for

topical gel formation. Liposomes exhibited zero-order kinetics, whereas ethosomes show kinetics of first order with a non-fickian diffusion mechanism (29). The two most widely applied gel preparation agents in ethosomal preparation are hydroxypropyl methylcellulose and Carbopol, across each of its relevant grades. It has been demonstrated that these polymers function good with ethosomal preparations, offering necessary(13). Hidha Fathima et al. 2023 prepared Ethosomal Gel of vildagliptin to treat diabetes mellitus by adding Carbopol 934 in the low distilled water content for 180 mins. They converted it into a homogeneous solution using a magnetic stirrer and added the prepared ethosomes to it at 100 rpm. They mixed it until they got a uniform ethosomal gel. In the evaluation of ethosomal gel, they found satisfactory responses in drug release, stability and spreadability(30).

Ethosomal patches: Ethosomal patches have an innovative advancement in transdermal drug delivery systems, combining ethosomal carriers with patch technology to enhance drug permeation through the skin(31). When ethosomes are adjusted into a patch system, ethosomes provide sustained and controlled drug release, improving bioavailability and patient compliance(28). The composition of ethosomal patches mainly contains ethosomes, a backing layer for protection, an adhesive layer for skin attachment, a rate-controlling membrane to regulate drug release, and a release liner to safeguard the patch prior to application(32)(3). The mechanism of action involves ethanol-induced fluidization of the stratum corneum lipids, enhancing permeability, while the deformable ethosomal vesicles traverse intercellular spaces to deliver drugs into deeper skin layers(27). This system offers several advantages, such as improved drug permeation, sustained release profiles, non-invasive administration, reduced systemic side effects, and ability to compose amphiphilic drugs(28). Applications of ethosomal patches span various therapeutic areas, including pain management, hormonal therapies, anti-inflammatory treatments, and neurological disorders (3). Denize Ainbinder and Elka Touitou 2008 prepared an ethosomal patch to deliver the testosterone and also compared the prepared patch with commercially available testosterone patch, Testoderm^{TM} (Alza, USA). They found that the prepared ethosomal patch was performed better and deeper skin permeation (32). Thus, ethosomal patches can hold significant promise for advancing transdermal drug delivery by combining the benefits of ethosomal carriers with the convenience and efficacy of patch systems.

Ethosomal creams: These are the semi-solid preparations having ethosomes. When incorporated into a cream base, ethosomes retain their characteristics of composing the amphiphilic drugs by maintaining the sustained release and improved skin retention (33). Ethosomal creams have benefits to treat of dermatological conditions such as psoriasis, where localization and increment in

drug action is desired(8)(34). Researchers found that the ethosomal creams containing antifungal drugs such as ketoconazole are effective, with better penetration and therapeutic results than the traditionally available creams. Furthermore, ethosomal creams containing natural antioxidants like quercetin and curcumin have demonstrated promising approach in the treatment of skin related conditions linked to oxidative stress(35)(34). Considering their promising approach, issues including production scalability and stability in a range of storage conditions must be resolved to enable broad clinical implementation (20). In broad terms, ethosomal creams provide a flexible and efficient platform for topical medication delivery, encompassing the gap between sophisticated vesicular systems and formulations that are easy to use.

Comparative study of ethosomes and liposomes: As described in the title of this review, ethosomes are the modified-advanced form of liposomes having various advantages. Here is a comparison study of both. It will help to understand the advancement of ethosomes.

Table 1: Comparative study of Ethosomes and Liposomes:

S. No.	Characteristics	Ethosomes	Liposomes	References
1.	Structure	Multilaminar vesicles having high flexibility and fluidity due to ethanol	Rigid bilayer vesicles with less flexibility	(7,33)
2.	Composition	Similar to liposomes but with a significant amount of ethanol integrated into the bilayer.	Composed primarily of phospholipids and cholesterol, forming bilayer vesicles.	(7,71)
3.	Mechanism of penetration	Lipolysis, fusion, and diffusion	Disorders of skin lipids	(4,27)
4.	Drug loading capacity	Higher drug encapsulation ability, especially to the lipophilic drugs, due to the ethanol-induced fluidization of the lipid bilayer.	Suitable to encapsulate amphiphilic drugs but with limitations in loading efficiency.	(71)
5.	Administration routes	Transdermal and topical	Topical, transdermal, parenteral, and oral	(4)
6.	Stability	long-term instability problems like fusion and aggregation.	Enhanced stability as a result of sterilizing properties of ethanol and capacity to inhibit vesicle aggregation.	(21)

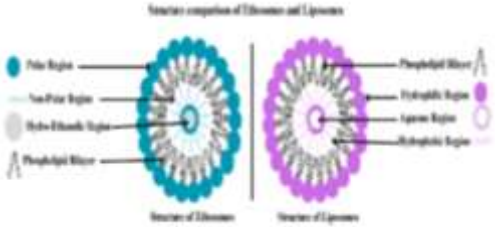

Figure 5: Structure comparison of Ethosomes and Liposomes

EVALUATION OF ETHOSOMES:

Physicochemical characterization:

Vesicle shape, size and distribution analysis:

These characteristics of ethosomes play an important role to influence stability, entrapment efficiency, drug delivery efficacy and skin penetration. According to Touitou et al. 2000, transmission electron microscopy or scanning electron microscopy (SEM) can be apply in the examination of spherical or nearly spherical morphology that ethosomes often display(7). Ethosomal vesicles size range typically from 50 nm to several hundred nanometers, based on formulation parameters such ethanol content, phospholipid concentration, and manufacturing technique. The polydispersity index can show uniformity in vesicle population, and particle size distribution are frequently ascertained by dynamic light scattering (DLS)(8). Consistent medication release and absorption are facilitated by an evenly distributed size distribution, particularly is indicated by a reduced PDI value (<0.3). Furthermore, the size distribution and concentration of vesicles in suspension can be determined by nanoparticle tracking analysis (NTA). Higher absolute values of zeta potential measurements often indicate more electrostatic repulsion and stability in suspension, which aids in evaluating the colloidal stability of ethosomes(36). Combining these analytical methods ensures the characteristics of ethosomal vesicles, which is essential for refining their composition for improved transdermal drug administration.

Zeta Potential:

It refers to the surface charge of vesicles, encompassing its ionic atmosphere (37). It is important to the drug stability; the values of zeta potential are more negative, typically below -40 mV, due to bearing the ethanol and negatively charged phospholipids. Increasing ethanol concentration causes the increase of negative zeta potential values and soya phosphatidylcholine changes in opposite directions. The more negative zeta potential is said to enhance permeation(37,38). The negative zeta potential values observed in the vesicles can be due to the characteristics of edge activators or permeation enhancers(10). Edge activators may influence the values of zeta potential, excess amount of edge activators increase the surface charge of the vesicles. Surface charge is important for predicting the reliability of drug delivery systems based on nanotechnology. Better stability is indicated by higher values. The zeta potential can be influenced by parameters like charge inducers, ethanol, and surfactants, which can hinder the capacity of vesicles to penetrate. Ethosomes that are negatively charged are more readily absorbed by the skin than those that are

positively charged (39). Zetasizer or Dynamic Light Scattering is widely applied to examine the surface charge potential of ethosomes.

Entrapment Efficiency:

Entrapment efficiency is a critical parameter in ethosomal formulations, as it determines the portion of a drug successfully entrapped within the vesicles relative to the total drug added. A high percentage of entrapment efficiency ensures better drug loading, stability, and controlled release(40). Compared to traditional liposomes, ethosomes have a higher entrapment efficiency. This is mainly because ethanol makes both hydrophilic and hydrophobic medications more soluble in lipid bilayers. While hydrophobic medications integrate into the phospholipid layers of ethosomes, hydrophilic drugs are retained inside the aqueous core. Ethanol serves as a solubilizing solvent to optimize drug loading. Studies show that, depending on variables including phospholipid concentration, ethanol percentage, and drug physicochemical characteristics, entrapment efficiency values for a variety of medicinal agents—including antifungal medications (like ketoconazole) and antioxidants (like curcumin), can surpass 80–90%. High entrapment efficacy of ethosomes is explained by their adaptable bilayer structure, which allows a variety of drug compounds to be incorporated without leaking and guarantees targeted administration and prolonged release(41)(7). The commonly known method used to examine the encapsulation efficiency is Ultra centrifugal technique.

Drug release and skin penetration:

***In-Vitro* drug permeation studies:**

These studies play a significant role in determination of potential of ethosomes to improve transdermal drug delivery. To mimic real-world permeation dynamics, these investigations usually include Franz diffusion cells fitted with artificial cellophane membrane (42)(7). In comparison to traditional liposomes or non-vesicular formulations, high ethanol content of ethosomes (20–45%) causes the lipid matrix of stratum corneum to be disrupted, allowing for deeper and more effective drug penetration (43) For example, research on antifungal drugs such as ketoconazole showed that ethosomal formulations significantly deposited in the epidermis and dermis and obtained 2–3 times higher cumulative drug permeation over 24 hours than liposomes (44)(43). Similarly, ethanol-mediated lipid fluidity and better drug solubility allowed hydrophobic medications like quercetin and curcumin to exhibit higher penetration rates in ethosomal systems (45). Permeation efficiency is measured by parameters including lag time, permeability coefficient, and steady-state flux, and ethosomes routinely exceed conventional carriers. Further confirming their mechanistic advantage are ethosomes loaded with fluorescent dye and confocal microscopy, which visually verify deeper

penetration of the epidermal layer(46)(47). The promise of ethosomes for targeted, long-lasting medication administration is highlighted by these in vitro results, especially for localized dermatological diseases.

***In-Vivo* drug permeation studies:**

To assess the manner in which drugs cross biological barriers including skin, mucosal membranes, or gastrointestinal tracts and reach systemic circulation, *in-vivo* drug permeation investigations of ethosomes are carried out within organisms(48). Because they take into consideration dynamic physiological aspects including blood flow, metabolic activity, and immunological responses, all of which are difficult to mimic *in-vitro* systems, these investigations are essential for evaluating bioavailability, therapeutic efficacy, and safety(49)(50). In this regard, researchers frequently use animal models, like rodents or pigs, to mimic human drug absorption. They use methods like micro dialysis, imaging (like PET or fluorescence), or biomarker analysis to measure the amount of drugs in blood or tissues over time (51)(52)(50). The pharmacokinetic information is used to optimize drug formulations for topical or transdermal drug delivery (53). *In-vivo* drug permeation studies of ethosomes continue to be essential in clinical research despite ethical concerns, interspecies heterogeneity, and high costs due to they provide a comprehensive understanding of drug behaviour and connect preclinical findings to clinical outcomes(53)(50).

***Ex-vivo* drug permeation studies:**

Usually Fresh detached skin tissues from the sacrificed or slaughter house, such as rat or pig skin, are usually used in these investigations and placed in diffusion cells, such as Franz diffusion cells. Analytical methods like HPLC or UV spectroscopy are used to measure the amount of medication that permeate the skin into the receptor compartment after ethosomes are introduced to the donor compartment. Ethanol fluidizing impact on the lipid bilayers of the skin and the tiny ethosomes vesicle size have been found to greatly improve drug penetration when compared to regular liposomes or free drug solutions(54)(55)(7). *Ex-vivo* studies have proven ethosomes to have higher efficacy in transporting different types of drugs than conventional liposomes(54)(55). These findings underscore the possibility of ethosomes use in transdermal application of drugs, particularly those that are difficult to absorb. However, different skin models and laboratory conditions may affect the outcome, thus procedures need to be meticulously designed(54).

Toxicology and safety:

Studies on Skin Irritation and sensitization:

Analysis of ethosomes possible skin irritation and sensitization indicates that they have a positive safety profile. Ethosomal formulations have been shown to cause significantly less irritation than

traditional liposomes or free drugs owing to the high biocompatibility and increased penetration efficiency, which decrease surface toxicity in vitro using reconstructed human skin epidermis (RhE) models like EpiDerm™(13). These outcomes are corroborated by other in vivo experiments, like the Draize skin test on rabbits, which proves that ethosomes produce no erythema or edema(7). Concerning other comparative studies, ethosomes show less damage to the skin compared to other vesicular systems. This is likely due to increased concentration, soft and pliable structures that mitigate the chances of erosion of the stratum corneum(56). Research shows that sensitization assays such as local lymph node test (LLNA) have further proved its safety with no appreciable immune response (11). They also comply with the OECD guidelines applicable to topical formulations, which strengthen possible clinical applications of ethosomes.

Cell Toxicity Studies:

Studies focusing on ethosomes, nanocarriers made of phospholipids for transdermal drug delivery, and cytotoxicity have illustrated low toxicity and biocompatibility in a multitude of cell models. Ethosomes show no cytotoxicity at therapeutic concentrations for prolonged periods of time. In vitro studies utilizing human cell lines such as keratinocytes (HaCaT), fibroblasts, and other cellular assays like MTT and CCK-8 showed no signs of strained cytotoxicity. Ethosomes laden with hydrophobic drugs did not significantly reduce cell viability in comparison with free drugs or ordinary liposomes(57)(13). The dose-dependent actions of ethanol, an integral part of ethosomes, are known. Ethanol concentrations lower than 20% include little cytotoxicity, and larger amounts can damage membranes(58)(13). Due to their controlled release kinetics and maintained bilayer structure, ethosomes have better safety margins than other nanocarriers such as transferosomes(59). These findings were also corroborated with in vivo studies in mouse models, which showed no inflammation or any other histological lesions after the topical treatment(58). As outlined in OECD's guidelines for the testing of nanomaterials, the basic criteria of cytotoxicity are still dose and formulation based (OECD, 2020). This still indicates the need for uniform approaches to ensure clinical safeguards are in place.

Stability studies:

To preserve the long-term efficacy and ethosomes safety, stability studies are required. These studies examine the physical, chemical, and biological stabilities within light, pH, temperature, and other different environmental conditions of ethosomal formulations. The stability studies are measured using the drug release profile, zeta potential, encapsulation efficiency, and particle size over a designated time period for the assessment of appropriate conditions of storage and determine shelf life for the ethosomes formulation. These studies are usually done in a controlled

manner to comply with international regulations, such as the International Council for Harmonization ICH Q1A regulations. Stable testing for ethosomal formulation which is meant for transdermal distribution is as vital for assurance of compliance with regulations as for enabling constant performance(7)(60).

Figure 6: Evaluation of Ethosomes

Advantages of Ethosomes: Ethosomes exhibits key benefits over alternative transdermal and dermal delivery methods, making it a prime alternative for transdermal administration of drug. So various benefits of ethosomes are described below.

Small vesicle shape and size: Ethosomes have smaller shape and size, which helps to improve skin permeation and increase surface area results in improved bioavailability(61).

Better penetration: Ethosomes are a good alternative for transdermal drug delivery which tends to improve drug penetration through the skin. Ethosomes, unlike conventional liposomes, improve drug administration via the skin under both occlusive and nonocclusive conditions(62).

Ideal for large and diverse groups: These are the vehicle for the administration of a broad spectrum of drugs. Ethosomes have ability to capsulate all kind of drugs, such as hydrophilic, lipophilic, or amphiphilic peptides and protein molecules(63)(64).

Safe and Non-toxic: Ethosomes are mainly prepared using non-toxic raw materials. Which plays an important role in being non-toxic(62).

Simple preparation process: Ethosomes can be prepared or produced relatively easily, doesn't require complex technical investments, and is ready for quick commercialization(40).

Provides good patient compliance: Ethosomal gels, creams and patches have characteristics such as flexibility, non-irritating and non-invasive, which helps them to provide a good patient compliance(65).

Improved stability and solubility: Ethosomes can increase stability and solubility of the drug in comparison to traditional vesicles by encapsulating them(66).

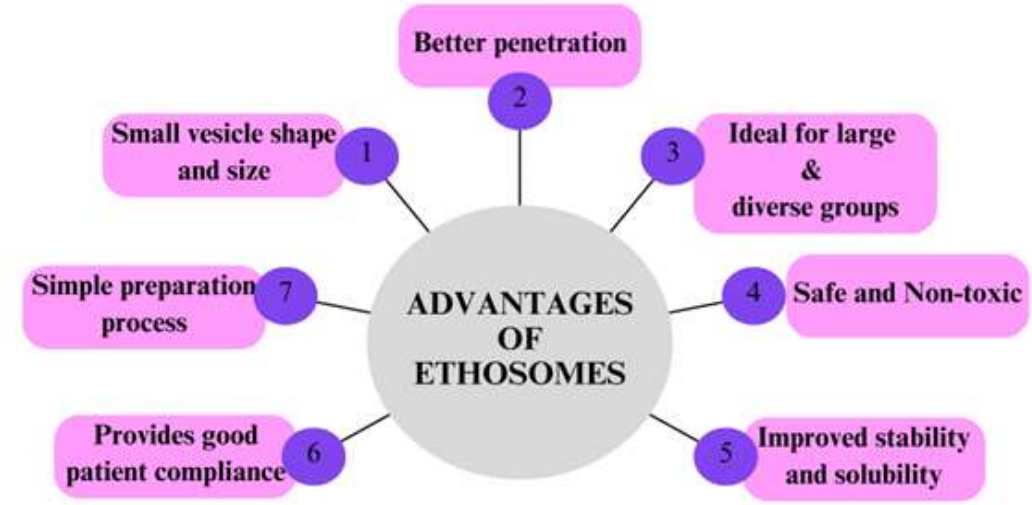

Figure 7: Advantages of Ethosomes

Applications of Ethosomes: In the comparison of traditional liposomes, these have tendency to improve skin penetration and improve bioavailability. Due to this, these can be widely used in transdermal and dermal delivery of drugs.

Topical drug delivery: The application of medications to the skin or mucosal surfaces for either localized or systemic effects is recognized as topical drug delivery. Ethosomes have transformed topical drug administration by greatly increasing drug permeation into and via skin, because of their special structure made up of phospholipids and ethanol(67).

Transdermal drug delivery: Ethosomes have high ethanol content and elastic vesicular structure which results to facilitate the permeation of bioactive substances via skin. They enhance the distribution of hydrophilic and lipophilic drugs within the stratum corneum, including antifungal, antioxidant, and anti-inflammatory drugs(45)(7).

Antifungal drug therapy: Ethosomes have been used to deliver antifungal agents like fluconazole, terbinafine and griseofulvin demonstrating superior skin penetration and retention compared to conventional formulations. This improves efficacy in treating cutaneous fungal infections(47)(12).

Anti-Inflammatory and Analgesic delivery: Targeted delivery to inflammatory areas via ethosomes containing NSAIDs (like diclofenac) or natural substances (like curcumin) improves localized therapy of dermatitis and arthritis while lowering systemic side effects(68)(69).

Vaccine Delivery: Ethosomes facilitate needle-free vaccination by acting as transdermal transporters of vaccine antigens. They hold promise for the creation of intradermal vaccines due to their capacity to transport macromolecules such as proteins and DNA(70).

Cosmeceutical Applications: Ethosomes are used in moisturizing, antioxidant, and anti-aging cosmetic products. They improve skin texture and lessen wrinkles by facilitating absorption via deep layers of the skin of vitamins, peptides, and plant extracts(4).

Figure 8: Applications of Ethosomes

Limitations of Ethosomes: Ethosomes exhibit key benefits over alternative transdermal and dermal delivery methods but these also have various limitations as described below.

Unsuitable to deliver bolus dose: Typically, ethosomal drug delivery systems are designed for gradual, sustained release rather than for quick delivery of bolus-type drug administration(61).

Limited drug loading capacity: Ability of ethosomes to encapsulate medications is limited, especially for hydrophobic or high molecular weight medications(63).

Temperature sensitive: Ethosomes are sensitive to changes in temperature, which can influence their stability and the way drugs are released(43).

Limited clinical data: There is a insufficiency of clinical information about the longstanding ethosomes safety and efficacy in people, despite encouraging preclinical outcomes(56).

DISCUSSION:

Ethosomes are a major innovation in transdermal and topical administration of drug in the comparison to conventional liposomes, as they have several benefits over them. Their distinctive

composition, characterized by a high level of ethanol, increases the permeability and flexibility of the vesicles so that they can permeate the skin deeply. This property makes the administration of amphiphilic drugs more advantageous, thus ethosomes are versatile drug carriers for various therapeutic agents. Several techniques apply to develop ethosomes, e.g., thin-film hydration, hot, cold, and injection methods, allow flexibility in formulation design according to different drug properties and desired release profiles. Ethosomes have proved to possess high drug loading capability, stability, and controlled release behaviour over simple liposomes that tend to reside in the upper layer of the skin. In addition, the option of formulating ethosomes in gels, creams, and patches increases the compliance of the patient since such formulations are minimally invasive and convenient to administer. In spite of these benefits, there are limitations like temperature sensitivity, low drug loading capacity for some molecules, and unavailability of extensive clinical data. Nevertheless, research and development in ethosomal technology are constantly overcoming these drawbacks, opening doors to wider applications in pharmaceutical and cosmeceutical fields. In general, ethosomes are very promising as a good and novel drug delivery system, especially for transdermal and topical uses.

CONCLUSION:

Ethosomes are a very effective and innovative lipid-based nanocarrier system. Their distinctive composition, mainly described by higher concentration of ethanol, enhance vesicular bilayer fluidity, disturbs the lipid structure of skin, and greatly improve permeation of drug through the stratum corneum. This systematic review of ethosomes presents their advantages, like high capacity of drug loading, increased permeation across the skin, and applicability for both lipophilic and hydrophilic drugs. Different preparation procedures, such as cold method, hot method, thin-film hydration, and injection method, have been studied elaborately to ensure optimal ethosomal formulations for pharmaceutical and cosmeceutical uses. The evaluation criteria, such as vesicle size, zeta potential, entrapment efficiency, in vitro permeation studies and in vivo permeation studies, support their effectiveness in targeted drug delivery. Ethosomes preparations, like gels, patches, and creams, have potential applications in curing dermatological diseases, systemic conditions, and cosmetic uses. Still, issues such as formulation stability, irritation caused by ethanol, and limitations in large-scale manufacturing need to be overcome for their acceptance in widespread clinical use. Future studies should concentrate on rational optimization of ethosomal formulations for extended drug release, enhancing their environmental stability, and performing thorough clinical trials to authenticate their therapeutic effectiveness. With progressive

developments, ethosomes have enormous potential to revolutionize transdermal drug delivery, providing an improved and patient-friendly alternative for transdermal therapeutics.

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