ETHOSOMES: A POTENTIAL CARRIER FOR ENHANCING TRANSDERMAL DRUG DELIVERY

Vaishnavi G. Bahe[1], Aman P. Sutarkar[2], Dr. Shagufta A. Khan[3]

[1,2,3]Department of Pharmaceutics, Institute of Pharmaceutical Education and Research, Borgaon (Meghe), Wardha, Maharashtra, India.

ABSTRACT

Topical delivery remains a significant challenge in pharmaceutical and cosmetic industries due to the skin's natural barrier properties. In recent years, ethosomes have emerged as promising carriers for enhancing the penetration of active ingredients through the skin. This review provides a comprehensive overview of ethosomes, exploring their potential in topical delivery applications. The introduction highlights the limitations of conventional topical formulations and the need for novel delivery systems. Ethosomes, composed of phospholipids, ethanol, and water, possess unique properties that enable efficient penetration through the skin barrier. Their structure comprises lipid bilayers with encapsulated aqueous compartments, providing stability and flexibility. Properties of ethosomes, including small size (20-200 nm), high encapsulation efficiency, and deformability, contribute to their enhanced permeation capabilities. Various types of ethosomes, such as conventional ethosomes, ultradeformable ethosomes, and elastic liposomes, offer versatility in formulation design to suit specific application requirements. The composition of ethosomes can be tailored by adjusting the lipid and ethanol content to optimize drug solubility and skin penetration. Ethosomes penetrate the skin through intercellular lipid pathways, facilitated by their small size and lipid bilayer structure. Applications of ethosomes span across pharmaceuticals, cosmetics, and dermatology, delivering drugs, vitamins, antioxidants, and other bioactive compounds. Safety profiles of ethosomes have been extensively studied, demonstrating biocompatibility and low irritation potential. Clinical studies highlight the efficacy of ethosomes in improving drug delivery efficiency and therapeutic outcomes for various dermatological conditions. In conclusion, ethosomes represent a promising platform for enhancing topical delivery, offering improved skin penetration, formulation flexibility, and biocompatibility, thereby addressing the challenges associated with conventional topical formulations. Further research and development are warranted to explore the full potential of ethosomes in diverse topical applications.

KEYWORD: Ethosomes, topical delivery, penetration mechanism, formulation design, application.

1. INTRODUCTION

Any drug delivery system's goal is to deliver an effective therapeutic dose to the intended site of action in the body in order to achieve the appropriate drug concentration as soon as possible and maintain it for the duration of the dosage. For many years, oral administration has been the most popular method of drug delivery; nevertheless, even using this approach, roughly 74% of pharmaceuticals are still not as effective as expected. [1] Oral drug delivery offers convenience and widespread acceptance, but it has several disadvantages compared to the transdermal route. Firstly, oral drugs can be affected by variable absorption rates due to factors like food, pH levels in the stomach, and enzymatic degradation, leading to inconsistent drug levels in the bloodstream. Additionally, some drugs are poorly absorbed in the gastrointestinal tract, requiring higher doses, which can increase the risk of side effects. Moreover, oral medications undergo first-pass metabolism in the liver, reducing their bioavailability and a tendency to cause fast blood level spikes (both high and low). [2] To get above these obstacles, it was imperative to comprehend and create novel medication delivery methods and systems, which can reduce the size and number of doses while boosting the therapeutic efficacy and safety of medications by precisely placing them in the body at the right time and place. This allows for the use of optimal dose concentrations. In order to accomplish these objectives and enhance such traits, transdermal medication delivery systems were developed. [3]

2. HISTORY OF TDDS

Although the name transdermal drug delivery system (TDDS) is relatively new, transdermal medication application is a well-established practice that dates back thousands of years. Many cultures have long been recognized to cure a wide range of illnesses with ointments, pastes, plasters, and intricate infusions. [4] For a considerable amount of time, people have used mustard plasters as a homemade treatment for severe chest congestion. In short, this procedure involved mixing powdered mustard (Brassica nigra) with warm water, spreading the resulting paste onto a flannel strip, and applying the plaster to the patient's chest. The plaster was then secured in place with a cloth binding wrapped around

the patient's body. The mustard's enzyme, myrosin, was triggered by the body's heat and moisture to hydrolyze a glycoside, sinigrin, releasing the strong active component, allyl isothiocyanate (CH2=CHCH2NCS).

Fleischer first stated that the skin is completely impermeable in 1877. This was an audacious and drastic claim that was not long lasting. [4] After nearly 80 years and a large number of collaborative studies, new concepts began to take shape. To find out what causes skin to have barrier qualities that stop molecules from penetrating, investigations were carried out.

Finally, Scheuplein and associates demonstrated how the SC restricted transdermal permeability by a passive mechanism. Michaels and colleagues investigated the apparent diffusion coefficients of model pharmaceuticals in the SC and demonstrated that certain compounds exhibited significant penetrability or permeability [5], despite the skin's strong barrier qualities. Substances/drugs cannot simply pass through the SC due to its nature, which is the primary barrier to percutaneous absorption, as demonstrated by the numerous studies that followed these ongoing, noteworthy setups. These results revealed a completely new field in which more research was necessary to fully understand the concept of treating the skin as an impermeable drug delivery membrane. [3]

Despite the low number of medications now administered by this method, considering the global economic situation, it is anticipated that transdermal product sales generate US$3 billion globally, with the USA accounting for 56% of this amount, Europe for 32%, and Japan for 7%. The formulation and clinical development of transdermal products for cardiovascular disease, neurological issues such as Parkinson's disease, Alzheimer's disease, depression, anxiety, attention deficit hyperactivity disorder (ADHD), or cancer, such as skin cancer or female sexual dysfunction, post-menopausal bone loss, and urinary incontinence, have advanced remarkably as a result of the overall tremendous surge in research and development. [6,7] Overall, these transdermal patches are very convenient, easy to use, and offer the option to eliminate treatment easily in case something goes wrong (like systemic toxicity). They also cause less pain when administering drug candidates, and given their economic viability and recent extremely sophisticated high-end developments, TDDS appears to have a lot of potential as an alternative drug delivery method. [8–11]

3. ADVANTAGES AND DISADVANTAGES OF TDDS
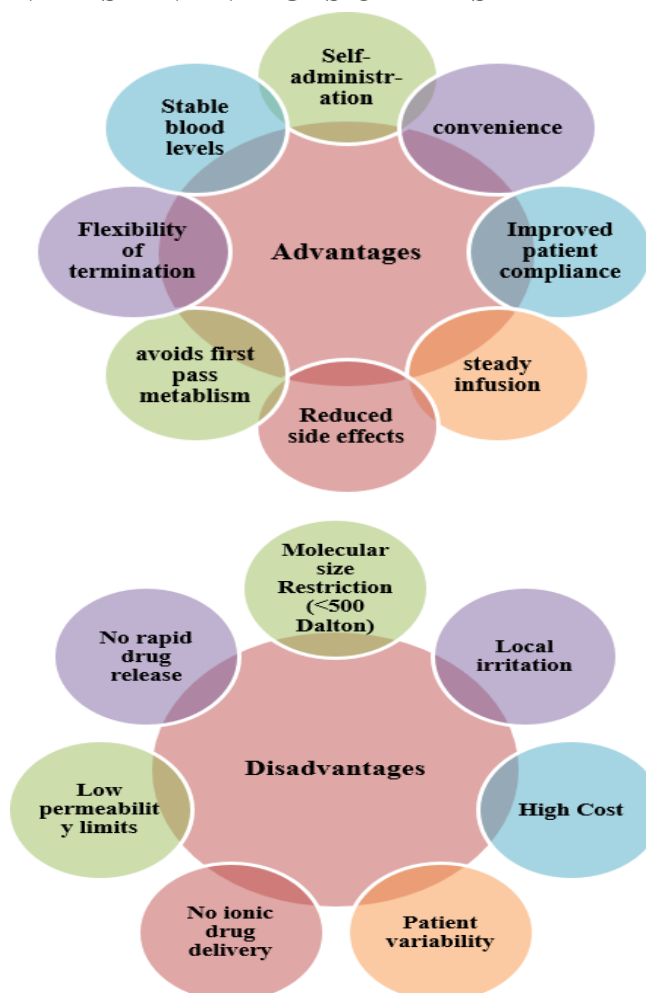
4. ANATOMY OF SKIN

The skin comprises three layers: the outer epidermis (50-200 μm thick) constantly exposed to the environment, requiring monthly renewal for optimal protection. The inner dermis, 5-20 times thicker and the innermost is hypodermis. [12]

The epidermis is composed of a stratified, squamous, keratinizing epithelium, with keratinocytes comprising over 90% of its cells, crucial for barrier function. Other cells like Melanocytes, Langerhans cells, and Merkel cells are present but don't directly contribute to the barrier. The epidermis is divided into five layers, with the Stratum Corneum (SC) forming the outermost layer, crucial for transdermal delivery by preventing water loss and blocking foreign substances. The SC consists of large, flat, keratin-filled dead cells lacking nuclei, continuously replenished by cells from the underlying layers. It contains 10-15 layers of corneocytes, varying in thickness from 10-15 μm in dry conditions to 40 μm when hydrated. The Stratum Corneum (SC) is described as "brick and mortar" structure, with dead, keratinized cells as the bricks and an intercellular matrix as the mortar, composed of various lipids. Molecules traveling through the SC can take two routes: transcellular, passing through keratinocytes, or intercellular, navigating through the lipid lamellae between cells. The intercellular route is favored for drug permeation due to lipids' greater solubility compared to keratinocytes' protein environment. [4,13]

The dermis houses microvasculature, sweat glands, and hair follicles. Dermis facilitates drug absorption into the circulatory system. Comprised mainly of connective tissue, it supports the epidermis and features the Dermal-Epidermal junction, a barrier for large molecules. Divided into papillary and reticular regions, it differs in fiber orientation.

The hypodermis, located between the dermis and muscle fasciae, stores fat and contains large blood and lymph vessels. It integrates with the dermis via nerve and vascular networks, comprising loose connective tissue. Its thickness varies across the body's surface. [3]

5. CHALLENGES TO TDDS

The skin acts as a formidable barrier to topical formulations due to its complex structure and composition, evolved to protect the body from the external environment while regulating water loss and maintaining homeostasis. Understanding the mechanisms behind this barrier is crucial for designing effective topical treatments and drug delivery systems.

A. Stratum Corneum (SC) Structure:

The primary barrier to absorption is the Stratum Corneum (SC), comprised of flattened, keratinized cells with high electrical resistance. This heterogeneous tissue becomes more impermeable towards its lower layers, suggesting a separate barrier.

The SC's horny cells lack nuclei and are relatively inactive. Penetration data, including controlled stripping experiments and electron microscopy, indicate that the keratin-phospholipid complex in the SC cells forms the barrier to penetration. Consequently, the SC presents the greatest resistance to molecule movement, making it the rate-limiting barrier for substances entering the skin from the environment. [3]

B. Lipid Barrier:

Lipids play a crucial role in skin barrier function. Lipid molecules such as ceramides, cholesterol, and fatty acids form lamellar structures between corneocytes, creating a hydrophobic barrier that repels water and prevents the penetration of hydrophilic molecules. Disruption of the lipid barrier can compromise the skin's ability to retain moisture and protect against external threats.

C. Cellular Barrier:

Corneocytes, the predominant cells in the SC, are highly specialized and lack nuclei and other organelles. This cellular architecture contributes to the SC's rigidity and stability, hindering the movement of molecules through intercellular spaces. Additionally, tight junctions between adjacent keratinocytes further reinforce the cellular barrier, preventing the passage of substances between cells.

D. Electrical Resistance:

The SC exhibits high electrical resistance due to the presence of densely packed keratinocytes and lipid bilayers. This resistance poses a significant barrier to the movement of charged molecules, such as ions and polar compounds, further limiting their penetration into the skin.

E. Barrier Function Regulation:

The skin's barrier function is dynamically regulated by various factors, including hydration status, pH, and lipid composition. Disruptions in these regulatory mechanisms, such as changes in skin pH or lipid levels, can compromise the integrity of the barrier and increase permeability to external substances.

F. Metabolic Barrier:

The skin also possesses enzymatic and metabolic processes that can metabolize and detoxify foreign substances. Enzymes such as cytochrome P450 and esterases present in the skin can biotransform and degrade certain molecules, reducing their bioavailability and potential toxicity. [12]

Finding the ideal balance between boosting active component penetration through the skin barrier and guaranteeing adequate retention to preserve therapeutic medication concentrations in the skin presents another difficulty. Another crucial challenge to address is increasing the selectivity by delivering bioactives to lesional skin preferentially. Not to mention, encapsulation may make it possible to distribute molecule classes like peptides, proteins, or nucleic acids that are susceptible to degradation and not yet suitable for topical treatments. [14]

There are several methods that offer alternatives to conventional creams or ointments, particularly for substances with poor skin penetration due to size or hydrophilicity. To enhance drug delivery through the skin barrier chemical agents can be utilized to disrupt skin structure, while techniques like low-frequency ultrasound, electroporation, laser ablation, and microneedle arrays create temporary or permanent pathways for drug penetration. [15–17]

Aforementioned methods have been developed in order to weaken and disturb the highly ordered intercellular lipids in an effort to improve medication transport through intact skin or to boost the force that propels drug penetration through this skin barrier. For a long time, the importance of vesicles in particle transportation and cellular communication has been widely acknowledged. Scientists have gained insight into the characteristics of vesicle architectures that can be used to enhance drug delivery within their cavities by labeling the vesicle for specific cell types.

Nano-vesicular carrier system

A vesicular carrier is a nanosize, intracellular sac with a membrane that serves the purpose for storage or movement of materials within a cell. In living things, several kinds of vesicles are spontaneously generated to store, transfer, or break down specific products and wastes. Scientists working in formulation utilized this occurrence to create liposomes, the primary structure of vesicular carriers. Vesicular carriers are drug delivery nanoparticles made of phospholipids in which, based on its physicochemical properties, the API is both integrated into the lamellar membrane and imprisoned inside an aqueous core. [18]

In addition to offering an alternative, nano-sized carrier systems may also be a potent supplement to the skin delivery technologies aforementioned, enabling topical drug delivery. In an ideal world, they would not interfere with the skin's natural barrier function in order to facilitate the flow of integrated or linked chemicals into the skin. [14]

A plethora of really intriguing clinical applications become possible when skin barrier components are tailored to interact differently with nanocarrier-based drug delivery systems. Particulate carriers might specifically assist certain transport processes that might not be applicable, for example, to dissolved bioactive compounds. Furthermore, drug depots may form in the skin for prolonged release; that is, the free drug may reach deeper skin layers following its release from a particle that is housed in a particular skin compartment. Consequently, uses comprise (i) penetration enhancement for already-established compounds, big molecules that are difficult to synthesize or penetrate deeply, (ii) the delivery of novel medication classes, or (iii) the targeted delivery to particular cell populations. [14] In addition, these systems offer better patient compliance, fewer dose intervals, and regulated medication distribution. With this delivery system, self-administration is also feasible because to its noninvasive application method. [19]

The first vesicular carriers to be created were liposomes, which are self-assembling spherical vesicles with an internal aqueous core surrounded by one (unilamellar) or many (multi-lamellar) lipid bilayers. [20] Typically, phospholipids with or without additional ingredients make up the lipid molecules i.e. vesicles. [21] The hydrophobic acyl chains, or tails, create the bilayer of the pospholipid molecules in solution, whereas the polar heads of the molecules self-assemble spontaneously to form vesicles in contact with both internal and external water. [22] The polar heads' less constrained motion at high alcohol concentration allows the phospholipid molecule to be freer in the bilayer configuration, increasing the membrane's cation permeability. In addition to liposomes, other significant vesicular carriers have also been created as innovative drug delivery systems, including transfersomes, ethosomes, phytosomes, also known as herbosomes, sphingosomes, virosomes , and invasomes. [18]

6. ETHOSOMES

One of the unique lipid vesicular systems with a comparatively high concentration of ethanol is the ethosome. Touitou created this ethanolic vesicular system in 1996. The primary components of enzymatic phagosomes include water, phospholipid, ethanol, and active pharmaceutical ingredients (API). [23] The structural components of ethosomal vesicles are an inner aqueous core containing medication and a phospholipid bilayer. The proportion of ethanol, vesicular bilayer fluidity, mechanism of skin penetration, production technique, and absence of negative effects distinguish ethosomes from other lipid nanocarriers. [19]

Ethosomes are flexible, malleable vesicles that range in size from several microns to 30 nm. According to a publication [23], when ethosomes are made using the same procedure without utilizing a size reduction step, their size is less than liposomes'. The high alcohol level is the cause of this size reduction, and size drops as ethanol concentration rises to 20–45%. The vesicles acquire a net negative charge from the ethanol, which reduces their size.

STRUCTURE OF ETHOSOMES:

Ethosomes are modified form of liposomes so they are little bit similar in structure. Ethosomes and liposomes differ primarily in their composition, particularly in the presence of alcohol and its concentration. Ethosomes are composed of various phospholipid structures, water, and high concentrations of low molecular weight alcohol, such as ethanol or isopropyl alcohol. This high alcohol concentration gives ethosomal lipids a more fluid state compared to liposomes containing the same ingredients but without ethanol. [24]

The inclusion of ethanol in ethosomes serves multiple purposes. Firstly, ethanol acts as a mixing agent for lipid vesicles, enhancing their malleability and allowing for increased distribution in different skin layers. This softness characteristic of ethosomes enables them to penetrate deeper into the skin compared to conventional liposomes. Despite their high ethanol concentration, ethosomes maintain equivalent solidity to conventional vesicles, facilitating enhanced drug distribution ability within the stratum corneum lipids. Furthermore, the presence of ethanol in ethosomes can lead to improved drug loading and encapsulation efficiency, particularly for drugs with high solubility. The ethanol acts as a solubilizing agent, aiding in the incorporation of hydrophobic drugs into the ethosomes structure. Interestingly, the concentration of ethanol in ethosomes can impact their size, making them unique. Decreasing ethanol concentration within the range of 20% to 45% results in an increase in the size of ethosomes. This variation in ethanol concentration offers flexibility in tailoring the size and properties of ethosomes to optimize drug delivery based on specific therapeutic requirements. [24]

PENETRATION MECHANISM OF ETHOSOMES:

The mechanism underlying the penetration of ethosomes into and through the skin remains incompletely unclear. There have been two simultaneous mechanisms of action proposed for

A. Ethanol: a fluidization effect on the stratum corneum lipids and a fluidization effect on the ethosomal lipids. Deformability of the produced vesicles is rising due to the usage of ethanol in the ethosomes preparation process. Ethanol and phospholipids synergistically enhance drug permeation in ethosomal formulations. Ethanol fluidizes both the ethosomal vesicle and stratum corneum lipid bilayers, reducing lipid density and altering their arrangement. This facilitates ethosomal vesicle penetration through the skin. [25]

B. Furthermore, it is anticipated that the high alcohol concentration will disturb some of the stratum corneum lipids. Ethosomes' increased intracellular and intercellular permeability is caused by these activities. The very pliable vesicles have the ability to create pathways through the disorganized stratum corneum, ultimately releasing medication into the skin's deeper layers. As a result, it is reasonable to anticipate the formation of a passage through the skin, which will allow ethosomes to fuse with cells from the deepest layers of the skin.

TYPES OF ETHOSOMES:

A. **Classical ethosomes:**

Classical ethosomes consist of phospholipids, water, and a high concentration of ethanol up to 45% w/w. They are a modification of classical liposomes. For transdermal drug delivery, it has been claimed that classical ethosomes, with their smaller size, negative β-potential, and better entrapment efficiency, are superior to classical liposomes. Furthermore, when compared to classical liposomes, classical ethosomes demonstrated superior skin penetration and stability profiles.6–8 Medications included within traditional ethosomes have had molecular weights ranging from 130.077 Da to 24 kDa.

B. Binary ethosomes:

Zhou et al. introduced binary ethosomes.In essence, they were created by combining the classical ethosomes with a different kind of alcohol. Propylene glycol (PG) and isopropyl alcohol are the most often utilized alcohols in binary ethosomes (IPA).

C. Transethosomes:

Song et al. (2012) first described transethosomes, the next generation of ethosomal systems.17 The fundamental elements of classical ethosomes are present in this ethosomal system, along with an extra component that may be an edge activator (surfactant) or penetration enhancer. In an attempt to create transethosomes, these unique vesicles combined the benefits of deformable liposomes (transfersomes) and traditional ethosomes in a single formulation. Transethosomes have been shown to have better qualities than standard ethosomes by numerous researches.17–30 Various kinds of penetration enhancers and edge activators have been studied to create ethosomal systems with improved properties. It has been observed that medicines with molecular weights ranging from 130.077 Da to 200–325 kDa are entrapped by transethosom
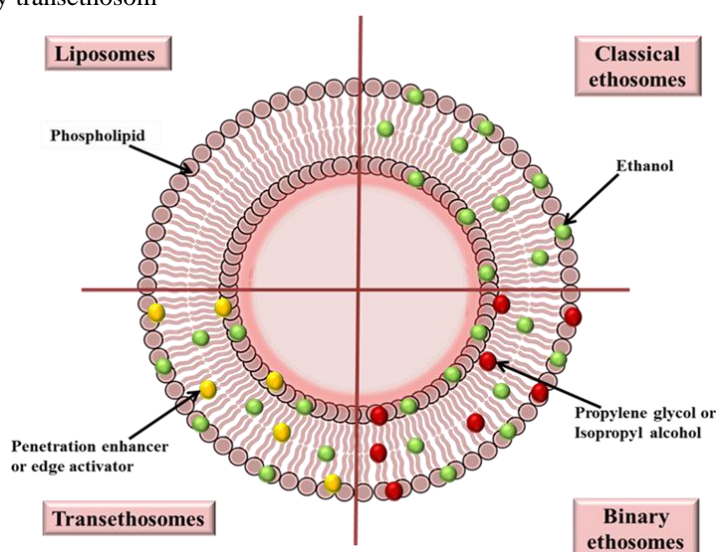

Figure 1. Structure of different types of ethosomes.

7. ADVANTAGES AND DISADVANTAGES OF ETHOSOMES

DISADVANTAGES

- Very low yield so may not be economical.
- Percutaneous absorption depends on the molecular size of the drug which should be rational.
- Ethosomal drug delivery system is limited to potent drugs and not for drugs that require high blood levels.
- Skin irritation or dermatitis may occur in some patients due to permeation enhancer or the excipients used.
- Ethosomal administration is not a means to achieve rapid bolus type drug input, rather it is usually designed to offer slow, sustained drug delivery.
- Adequate solubility of the drug in both lipophilic and aqueous environments to reach dermal microcirculation and gain access to the systemic circulation.
- Release of product during transfer from organic to water media.

COMPOSITIONS OF ETHOSOMES:

ETHANOL:

Ethanol serves as a potent penetration enhancer in ethosomal systems, imparting unique characteristics such as size, ζ-potential, stability, entrapment efficacy, and enhanced skin permeability. Concentrations of ethanol in ethosomal formulations typically range from 10% to 50%. Studies have shown that an optimal ethanol concentration enhances vesicle properties, with formulations containing 40% ethanol exhibiting smaller mean vesicle diameter compared to classical liposomal formulations.

However, exceeding the optimal ethanol concentration can compromise vesicle integrity, leading to increased leakage, larger vesicle size, and decreased entrapment efficacy. Ethanol-induced modifications in the net charge of ethosomal systems contribute to steric stabilization, reducing mean vesicle size and preventing aggregation through electrostatic repulsion. The high ethanol concentration in ethosomes shifts vesicular charge from positive to negative, further aiding in preventing aggregation.

Moreover, ethanol exerts stabilizing effects on ethosomal systems, contributing to their overall stability. It significantly influences entrapment efficiency, with higher ethanol concentrations generally correlating with increased efficiency. [25]

PHOSPHOLIPID:

The choice of phospholipid type and concentration is crucial in ethosomal system formulation as they impact various properties such as size, entrapment efficacy, zeta-potential, stability, and penetration capabilities of the vesicles. Different phospholipid sources, including Phospholipon 90H, 80H, and soy phosphatidylcholine, have been utilized, with each affecting the ethosomal size differently but not significantly altering entrapment efficiency. Incorporating DPPG leads to the production of highly negatively charged vesicles, while cationic lipid DOTAP results in cationic ethosomal vesicles. Generally, phospholipid concentrations in ethosomal formulations range from 0.5% to 5%. Increasing phospholipid concentration tends to slightly or moderately increase vesicular size but significantly enhances entrapment efficiency. Understanding the influence of phospholipid type and concentration is essential for optimizing ethosomal formulations to achieve desired drug delivery outcomes. [25]

Table 1 : Phospholipid used in ethosomes fabrication.

Phospholipid	Composition	Sources	References
Phospholipon 90H	90% Hydrogenated soybean phosphatidylcholine (PC)	Soybean derived	(26)
Phospholipon 90G	90% Hydrogenated soybean phosphatidylcholine (PC)	Soybean derived	(27)
Phospholipon 80H	80% Hydrogenated soybean phosphatidylcholine (PC)	Soybean derived	(28)
Phospholipon 50	50% phosphatidylcholine	Soybean + egg derived	(29)
NAT 8539	73% to 79 phosphatidylcholine, with lysophospha-tidylcholine comprising up to 6%, cephalin up to 4%, and phosphatidic acid up to 6% of the dry residue. Additionally, natural oils and sterols can make up to 6% of the composition, along with ethanol constituting 23% to 27% of the total mixture.	-	(30)
Dipalmitoylphosphotidylcholine (DPPC)	Phosphatidylcholine where both fatty acid chains are palmitic acid (16:0).	Egg yolk	(31)
Lipoid S100	Hydrogenated soy phosphatid-ylcholine with around 100% phosphatidylcholine	Soyabean derived	(32)

SPC 50	Phosphatidylcholine content (50.3%), from soybean	Soyabean derived	(33)
Lipoid S75	Hydrogenated soy phosphate-dylcholine with 75% phosphatidylcholine.	Soyabean derived	(34)
Lipoids E80	80% phosphatidylcholine.	Egg yolk	(35)
Phosphatidylethanolamine	Phospholipid with ethanolamine as the head group.	Derived from egg yolk, soyabean and some meat	(36)
POPC (1-palmitoyl-2-oleoyl-sn-glycero-3-phosphocholine)	palmitic acid (16:0) and oleic acid (18:1) as its fatty acid chains.	Soyabean derived	(37)
DOTAP (1,2-dioleoyl-3- tri-methylammonium-propane [chloride salt])	cationic lipid having dioleoyl-phosphatidylcholine (DOPC) with a trimethylammonium head group.	Synthesized in laboratory.	(38)

CHOLESTEROL:

In ethosomal systems, cholesterol, a rigid steroid molecule, improves stability and entrapment of drug. It decreases vesicular fusion and permeability while preventing leakage of drug. It is typically added at a concentration of 3%, although in certain formulations, it has been added up to 70% of the formulation's total phospholipid concentration. According to a number of studies, higher concentration of cholesterol leads vesicles of larger size. However, high cholesterol concentrations in ethosomes hinder skin permeation due to increased rigidity, as observed in Franz diffusion cell and confocal microscopy studies. Other research confirms that cholesterol addition decreases ethosomal vesicle elasticity, indicating enhanced rigidity. [25]

OTHER ALCOHOLS:

Additional alcohols like propylene glycol (PG) and isopropyl alcohol (IPA) are employed in conjunction with ethanol in the formulation of binary ethosomes.

A. **Propylene glycol** (PG) is a common penetration enhancer used in binary ethosome formulations at concentrations of 5%-20%. Its inclusion influences ethosomal properties, reducing particle size and enhancing entrapment efficiency, permeation, and stability. Increasing PG concentration from 0% to 20% v/v led to a significant decrease in particle size.

B. **IPA** can also be used in fabrication of ethosomes. It was observed that, IPA had a minor impact on drug release but, it had a significant influence on entrapment efficiency. [25]

EDGE ACTIVATOR OR PENETRATION ENHANCER:

Since edge activators and penetration enhancers have a significant impact on the characteristics of the ethosomal system, choosing the right one is essential when formulating transethosomes.

A. **N-decylmethyl sulfoxide:** One well-known penetration enhancer for topical medicinal formulations is dimethyl sulfoxide.69 Instead of using 1% w/w N-decylmethyl sulfoxide in Tumorep DS transethosomes containing 5-fluorouracil, Ainbinder and Touitou used 10% dimethyl sulfoxide. According to the in vitro study findings, transethosomes exhibited 2.9 times higher drug penetration (109.23±12.35 µg) and 2.3 times lower drug accumulation in the skin than conventional ethosomes (38±8.86 • g).

B. **Tweens and spans:** In the ethosomal system, tween 80 is employed at concentrations ranging from 10% to 50% of the total phospholipid concentration. It has been found that Tween 80 incorporation in ethosomal systems reduces vesicular size and improves skin-permeation and system stability. Tween 80 inhibits vesicle fusion and has solubilizing properties that mostly affect the ethosomal system. Ethosomes fabricated with tween 20 were found to be less stable. It was not possible to produce homogenous and stable transethosomes with Spans 80, 60, and 40.

C. **Oleic acid:** Oleic acid increases the fluidity of the stratum corneum, which affects vesicular size, elasticity, ε-potential, and skin-permeation qualities. Compared to transethosomes containing Tween 80 or sodium taurocholate, those containing oleic acid were smaller and more elastic. Additionally, there was an increase in the skin-permeation rate, negative αzeta potential, and drug disposition in the epidermis/dermis of the transethosomes containing oleic acid.

D. L-Menthol: It is added as a penetration enhancer in transethosomes of ascorbic acid at 5% concentration, led to increased drug release through human skin cadavers compared to classical ethosomes. The higher cumulative percentage of drug release (36.5% vs. 33.55%) was attributed to L-menthol's formation of a eutectic mixture with the drug, enhancing solubility and altering stratum corneum barrier properties, as observed in in vitro studies.

E. Sodium stearate: In an effort to improve system stability and elevate the vesicular surface's negative charge, sodium stearate was employed. Three primary impacts resulted from the addition of sodium stearate: 2) raised the negative surface charge; 1) decreased the vesicles that indicated the introduction of sodium stearate carbon chains into the lipid bilayers 3) enhanced the entrapment efficiency, which resulted from the medication and sodium stearate interacting in the lipid bilayers.

F. Cremophor: It is a range of nonionic polyethoxylated detergents, was utilized in ethosomal systems of testosterone propionate at concentrations of 0.5%-1.5% w/w. Cremophor EL-35 decreased vesicle size and increased drug solubility and entrapment efficiency. However, Cremophor RH-40 in transethosomes of artesunate and febrifugine led to unstable vesicles and needle crystal formation after 5 days.

C. Sodium dodecyl sulfate: An anionic surfactant called sodium dodecyl sulfate was utilized to prepare the transethosomes of imiquimod and ketoconazole26 at a dosage of 0.8% w/v. The findings demonstrate that sodium dodecyl sulfate markedly lowered the size, raised the β-potential and entrapment efficiency, and improved the ethosomal systems' in vitro and in vivo skin-permeation characteristics. [25]

Table 2. Components of ethosomes [25]

Ingredients	Uses	Example
Phospholipid	Phospholipon 90, Soya lecithin, Lipoid S100, Egg phosphatidyl choline, etc.	Vesicle forming lipid
Cholesterol	Cholesterol	For providing rigidity and stability to vesicles.
Ethanol	Ethanol, Isolpropyl alcohol.	For providing flexibility and softness to vesicles. As a penetration enhancer.
polyglycol	Propylene glycol, Transcutol P.	As a penetration enhancer.
Dye	Rhodamine-123, Rhodamine red, etc.	Foe characterization test.
Vehicle	Carbopol 934, Poloxamer 470	As a gel former.

8. METHOD OF PREPERATION OF ETHOSOMES

A. Cold method:

This method, widely used for preparing ethosomal systems, was pioneered by Touitou in 1996. It involves two phases: organic and aqueous. In the organic phase, phospholipids are dissolved in ethanol or a mixture of solvents (such as ethanol and propylene glycol for binary ethosomes) at room temperature or 30°C. The aqueous phase, comprising water, buffer solution, or saline solution, is then added to the organic phase slowly, either drop by drop or via a syringe pump at a constant rate. The mixture is stirred vigorously for 5 to 30 minutes at speeds ranging from 700 to 2,000 rpm using either an overhead or magnetic stirrer. This process yields the desired ethosomal suspension. Depending on its properties, the drug to be incorporated can be dissolved in either the aqueous or organic phase.

B. Hot method:

In 1996, the creator of ethosomes published the first description of this technique. Phospholipid is dissolved in water in one vessel, and it is subsequently submerged in a water bath at 40°C to produce a colloidal suspension. After heating ethanol to 40°C in a different jar, dropwise additions of ethanol are made to the phospholipid dispersion while it is continuously mixed with a mechanical or magnetic stirrer. Depending on whether the medication is hydrophilic or hydrophobic, it dissolves in either the organic or aqueous phase.

C. Thin film hydration method:

This method is an expansion of conventional liposome preparation, where a hydroethanolic solution hydrates the lipid film. Initially, phospholipids dissolve in chloroform or a chloroform-methanol mixture, then solvents are evaporated using a rotary vacuum evaporator. Residual solvents are further removed under vacuum overnight. The resulting lipid film is hydrated with a water-ethanol or phosphate buffered saline-ethanol solution while being rotated and heated at specific temperatures for 30 minutes to 6 hours, depending on phospholipid characteristics. This process ensures efficient hydration and formation of liposomes.

D. Reverse phase evaporation method:

This technique, which produces huge unilamellar vesicles, is the least used. To create a water-in-oil emulsion, the organic phase is made by dissolving the phospholipid in diethyl ether and combining it with the aqueous phase at a 3:1 v/v ratio in an ultrasonic bath set at 0°C for five minutes. After the organic solvent is extracted under low pressure, a gel is created. This gel then undergoes intense mechanical agitation to become a colloidal dispersion.

E. Transmembrane ph-gradient method:

In conventional ethosomal preparation methods, drugs are added passively to either the organic or aqueous phase. However, the transmembrane pH-gradient method enables active drug loading by exploiting the pH difference between the acidic interior and basic exterior of the ethosomal system. Initially used for liposomes, this technique has been adapted for ethosomal systems by researchers like Zhou et al and Fan et al. It's applicable for water-soluble drugs with protonizable amine functions. The process involves three stages: preparing the empty ethosomal suspension with an acidic buffer, actively loading the drug into the suspension while adjusting external pH to establish a pH gradient, and incubating the system at a specific temperature for a defined duration. Factors like drug properties, pH of phases, and incubation conditions must be considered before application. This method enhances drug entrapment efficiency by facilitating the active transport of unionized drugs across ethosomal bilayers.

F. The ethanol injection-sonication method:

Using a syringe system and a flow rate of 200 µL/min, the organic phase containing the phospholipid which has been dissolved in ethanol is introduced into the aqueous phase in this approach. The mixture is then homogenized for five minutes using an ultrasonic probe. [25]

9. CHARACTERIZATION OF ETHOSOMES

Characterizing ethosomes involves a comprehensive assessment of their physical, chemical, and structural properties to understand their behavior, stability, and suitability for drug delivery. Here are some key methods used for the characterization of ethosomes:

A. Particle Size and Size Distribution:

Determining the size of ethosomes is crucial as it influences their penetration and drug delivery properties. Techniques such as dynamic light scattering (DLS) or laser diffraction are commonly employed to measure the average particle size and size distribution of ethosomes.

B. Morphology:

Electron microscopy techniques such as transmission electron microscopy (TEM) or scanning electron microscopy (SEM) are used to visualize the morphology and shape of ethosomes. This helps in assessing their structural integrity and uniformity.

C. Zeta Potential:

Zeta potential provides information about the surface charge of ethosomes, which affects their stability and interaction with the skin. Measurement of zeta potential using techniques like electrophoretic light scattering helps in predicting the stability of ethosomes in dispersion.

D. Encapsulation Efficiency:

Encapsulation efficiency indicates the amount of drug encapsulated within ethosomes relative to the total amount used during formulation. Various methods like ultracentrifugation, dialysis, or centrifugation with filtration are employed to separate and quantify the free and encapsulated drug, thus determining encapsulation efficiency.

E. Drug Loading Capacity:

This parameter indicates the amount of drug loaded per unit mass or volume of ethosomes. It is determined by measuring the concentration of the drug in the ethosomes dispersion and calculating the amount of drug loaded based on the volume or weight of ethosomes.

F. Physical Stability:

Stability studies involve assessing changes in particle size, morphology, drug content, and leakage over time under different storage conditions (e.g., temperature, humidity). Stability is typically evaluated using techniques such as DLS, TEM, and high-performance liquid chromatography (HPLC).

G. In vitro Drug Release:

In vitro drug release studies are conducted to evaluate the release profile of the drug from ethosomes over time. Various diffusion cells (e.g., Franz diffusion cells) or dialysis membranes are used to mimic the skin barrier, and the amount of drug released is quantified using suitable analytical methods.

H. Skin Permeation Studies:

Skin permeation studies involve assessing the ability of ethosomes to deliver drugs across the skin barrier. Techniques such as Franz diffusion cells with excised animal or human skin are commonly used to measure drug permeation and penetration depth.

I. Confocal Scanning Laser Microscopy:

Confocal Scanning Laser Microscopy (CSLM) is utilized to examine the depth and mode of skin penetration by ethosomal formulations. It allows optical scanning of skin thickness along the z-axis.

J. XRD: Vesicular carrier formulations may be subjected to X-ray diffraction (XRD) research to determine if the carriers are crystalline or amorphous.

K. Degree of deformability or elasticity:

The flexibility of the membranes during the passage of vesicles through membrane holes or intercellular spaces determines their elasticity or degree of deformability. An essential component for improving skin penetration or permeation is the deform-ability index. [18] By employing these characterization techniques, researchers can gain insights into the physical properties, stability, drug-loading capacity, and drug delivery efficiency of ethosomes, thus guiding their formulation and optimization for various pharmaceutical and cosmetic applications.

ETHOSOMAL DOSAGE FORMS:

Most studies focus on initial ethosomal suspensions, rich in alcohol. However, incorporating these systems into suitable dermal/transdermal vehicles offers advantages: preventing ethanol evaporation, prolonging skin contact, enhancing drug efficacy, improving stability, shelf life, and patient compliance. Ethosomal systems have been integrated into various formulations like ethosomal gels, transdermal patches, and creams, expanding their pharmaceutical applications. [25]

A. Ethosomal gels:

Ethosomal gels are evaluated based on pH, viscosity, spreadability, and extrudability. Common gel-forming agents include Carbopol and hydroxypropyl methylcellulose, known for their compatibility with ethosomal systems and ability to provide required viscosity and bioadhesive properties. Researchers have explored various polymer types and concentrations for ethosomal gel preparation. Studies compare skin-permeation and disposition properties of drugs from ethosomal gels to traditional formulations. For instance, Puri and Jain found 5-fluorouracil transdermal flux from ethosomal gels to be 4.9-fold higher than marketed creams, with 9.4-fold higher skin disposition. Similarly, aceclofenac flux from ethosomal gel exceeded that of Zynac gel. Other authors reported ethosomal gels' superiority over traditional counterparts for diverse drugs. Interestingly, ethosomal suspension exhibited faster drug release than ethosomal gel due to the latter's higher viscosity. Such findings underscore ethosomal gels' potential in enhancing transdermal drug delivery efficacy and highlight the importance of formulation optimization for specific therapeutic goals. [25]

B. Ethosomal patches:

Preparation of ethosomal patches involves the use of molds, making it more complex than for ethosomal gels. Only seven research articles have reported ethosomal patch formulations for various drugs like testosterone, artesunate, febrifugine, ligustrazine, valsartan, tizanidine hydrochloride, and insulin. Different polymers such as polyvinylpyrrolidone/vinyl acetate, acrylic resin, and hydroxypropyl methylcellulose E15 were utilized along with triethyl citrate as a plasticizer. In vitro and in vivo studies comparing ethosomal patches with non ethosomal patches showed significantly higher transdermal delivery and skin deposition of drugs from ethosomal patches. Ethosomal patches offer advantages over gels and creams by facilitating ethosomes application under occlusive conditions, potentially enhancing permeation. In vitro studies by Godin and Touitou on bacitracin ethosomes using human cadaver skin showed comparable drug permeation regardless of occlusive or non-occlusive application. This suggests occlusion minimally impacts ethosomes mediated drug permeation. Similar research supports ethosomes' ability to enhance dermal drug delivery under both occlusive and non-occlusive conditions. Overall, ethosomal patches provide a promising avenue for efficient drug delivery through the skin, offering flexibility in application methods while maintaining efficacy. [25]

C. Ethosomal creams:

Till today only few studies have reported on ethosomal cream formulations, both involving Curcuma longa extract-loaded ethosomal systems for photo-protection and anti-wrinkle effects. Results from human volunteer trials showed promising outcomes. Overall, incorporating ethosomal systems into vehicles like gels, patches, and creams enhances skin permeation. Gels are deemed the most suitable, while ethosomal creams are preferred for cosmetic applications. [25]

INTERNATIONAL JOURNAL OF PROGRESSIVE RESEARCH IN ENGINEERING MANAGEMENT AND SCIENCE (IJPREMS)

www.ijprems.com
editor@ijprems.com

Vol. 04, Issue 05, May 2024, pp: 1062-1078

e-ISSN : 2583-1062

Impact Factor: 5.725

APPLICATIONS OF ETHOSOMES:

A. Ethosomes for bacterial and viral skin infections

Ethosomes have demonstrated significant efficacy in delivering antibiotics for skin infections. In murine models, ethosomal erythromycin effectively healed Staphylococcus aureus infections, outperforming hydroethanolic solutions. Research on bacitracin permeation, using fluorescently labeled ethosomes, confirmed enhanced skin penetration via CLSM and FACS analysis. Additionally, a gel formulation combining clindamycin and salicylic acid notably improved mild to moderate acne vulgaris. For vancomycin hydrochloride, a nanoethosome-iontophoresis combination enhanced transdermal flux, reducing bacterial count in S. aureus-induced mediastinitis in rats. This highlights ethosomes' stability and efficacy in transdermal drug delivery, particularly in combating bacterial infections, suggesting promising applications in dermatology. [19]

Ethosomal delivery of fluorescently labeled bacitracin demonstrated deep penetration into rat skin layers, monitored by CLSM following topical application. Co-loading FITC-Bac with RR in ethosomes proved significantly more efficient in permeating human cadaver skin compared to classic liposomes, delivering both molecules to a depth of 200 microns. Co-localization of drug and phospholipid confirmed ethosomal penetration mechanism, contrasting with negligible delivery from classic liposomes. These findings underscore the efficacy of ethosomes in facilitating deep skin penetration of bacitracin and co-loaded molecules, highlighting their potential for enhanced transdermal drug delivery. [39]

B. Ethosomes in fungal infections

Ethosomes offer enhanced delivery of Acyclovir (ACV) for herpes labialis, reducing side effects and improving percutaneous penetration into the basal epidermis, as demonstrated in clinical studies. Griseofulvin ethosomes exhibit superior antifungal activity compared to liposomes. Deformable liposomes prove more effective than other lipid-based nanocarriers for ketoconazole targeting in Candida albicans-induced fungal infection. Econazole nitrate-loaded ethosomal gel effectively treats deep fungal infections. Ethosomal formulations of fluconazole and itraconazole also show efficacy against fungal infections. Transethosomes of voriconazole enhance skin deposition in hairless mice. A Cavamax W7 composite ethosomal gel enhances clotrimazole delivery across the epidermis, exhibiting superior antifungal activity against Candida albicans and Aspergillus Niger, as observed through in vitro and in vivo studies, indicating promising applications in fungal infection treatment. [19]

C. Testerone ethosomes for hormonal deficiency

Because of hepatic first pass metabolism, testosterone, a significant male sex hormone that is primarily administered in male hypogonadism, is inefficient when taken orally. While intramuscular injection frequently results in supra-physiological or sub-physiological testosterone levels, direct testosterone injection is extremely uncomfortable. Thus, it is discovered that transdermal distribution of testosterone offers an alternate means of avoiding issues related to the parenteral route and the hepatic first pass metabolism.

Testoderm^{TM} (Alza, USA), a testosterone patch that is sold commercially, was tested for efficacy against an ethosomal patch containing testosterone called Testosome using both in vitro and in vivo methodologies. Testosome demonstrated 30 times higher levels of testosterone skin penetration in in vitro experiments compared to Testoderm^{TM}, and Testosome-derived skin contained seven times more of the substance. Testosome exhibited 2.2 and 2.4 times higher area under the curve (AUC) and maximum concentration (Cmax) values in vivo as compared to Testoderm^{TM}.

An additional testosterone-containing ethosomal gel formulation was created and assessed against the commercially available Androgel® (Unimed) testosterone gel. Rats used in in vivo clinical investigations revealed that Testosterone ethosomal gel had 6.4 times higher drug skin penetration than Androgel®. [19]

D. Ethosomes for menopausal syndrome

Buspirone hydrochloride (BH) has low oral bioavailability due to extensive first-pass metabolism (3.9%) and a short half-life (2.5 hours). Its hydrophilic nature limits skin penetration. Ethosomes are designed to enhance BH permeability, evaluated for treating menopausal syndrome in animal models. BH ethosomes prove effective and safe, offering potential for alleviating menopausal symptoms like hot flashes and anxiety. [19]

E. Ethosomes for erectile dysfunction

In a pilot clinical research involving 15 patients who experienced 17 erectile episodes, ethosomal prostaglandin E1 (PGE1) was produced and assessed for the treatment of erectile dysfunction. The test measured the patients' erectile response following topical application of ethosomal PGE1 to the glans penis in order to assess their capacity for sexual activity. After ethosomal PGE1 was applied topically just once, it was shown that 12 out of 15 patients

exhibited increased penile stiffness and increased systolic peak velocity. The erection lasted for ten to sixty minutes. The trial participants did not experience any side effects, including penile erythema, with the ethosomal PGE1.

A study developed a nanoethosomal vardenafil formulation using a thin layer evaporation technique, guided by the Box-Behnken design, for treating erectile dysfunction. Administered topically as a film, it bypassed hepatic first-pass metabolism. In male Wistar rats, the transdermal nanoethosomal formulation exhibited twice the bioavailability of oral suspension. It facilitated effective transdermal permeation, offering prolonged management of impotence.[19]

F. Delivery of peptides through ethosomes

Peptides and proteins, due to their large size, typically do not permeate the skin's stratum corneum (SC), leading to low oral bioavailability. Therefore, they are usually administered via intravenous (I.V.) or subcutaneous (S.C.) routes. Insulin, an oligomeric protein with a molecular weight of 6000 Dalton per monomer, is typically given via the I.V. route for insulin-dependent diabetes mellitus. Researchers have explored non-passive delivery methods like iontophoresis and phonophoresis for delivering insulin through the skin. Additionally, passive delivery enhancement using deformable phospholipid vesicles has shown efficacy in facilitating percutaneous absorption of insulin. An ethosomal insulin patch has been developed and tested in vivo on normal and diabetic rats, demonstrating a significant (up to 60%) reduction in blood glucose levels and a prolonged effect lasting at least 8 hours compared to a non-ethosomal insulin patch. [19]

G. Ethosomes for parkinsonism disease

Trihexphenidyl (THP) is a cationic drug used to treat Parkinsonism disease, affecting over 2% of the elderly population. Its short half-life of 3 hours necessitates frequent oral dosing, challenging for Parkinson's patients with swallowing difficulties. Topical delivery offers an alternative, with ethosomes designed to enhance transdermal delivery. Characterized by small size (109 ± 2 nm) and high drug entrapment efficiency (75 ± 0.5%), THP ethosomes significantly outperformed liposomes. In dorsal skin experiments on nude mice, THP flux from ethosomes was 51 times greater than from liposomes, with higher skin accumulation over 18 hours. Moreover, THP ethosomes exhibited stability for over 2 years. [19]

H. Minoxidil ethosomes for hair loss

Many people worldwide are currently afflicted with hair diseases such as acne, seborrhea, and excessive hair loss. Therefore, for the efficient treatment of pilosebaceous illnesses, focused distribution of the specific medicine to the hair follicles is crucial. A lipophilic medication called minoxidil is applied topically to the scalp to cure hair loss. The preparation of minoxidil ethosomes and their in vivo assessment in hairless rats were conducted to explore the targeting of minoxidil to pilosebaceous units via ethosomes. The findings revealed that minoxidil was localized in the pilosebaceous units, indicating that ethosomal carriers were used to transport minoxidil at a higher rate. [19,39]

I. Anti-inflammatory and anti-arthritis ethosomes

Various ethosome formulations have been developed to enhance the transdermal delivery and anti-inflammatory effects of drugs. Ammonium glycyrrhizinate (AG) ethosomes demonstrated significant reduction in erythema intensity and duration compared to hydroethanolic solutions. Cannabidiol (CBD) ethosomes showed enhanced skin accumulation and suppressed carrageenan-induced paw edema. Triptolide-loaded ethosomes exhibited rapid erythema reduction without delay. Propylene glycol liposomes provided the most prolonged inhibition of edema, followed by ethosomes, in carrageenan-induced paw edema models. Carbopol-loaded meloxicam nanoethosomal gel showed higher edema inhibition compared to oral formulations. Lycopene ethosomes effectively reduced Anthralin-induced ear swelling. Matrine ethosomes exhibited significant anti-inflammatory effects and permeation across rat skin. Diclofenac ethosomal gel demonstrated significant paw edema inhibition compared to liposomes and plain hydrogel. Tetrandrine ethosomes showed superior ex vivo permeation and therapeutic efficacy on arthritis models compared to liposomes. Ethosomes prove promising for improving drug delivery and anti-inflammatory outcomes. [19]

Recently, a medication candidate called cannabidol (CBD) was created to treat rheumatoid arthritis. Lodzki et al. have created a transdermal administration of CBD-ethosomal formulation. The results indicate a significant increase in its skin penetration and, consequently, activity. [24]

J. Ethosomes for vaginal delivery

Metronidazole, an antifungal drug, was developed and tested for vaginal administration using pH-responsive ethosomes. Using a phosphate buffer pH 5.5 as the medium and a regenerated cellulose semi-permeable membrane, the Franz diffusion cell was used for the in vitro permeation investigation. According to the study, metronidazole may be delivered continuously using ethosomal gel at a maximum flow of 143.67 +2.73 µg/cm2/h. [19]

K. Ethosomes for analgesic and anti-pyretic action

In vivo testing of transdermal ibuprofen ethosomes demonstrated significant analgesic and antipyretic effects in animals. Fevered rats treated with ibuprofen ethosomal gel experienced a gradual decrease in body temperature, reaching normal levels within 3 hours and maintaining it for 12 hours. Oral administration led to a shorter duration of reduced body temperature. Additionally, ethosomal ibuprofen gel showed higher analgesic effects compared to oral ibuprofen treatment in mice, particularly at 120 and 360 minutes post-application. These findings highlight ethosomes as an effective carrier for transdermal ibuprofen delivery, potentially avoiding gastrointestinal adverse effects associated with oral administration. [19]

K. Space- Ethosomal system

Conventional methods struggle to deliver macromolecules like peptides, proteins, and nucleic acids into the skin. Skin penetrating peptides, like the "Space" peptide, offer promise for transdermal delivery. Incorporating Space peptide into lipid-based carriers, such as Space-Ethosomal Systems (SES), facilitates the delivery of hydrophilic macromolecules like hyaluronic acid (HA) into the skin. In vitro and in vivo studies confirmed enhanced penetration of HA into the epidermis and dermis using SES. Similarly, siRNA delivery into the skin faces challenges due to its large size and hydrophilicity. Utilizing Space peptide in a DOTAP-based ethosomal system (DOTAP-SES) successfully delivered siRNA into the skin, addressing difficulties in topical delivery of such macromolecules. These findings underscore SES as an effective formulation for transdermal delivery of challenging macromolecules. [19]

L. Anti-hypertensive Ethosomes

Topical delivery of Valsartan, an antihypertensive drug with low oral bioavailability, offers a solution to its first-pass effect and poor gastrointestinal absorption. Ethosomal and nanoethosomal formulations of Valsartan exhibited superior antihypertensive effects compared to oral administration in Wistar rats, with prolonged and significant reductions in blood pressure. Ethosomal Valsartan showed better efficacy and sustained antihypertensive activity compared to oral suspension, while nanoethosomal gel formulations achieved a notable 34.11% reduction in blood pressure in hypertensive rats, demonstrating their effectiveness in treating hypertension.

M. Delivery of troublesome drug molecule

Since oral distribution of these huge, biogenic molecules is quite challenging and has poor penetration, transdermal delivery is a convenient technique to administer peptides and proteins. Ethosomes are a fantastic idea to boost the therapeutic efficacy and penetration of the aforementioned compounds. [24]

Table 3. Commercially available ethosomal products [19]

Brand name	Active pharmaceutical ingredient (API)	Application and dosage form	Manufacturer
Noicellex	Numbers of API	Hair serum	Sinere, Germany
Supravir	Acyclovir	Cream	Trima, israel
Cellutight EF	Numbers of API	Cream	Hampden health, USA
Skin genuity	Numbers of API	Cream	Physonics Nottigham, Germany
Body shape	Numbers of API	Cream	Maccabi-care, israel

STABILITY OF ETHOSOMAL FORMULATION

Ethosomes, colloidal dispersions used for drug delivery, face stability challenges due to phospholipid hydrolysis and oxidation. Turbiscan Lab® Expert analyzes optical properties, distinguishing destabilization processes like coalescence or sedimentation. Ethosomes containing varying linoleic acid amounts showed no destabilization signs in long-term studies. Minoxidil, testosterone, and trihexphenidyl HCl ethosomal formulations remained stable for 2 years, while erythromycin gel was stable for 1 year. A 5-Fluorouracil transethosomal gel stayed stable for 2 months under accelerated conditions and 11 months at room temperature. Multilamellar and large unilamellar benzocaine-loaded ethosomes maintained stability, while small unilamellar vesicles experienced aggregation. Long-term stability studies beyond one year are essential due to limited research. Turbiscan optical analysis provides valuable insights into the stability of ethosomes, crucial for their pharmaceutical applications. [19]

SAFETY OF ETHOSOMAL FORMULATION

Ethosomes, as carriers for drug delivery, have been extensively studied for their skin tolerability and safety in various in vitro and in vivo experiments. These studies are crucial as they determine the suitability of ethosomes for topical

application, particularly on intact, wounded, infected, or damaged skin. In vitro investigations have shown no significant toxicity of ethosomes on cell cultures. For instance, ethosomes containing Phenyl ethyl Resorcinol did not induce acute dermal irritation in albino rabbits. Similarly, Valsartan ethosomes were evaluated for primary skin irritation in intact rat skin, demonstrating no erythema or edema post-application. These findings suggest the safety of ethosomes for topical use. Further studies have evaluated the safety of ethosomes in various formulations and applications. For example, cetrizine ethosomal formulation was found safe for topical use on mouse skin without any side effects. Additionally, histological examination post-application of buspirone ethosomes did not reveal any changes in skin structure or thickness of the horny layer. Ethosomes have also been investigated for their efficacy and safety in treating specific skin conditions. Transdermal psoralen ethosomes and liposomes were deemed safe and effective after seven days of evaluation on rat skin. Similarly, ethosomal suspension of melatonin demonstrated safety, less irritation, and good tolerability on rabbit skin in a long-term study. Moreover, ethosomes have shown promise in therapeutic applications. For instance, MTO ethosomal gel exhibited a tumor inhibitory effect on melanoma-bearing mice without severe side effects. Similarly, transdermal ethosomal ibuprofen treatment did not induce significant changes in kidney, liver, or muscle function parameters, indicating safety in biochemical and hematological analyses. Dermal irritation studies of lidocaine ethosomes on guinea pigs revealed no signs of irritation or histopathological changes in the skin. Human studies further support the safety and tolerability of ethosomes. Reflectance spectrophotometry was used to evaluate the tolerability of ethosomes in healthy volunteers, showing no adverse reactions. Clinical trials have also demonstrated the efficacy and safety of ethosomes in treating various skin conditions. For instance, ethosomes containing clindamycin phosphate and salicylic acid significantly improved acne vulgaris without worsening symptoms or side effects. Similarly, acyclovir ethosomes improved clinical parameters in patients with Herpes Labialis without inducing skin irritation or toxicity. In vivo antifungal activity studies of griseofulvin-loaded ethosomes showed complete cure of dermal fungal infections with good tolerability and safety. Overall, the extensive research on ethosomes highlights their safety and efficacy for topical application on intact and damaged skin. These findings underscore the potential of ethosomes as promising carriers for transdermal drug delivery with minimal irritation and adverse effects. Further studies are warranted to explore the full therapeutic potential and optimize formulations for clinical use. [19]

CLINICAL ASPECTS FOR DERMAL USE OF ETHOSOMES

With the advancements in nanotechnology, the focus in medicine has shifted towards specifying medical indications, application methods, and carrier architectures with precision. Ethosomes offer a promising avenue for enhancing the delivery of therapeutic agents, addressing challenges such as short half-life, first-pass metabolism, and gastrointestinal irritation. Studies have demonstrated that ethosomes facilitate higher permeation flux of drugs across biological barriers compared to traditional liposomes or hydroalcoholic solutions. However, the stability of ethosomes poses a challenge due to lipid/phospholipid oxidation, necessitating refrigerated storage for optimal stability. Other strategies for stability improvement include the formulation of provesicles and gel formulations to enhance viscosity and prolong residence time. Ethosomes and other vesicular carriers can be prepared using solvent evaporation or mechanical dispersion methods and characterized based on various parameters. Ethosomes hold potential applications in transdermal drug delivery and nanomedicine, offering solutions for drugs with solubility and permeability issues. They can incorporate features like formability, biodegradability, and pH sensitivity, making them suitable for diverse therapeutic needs. External and endogenous triggers can be utilized for targeted drug delivery, catering to specific conditions such as microbial therapy, wound healing, and cancer treatment. While ethosomes show promise in preclinical investigations, translating experimental approaches into clinical applications poses challenges. Preclinical models need to accurately predict skin penetration, tissue concentration, and biological efficacy to assess the clinical value of new carrier systems. Moreover, the selection of carrier systems and indications must consider patient benefits, health economics, and competition with existing therapies.

Factors such as particle size, loading capacity, and release kinetics need to be carefully evaluated for each application. Tailoring carrier systems to specific needs, such as hair follicle targeting or intracellular delivery, requires a nuanced approach. Translational efforts are essential to bridge the gap between academic research and industrial development, ensuring compliance with good manufacturing practices and demonstrating clinical efficacy. In summary, ethosomes represent a promising platform for drug delivery, offering solutions to complex challenges in medicine. However, their clinical translation requires rigorous evaluation, strategic planning, and collaboration between academia and industry to realize their full potential in improving patient outcomes.[19]

10. CONCLUSION

The development of ethosomes represents a promising advancement in enhancing the bioavailability of therapeutic agents, offering improved delivery for a wide range of compounds, including both small and large molecules, soluble and insoluble substances, as well as phytomedicines. Ethosomes address various physiological and drug-related delivery challenges, such as short half-life, first-pass metabolism, and gastrointestinal irritation, which can limit the effectiveness of conventional delivery methods like hydroalcoholic solutions. Studies have consistently demonstrated that ethosomes facilitate higher permeation flux of drugs across biological barriers compared to traditional liposomes or ordinary solutions. However, a key challenge in utilizing ethosomes and other vesicular carriers lies in maintaining their stability, particularly due to lipid/phospholipid degradation through oxidation. To mitigate this, storage under refrigeration at 4–8°C is often recommended for optimal stability. Additionally, formulation strategies such as provesicles and gel formulations have been explored to enhance stability and viscosity, thereby prolonging residence time at the application site. Methods for preparing ethosomes and other vesicular carriers generally fall into two categories: solvent evaporation and mechanical dispersion. Ethosomes can be characterized through various parameters including encapsulation efficiency (EE), loading capacity (LC), vesicle yield (VY), morphology, polydispersity, zeta potential, viscosity, surface tension, pH, biodistribution, and lipid, alcohol, and drug contents. Ethosomes hold significant potential in transdermal drug delivery (TDD) and nanomedicine, offering solutions for drugs with solubility and permeability issues. They have versatile applications in targeted drug delivery, allowing for precise delivery to specific sites within the body. In summary, ethosomes represent a promising avenue for improving drug delivery, overcoming various challenges associated with conventional methods. Their enhanced permeation capabilities and potential for targeted delivery make them valuable tools in pharmaceutical research and development. However, addressing stability concerns and further optimizing formulation techniques are crucial for maximizing their therapeutic potential in clinical settings.

11. REFERENCES

[1]   Ahmed A. Transdermal drug delivery systems: an overview. International Journal of Biomedical and Advance Research. 2011;2(1):38–56.

[2]   Alexander A, Dwivedi S, Ajazuddin, Giri TK, Saraf S, Saraf S, et al. Approaches for breaking the barriers of drug permeation through transdermal drug delivery. Vol. 164, Journal of Controlled Release. 2012. p. 26–40.

[3]   Pal K. Transdermal Drug Delivery System (TDDS) - A Multifaceted Approach For Drug Delivery. J Pharm Res [Internet]. 2014;8(12):1805–35. Available from: http://jprsolutions.info

[4]   Chen HY, Fang JY. Chen & Fang Therapeutic patents for topical and transdermal drug delivery systems Therapeutic patents for topical and transdermal drug delivery systems [Internet]. Vol. 10, Exp. Opin. Ther. Patents. 2000. Available from: http://www.ashley-pub.com

[5]   Daniels R. . Strategies for skin penetration enhancement. 2004 Oct (Vol. 37, No. 1, pp. 50-55). In Skin Care Forum. 2004 Oct;37(1):50–5.

[6]   Gaur PK, Mishra S. Transdermal drug delivery system: a review. . Asian Journal of pharmaceutical and clinical Research. 2009 Jan;2(1):14–20.

[7]   Giri TK, Thakur A, Alexander A, Ajazuddin, Badwaik H, Tripathi DK. Modified chitosan hydrogels as drug delivery and tissue engineering systems: Present status and applications. Vol. 2, Acta Pharmaceutica Sinica B. Chinese Academy of Medical Sciences; 2012. p. 439–49.

[8]   Goswami DS, Uppal N, Goyal S, Mehta N, Gupta AK. Permeation Enhancer for TDDS from Natural and Synthetic Sources: A Review. Vol. 2, REVIEW ARTICLE Journal of Biomedical and Pharmaceutical Research. 2013.

[9]   Gupta V, Yadav SK, Dwivedi AK, Gupta N. INTERNATIONAL JOURNAL OF PHARMACY & LIFE SCIENCES Transdermal drug delivery: Past, present, future trends. Int J of Pharm & Life Sci (IJPLS). 2011;2(9):1096.

[10]   Harsoliya MS, Patel VM, Modasiya M, Pathan JK, Chauhan A, Parihar M, et al. Development and Evaluation of Transdermal Drug Delivery System of naproxen Drug with Chitosan for Treatment of Arthritis [Internet]. Vol. 3, International Journal of Pharmaceutical & Biological Archives. 2012. Available from: www.ijpba.info

[11]   Hasan MK, Rahman MA, Shahin SM, Anwar M, Islam U. In Vitro and In Vivo Evaluation of a Rosiglitazone Maleate-loaded HPMC-PVA Blend Patch.

[12]   Cevc G. Drug delivery across the skin. Exp Opin Invest Drugs. 1997;6(12):1887–937.

[13]   Benson HAE. Transdermal Drug Delivery: Penetration Enhancement Techniques. Vol. 2, Current Drug Delivery. 2005.

[14] Vogt A, Wischke C, Neffe AT, Ma N, Alexiev U, Lendlein A. Nanocarriers for drug delivery into and through the skin — Do existing technologies match clinical challenges? Journal of Controlled Release. 2016 Nov 28;242:3–15.

[15] Azagury A, Khoury L, Enden G, Kost J. Ultrasound mediated transdermal drug delivery. Vol. 72, Advanced Drug Delivery Reviews. Elsevier; 2014. p. 127–43.

[16] Blagus T, Markelc B, Cemazar M, Kosjek T, Preat V, Miklavcic D, et al. In vivo real-time monitoring system of electroporation mediated control of transdermal and topical drug delivery. Journal of Controlled Release. 2013;172(3):862–71.

[17] Sklar LR, Burnett CT, Waibel JS, Moy RL, Ozog DM. Laser assisted drug delivery: A review of an evolving technology. Lasers Surg Med. 2014;46(4):249–62.

[18] Mbah CC, Builders PF, Attama AA. Nanovesicular carriers as alternative drug delivery systems: Ethosomes in focus. Vol. 11, Expert Opinion on Drug Delivery. Informa Healthcare; 2014. p. 45–59.

[19] Nainwal N, Jawla S, Singh R, Saharan VA. Transdermal applications of ethosomes–a detailed review. Vol. 29, Journal of Liposome Research. Taylor and Francis Ltd; 2019. p. 103–13.

[20] Preiss MR, Bothun GD. Stimuli-responsive liposome-nanoparticle assemblies. Vol. 8, Expert Opinion on Drug Delivery. 2011. p. 1025–40.

[21] Elsayed MMA, Abdallah OY, Naggar VF, Khalafallah NM. Lipid vesicles for skin delivery of drugs: Reviewing three decades of research. Vol. 332, International Journal of Pharmaceutics. 2007. p. 1–16.

[22] Alberts B, Johnson A, Lewis J. The lipid bilayer. Mol Biol Cell. 2002; 09 MU Og. 1996.

[23] Razavi H, Janfaza S. Ethosome: A nanocarrier for transdermal drug delivery. Vol. 6, Journal of Paramedical Sciences (JPS) Spring. 2015.

[24] Abdulbaqi IM, Darwis Y, Khan NAK, Assi RA, Khan AA. Ethosomal nanocarriers: The impact of constituents and formulation techniques on ethosomal properties, in vivo studies, and clinical trials. Vol. 11, International Journal of Nanomedicine. Dove Medical Press Ltd.; 2016. p. 2279–304.

[25] Bhosale SS, Avachat AM. Design and development of ethosomal transdermal drug delivery system of valsartan with preclinical assessment in Wistar albino rats. J Liposome Res. 2013 Jun;23(2):119–25.

[26] Bendas ER, Tadros MI. Enhanced Transdermal Delivery of Salbutamol Sulfate via Ethosomes [Internet]. 2007. Available from: http://www.aapspharmscitech.org

[27] Prasanthi D, Lakshmi PK. Development of ethosomes with taguchi robust design-based studies for transdermal delivery of alfuzosin hydrochloride [Internet]. Vol. 2012, International Current Pharmaceutical Journal. 2012. Available from: http://www.icpjonline.com/documents/Vol1Issue11/06.pdf

[28] Caddeo C, Sales OD, Valenti D, Saurí AR, Fadda AM, Manconi M. Inhibition of skin inflammation in mice by diclofenac in vesicular carriers: Liposomes, ethosomes and PEVs. Int J Pharm. 2013 Feb 25;443(1–2):128–36.

[29] Verma DD, Fahr A. Synergistic penetration enhancement effect of ethanol and phospholipids on the topical delivery of cyclosporin a. Journal of Controlled Release. 2004 May 31;97(1):55–66.

[30] Bodade SS, Shaikh KS, Kamble MS, Chaudhari PD. A study on ethosomes as mode for transdermal delivery of an antidiabetic drug. Drug Deliv. 2013;20(1):40–6.

[31] Fan C, Li X, Zhou Y, Zhao Y, Ma S, Li W, et al. Enhanced topical delivery of tetrandrine by ethosomes for treatment of arthritis. Biomed Res Int. 2013;2013.

[32] Shen S, Liu SZ, Zhang YS, Du MB, Liang AH, Song LH, et al. Compound antimalarial ethosomal cataplasm: Preparation, evaluation, and mechanism of penetration enhancement. Int J Nanomedicine. 2015;10:4239–53.

[33] Shen LN, Zhang YT, Wang Q, Xu L, Feng NP. Enhanced in vitro and in vivo skin deposition of apigenin delivered using ethosomes. Int J Pharm. 2014 Jan 2;460(1–2):280–8.

[34] Fathi-Azarbayjani A, Ng KX, Chan YW, Chan SY. Lipid vesicles for the skin delivery of diclofenac: Cerosomes vs. other lipid suspensions. Adv Pharm Bull. 2015;5(1):25–33.

[35] Fang YP, Huang Y Bin, Wu PC, Tsai YH. Topical delivery of 5-aminolevulinic acid-encapsulated ethosomes in a hyperproliferative skin animal model using the CLSM technique to evaluate the penetration behavior. European Journal of Pharmaceutics and Biopharmaceutics. 2009 Nov;73(3):391–8.

[36] Madsen JT, Vogel S, Johansen JD, Andersen KE. Encapsulating contact allergens in liposomes, ethosomes, and polycaprolactone may affect their sensitizing properties. Cutan Ocul Toxicol. 2011 Jun;30(2):116–23.

[37] Chen M, Zakrewsky M, Gupta V, Anselmo AC, Slee DH, Muraski JA, et al. Topical delivery of siRNA into skin using SPACE-peptide carriers. Journal of Controlled Release. 2014 Apr 10;179(1):33–41.

[38] Godin[1] B, Touitou[1] [2] E. Ethosomes: New Prospects in Transdermal Delivery [Internet]. 2003. Available from: www.begellhouse.com