A review article on: Comparative study between Ethosomes and Conventional liposomes

Shubham Yadav[1]*, Janaki Devi Sirisolla[2]

1. M Pharmacy student, Department of Pharmaceutics, GITAM Institute of Pharmacy, GITAM (Deemed to be University), Rushikonda, Visakhapatnam, Andhra Pradesh, India, PIN-530045.

2. Assistant Professor, Department of Pharmaceutics, GITAM Institute of Pharmacy, GITAM (Deemed to be University), Rushikonda, Visakhapatnam, Andhra Pradesh, India, PIN-530045.

Corresponding author Email ID: syadav4@gitam.in

ABSTRACT

Transdermal drug delivery's main drawback is that most drugs only penetrate the skin poorly into the human skin. The skin's basic defence mechanism is contained within the stratum corneum, being the top layer (SC). Various approaches have been created to make the skin barrier more vulnerable. Ethosomes and Liposomes in cosmetics and pharmaceuticals are used for skin penetration. Ethosomes are elastic nanovesicles made of phospholipids and include ahigh ethanol concentration (20–45%). It is well known that ethanol effectively improves permeability, drug deposition, strong flexibility and deformability which have been included in vesicular systems to create elastic nanovesicles. Compared to hydroalcoholic solutions or standard liposomes, the ethosomal systems are significantly very effective in chemical delivery to the skin regarding amount and depth. Transdermal delivery has become simple due to their distinct structures and high levels of ethanol, which has improved the efficacy of the drugs and patient compliance. Ethosomal dispersions are included in creams, patches, and gels for convenient use and stability. Because of their better composition, ethosomes have a number of advantages over conventional liposomes when it comes to therapeutic drug delivery for conditions like alopecia, acne, skin infections, psoriasis and hormone imbalances, respectively. This article offers a thorough analysis of the ethosomal system and a prediction of its effectiveness as a nanocarrier for transportation of active substances to the skin.

KEY WORDS

Liposomes, ethosomes, transdermal delivery, permeability, ethanol

INTRODUCTION

One of the key subjects of pharmaceutical research is the controlled delivery of drugs into the body. Effective drug delivery is just one aspect of optimal therapy, in addition to proper drug selection.[1-3] When compared to the conventional oral administration route, transdermal delivery exhibits additional benefits, including reduced presystemic metabolism, more contained delivery, avoidance of incompatible delivery caused by food interactions and prevention of drug breakdown inside the stomach's extremely acidic environment.[1-5] Naturally, altering the vehicle or using a drug carrier concept is the greatest way to increase drug penetration and/or localisation.[5-8] Paul Ehrlich and Bangham proposed the idea of using liposomes as a cure for treating numerous ailments. Drugs that are hydrophilic or lipophilic can be entrapped in the body by liposomes, which are tiny lipid vesicles that contain water. Many drugs used to treat locally or systemically spread diseases were delivered by liposomes via transdermal and topical delivery routes. Since they are non-toxic, eco-friendly, capable of enclosing lipophilic as well as water-soluble compounds, liposomes have many benefits as drug delivery vehicles. Different generations of liposomes are developed to subdue this barrier effect because conventional liposomes have various drawbacks regarding the ability of penetrating the stratum corneum barrier to the skin. Comparison of various vesicular carriers with various distinguishing characteristics is shown in **Table 1**. Developed by Touitou, ethosomes are the third generation of elastic lipid carriers. Ethosomes are ethanol-modified liposomes that serve as reservoir systems for the continuous delivery of medication to the target region. [7-12] Ethosomes are flexible vesicles that release drugs into several layers of skin by acting with ethanol effect and lipid penetration. Additionally, ethanol may provide vesicles soft, flexible qualities that make it easier for them to enter the skin's deep layers.[10-13] The skin delivery method has gained popularity and convenience over time as a more effective means of delivering drugs while avoiding the drawbacks of parenteral and oral route. Skin delivery is a simple, non-invasive option to parenteral route that offers a number of benefits, including avoiding presystemic metabolism, improved patient consent, resulting elevated plasma concentrations and minimizing systemic side-effects. Transdermal and topical drug delivery are both included in skin delivery. Without needing to focus on systemic circulation, topical delivery is designed for treating a local dermatological disease. Topical formulations include, for instance, local anesthetics, anti-fungal and anti-acne medications, anti-inflammatory drugs.[14] Different kinds of nanocarriers exist, such as lipid-formed, polymer-formed and surfactant-formed ones. Vesicular systems are more adaptable with the skin because they are made with lipids found in the skin. Due to their favourable qualities, including affordability, biodegradability and an easy creating procedure, liposomes have been the subject of substantial research. Liposomes, however, are typically confined to the SC or the top skin layers of the epidermis since they are unable to easily permeate through the skin layers. [16,17] Instead, because of their unique characteristics, such as their great deformability and flexibility, ethosomes are the vesicular carriers with improved skin delivery qualities. In addition to having a smaller vesicle size ranging from tens of nanometers to microns depending on the composition, ethanol also gives ethosomes great deformability, fluidity and stability. [18,19] Therefore, ethosomes appear to be better than traditional liposomes or traditional hydro-alcoholic solutions as to increasing the extent and effectiveness of skin penetration, according to a number of studies. [20,21]

Table 1: Different Characteristics between Conventional Liposomes and Ethosomes[8]

Characters	Liposomes	Ethosomes
Vesicles	Lipid bilayer vesicle	Stretchy lipid vesicle carriers of 3rd generation
Flexibility	Naturally rigid	Great deformability and elasticity due to ethanol
Administration Route	Parenteral, Transdermal, Topical and Oral	Transdermal and Topical
Characteristics	Microscopic Spheres (Vesicles)	Elastic Liposomes
Permeable Mechanism	Diffusion/Lipolysis/Fusion	Lipid Perturbation
Composition	Cholesterol and Phospholipids	Ethanol and Phospholipids
Skin Penetrating Extent	Low penetration rate between the stratum corneum due to hard shape and size	Ability to easily enter the paracellular space with the help of ethanol effect
Marketing Products	Doxil, Ambisome	Decorin Cream, Nanominox
Limitations	Unable to penetrate deep skin layers	No soluble drug in ethanol

ETHOSOMES AND CONVENTIONAL LIPOSOMES

ETHOSOMES

Ethosomes, which are further unique lipid carriers made of ethanol, phospholipids, and water, were created by Touitou in 1997. They are capable of enhancing skin delivery of many drugs.[22] An effective permeating enhancer, ethanol is thought to work by interfering with the stratum corneum's intercellular area. These soft vesicles are new vesicle carriers for improved skin delivery. Drugs can enter the systemic circulation and/or deeper skin layers thanks to ethosomes, which are non-invasive delivery vehicles. The ethosomes are special due to the high ethanol capacity because ethanol is well-known to distort the lipid bilayers structure in the skin. The ability to permeate the stratum corneum is thus provided when it is integrated into a vesicle's membrane. Additionally, despite having equal stability, the lipid membrane of the stratum corneum lipids is packed loosely than typical vesicles due to their high ethanol collection. This permits a more pliable shape and enhances the ability to distribute drugs in these lipids. In particular, multilamellar vesicles are produced when an aqueous solution is mixed with an ethanol solution of phosphatidylcholine.[23] Additionally, it provides the system with a negative surface charge that stops vesicles from aggregating and drugs from leaking. Ethanol and phosphatidylcholine interact to give vesicles their flexible nature. By substituting the hydrophilic head group, the solvent actually lowers the phase transition temperature of phosphatidylcholine, which facilitates the transition from the gel state to the high elastic liquid crystalline state.[24,25] In fact, ethanol's capability to operate as a penetration enhancer, encouraging deeper skin permeation or straight into systemic circulation, is its most crucial component in the formulation. First, ethanol alters the stratum corneum's normal arrangement, increasing the permeability of lipid membrane. The vesicles then create their own paths by moving through the disrupted stratum corneum due to their elastic nature.[26] The drug release with the transdermal absorption is finally produced by the fusing of ethosomes in

deep skin layers.[27] It should be noted that ethanol appears to have a considerable impact on the permeation flux; as a result, the permeation flux increases with increasing ethanol content.[28] The precise method of transdermal delivery is still being researched, despite the seamer route of skin penetration having been suggested as a potential mechanism of ethosome delivery.[29] Ethosomes have a number of advantages over other vesicular systems, including effectiveness in non-occlusive and occlusive conditions, improved intracellular, transdermal and dermal delivery, ultra-elastic structure, smaller vesicle size, ability of enclosing both water and non-water soluble drugs, increased stability, multidisciplinary application and good consent.[30-32] There are many ethosomal formulations present on the market now for cosmetic purposes, and ethosome delivery systems have recently been the subject of patent applications.[33,34] Although some authors have claimed that the formulation's residual ethanol concentration may cause skin irritation, other authors have shown their safety through an in vivo irritation research.[34,35] In specifically, the potential irritant reactions brought on by cutaneal application of nanoparticulate systems were assessed with a patch test carried out on 20 healthy patients, revealing that ethosomes are categorised as non-irritating when tested on human skin.

Diagrammatic representation of ethosomes is shown in **Figure 1**.

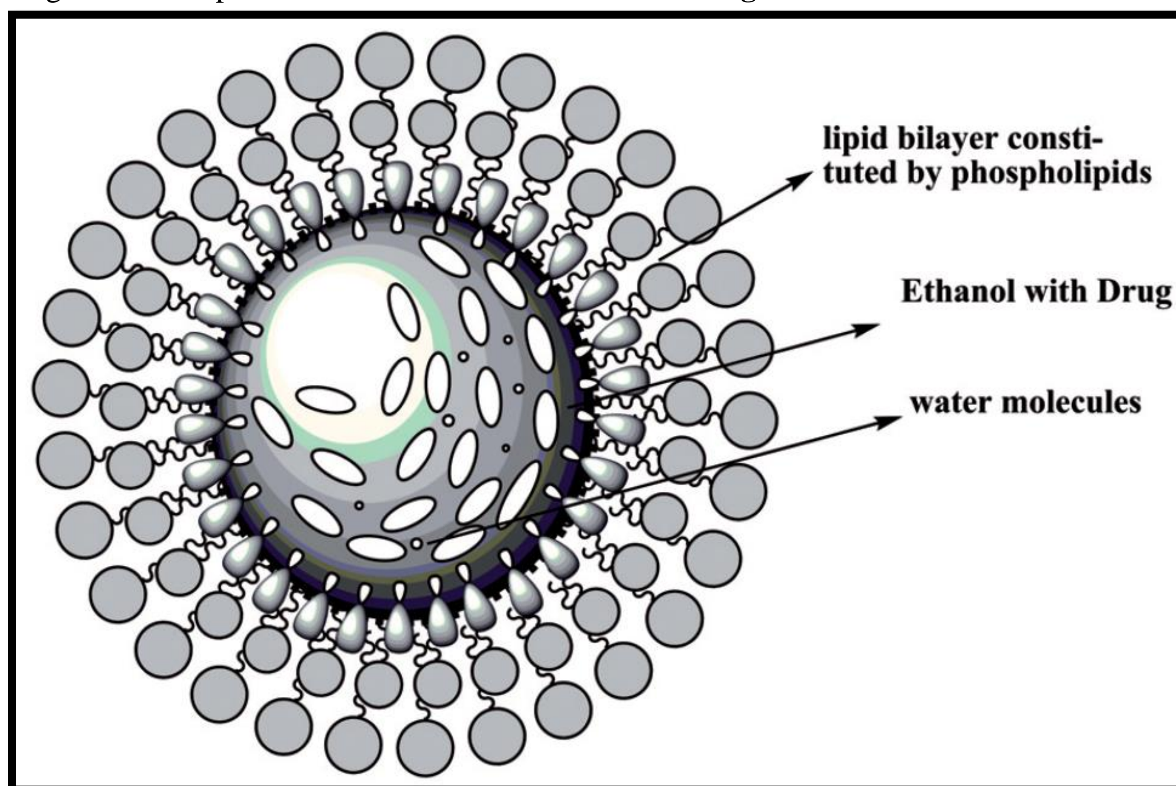

Figure 1: Diagrammatic Representation of Ethosomes [36]

LIPOSOMES

The Greek words “Lipos” (meaning “fat”) and “Soma” (meaning “body”) are the source of the name "liposome". Alec D. Bangham created the first liposomes in England in 1961 while researching phospholipids and blood coagulation[37] Alec Bangham was the first to explain how membrane particles, such as phospholipids, combine with water to create the distinctive structures that are called liposomes.[38] He discovered that when phospholipids and water were combined, the molecules instantly constructed a sphere because only one end of each molecule is soluble in water while the other end is not soluble in water. Drugs soluble in fats

are integrated into the phospholipid layers, while drugs soluble in water are introduced to a water trap inside the accumulation of hydrophobic ends. Vesicles called liposomes might have a lot, a little, or only one phospholipid bilayer inside of them. Polar medicinal molecules can be enclosed due to the liposomal core's polar nature. In the phospholipid bilayer, amphiphilic and lipophilic compounds are solubilized in accordance with their affinity for phospholipids. Liposomes can be created as a gel, cream, dry powder, aerosol and a suspension that is semisolid. They are given topically or parenterally in vivo. Liposomes are frequently detected as foreign molecules and endocytosed by Mononuclear Phagocytic System (MPS) cells, who primarily fixed Kupffer's cells in the spleen and liver.[39] By getting pharmaceuticals to their target sites and sustaining therapeutic drug level for extended periods of time, liposomes can enhance the drug safety and its therapeutic effects as a drug delivery method. Additionally, liposomes assist intracellular delivery by fusing with the plasma membrane, engaging in phagocytosis and receptor-mediated endocytosis. [40,41] Liposomes are becoming more and more popular as a potential drug delivery system for the human body. This is because these lipid vesicles have numerous specific advantages such as biodegradability, non-toxicity, targeting and biocompatibility. They can carry both water and oil soluble payloads, have an ideal specific gravity and be produced in a variety of sizes, are flexible and non-immunogenic for systemic along with non-systemic administrations, lower the exposure of delicate tissues to toxic drugs, stabilise proteins and provide regulated hydration.[42] Despite the developmental efforts and extensive research put into liposomes, only few liposomal products have so far received approval for usage in humans. This may be the result of a variety of factors, such as the following: 1) toxicity of several liposomal formulations, 2) fluidity of liposomal carriers, 3) Fusion and leakage of encapsulated drug or molecule, 4) lower solubility, 5) massive liposome production cost, especially on large-scale, 6) limited entrapment of molecules and compounds into liposomes, 7) phospholipid experiences oxidation and hydrolysis-like reaction respectively. Cross sectional diagrammatic representation of liposomes is shown in **Figure 2**.

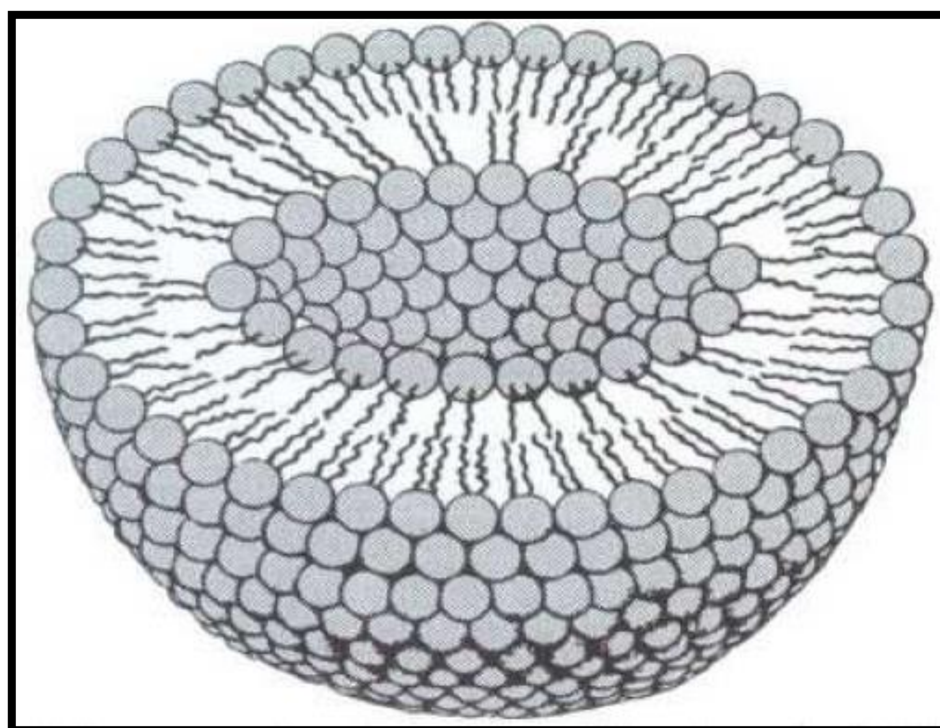

Figure 2: Cross sectional Diagrammatic Representation of a Liposome [43]

CONVENTIONAL LIPOSOMES

These are referred as liposomes since they are frequently made only of cholesterol and/or negatively or neutral charged phospholipids. This kind of liposome was used in the majority of early research on liposomes in the form of a drug-carrier system. A type of vesicular structures called conventional liposomes is built on lipid bilayers covering water sections. They can differ greatly in lipid constitution, size, number of phospholipid bilayers, fluidity, and their surface charge, among other physical characteristics. Conventional liposomes are rapidly coated with plasma proteins when administered intravenously, improving reticuloendothelial system (RES) cells' ability to phagocytose them. As a result, the systemic circulation is quickly removed. Nevertheless, this has been used to good effect in treatment of liver and spleen parasites.[44] They first gained attention as a delivery device, but their extremely short circulating half-life has put a stop to that. Increased circulation time has been achieved by modifying the surface of liposomes with antibodies, polymers, peptides, carbohydrates and proteins.[45] These constituted a significant advancement in the study of liposomal drug delivery. Changing the vesicle's lipid content, size, and charge results in long-circulating liposomes. Conventional liposomes' use in treating a wide range of disorders affecting other organs has been severely hampered by the liver and spleen macrophages' quick and effective removal of them from circulation. The development of new liposomal preparations that can stay in the bloodstream for prolonged periods of time has sparked renewed interest in liposomal delivery. Reduced RES uptake will allow liposomes to stay in the circulation for a lengthy time period, which is essential if they are to be targeted to extra reticuloendothelial system (RES) tissues.[46] The most common method at the moment for creating long-circulating liposomes is to covalently link the hydrophilic polymer polyethylene glycol (PEG) to the external surface. These PEG-coated liposomes are further referred to as “stealth” or “sterically stabilised” liposomes. Long-circulating liposomes' propensity to drift at body regions where the vascular wall is more permeable may be their most crucial fundamental feature. Diagrammatic representation of liposomes along with ethosomes are shown in **Figure 3.**

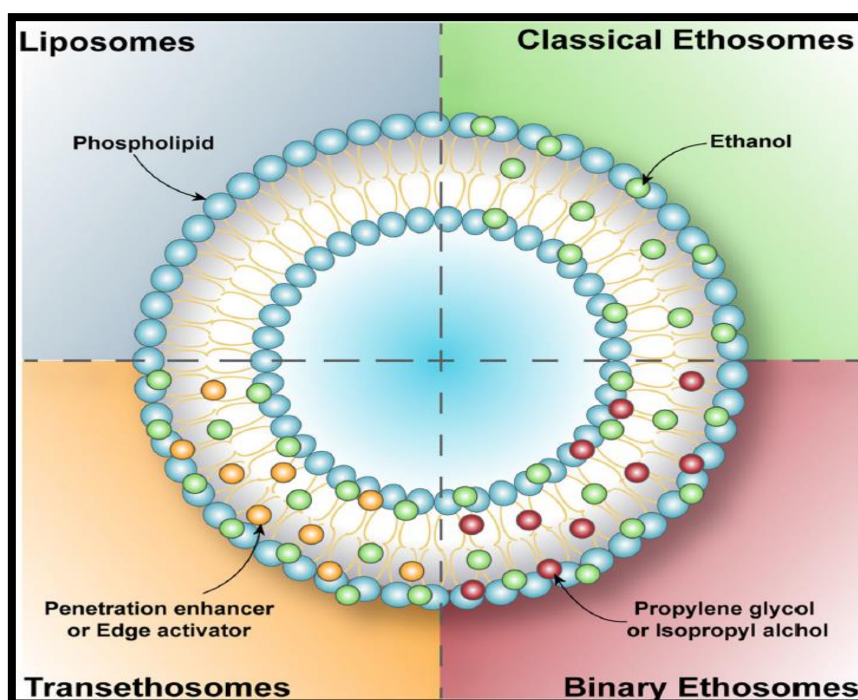

Figure 3: Diagrammatic Representation of Liposomes [47]

TYPES OF ETHOSOMES AND CONVENTIONAL LIPOSOMES

ETHOSOMES

Touitou (2000) was the first to describe classical ethosomes, a variant of the traditional liposomal formulation that are mostly composed of water, phospholipids and an ethanol content that is relatively high.[48] When compared to conventional liposomes, these vesicle nanocarriers have shown superior drug delivery due to having 1) smaller vesicle size, 2) negative zeta potential, 3) improved stability and (4) higher entrapment efficiency.[49] Binary ethosomes often contain isopropyl alcohol along with propylene glycol (PG) in addition to ethanol. [49-51] PG is a common penetration enhancer that has lower toxicity, less skin irritation, stronger viscosity and hygroscopicity than ethanol, as well as stability.[49-52]This boosts the drug's retention capacity in the skin's deep layers and improves the drug's affinity for the dermis layer.[50,53] In addition to reducing aggregation, the ethanol addition to other alcohols gives binary ethosomes improved stability, smaller vesicles, more skin permeability and increased entrapment efficiency.[50] However, in order to maximise drug permeability, it is crucial to modify the ethanol and PG ratio. Song developed a novel class of ethosomes in 2012, that aim to unite the benefits of deformable liposomes and ethosomes.[54]

Ethosomes are classified into three types as shown in **Figure 4**:

a) Classic Ethosomes

Classic ethosomes are a variant of classic liposomes which are made of water, phospholipids and ethanol at a high capacity (up to 45% w/w). For transdermal drug delivery, classical ethosomes were reportedly superior to classical liposomes because of their small size, negativeζ-potential, and greater entrapment efficiency. Furthermore, when correlated to classical liposomes, classical ethosomes shown greater skin penetration and stability profiles.[55-57]

b) Binary Ethosomes

Zhou developed binary ethosomes.[58] In essence, they were formed by mixing a rare form of alcohol with the classical ethosomes. Isopropyl alcohol (IPA) and propylene glycol (PG) are two alcohols that are most frequently utilised in binary ethosomes.[59-63]

c) Transethosomes

The next generation of ethosomal systems, known as transethosomes, was initially described by Song in 2012.[64] This ethosomal system includes a substance, such as a surfactant or a penetration enhancer, along with the fundamental elements of classical ethosomes. These unique vesicles were created in an effort to create transethosomes by fusing the benefits of classic ethosomes with deformable liposomes (transfersomes) into a single formulation. To create ethosomal systems with better properties, many types of penetration enhancer and edge activator have been studied. Correlation of binary ethosomes, transethosomes and classic ethosomes in their initial suspensions are shown in **Table 2**.

Table 2: Comparison of binary ethosomes, classical ethosomes and transethosomes [65]

Features	Classical Ethosomes	Binary Ethosomes	Transethosomes
Morphology	Spherical-shaped	Spherical-shaped	Regular or uneven spherical shapes
Entrapment efficiency	More than classical liposomes	Usually more than classical ethosomes	Usually more than classical ethosomes
Stability	Highly stable than classical liposomes	Highly stable than classical ethosomes	No distinct trend/shift detected
Size	Shorter/lower than classical liposomes	Equal to or lower/shorter than classical ethosomes	Size dependent on the type and content of permeation enhancer or edge activator employed
Skin permeation	Usually more than classical liposomes	Usually equal to or more than classical ethosomes	Usually more than classical ethosomes
Zeta(ζ)-potential	Negative-charged	Negative-charged	Positive or negative-charged
Composition	1. Stabilizer 2. Drug/agent 3. Ethanol 4. Water 5. Phospholipids 6. Charge inducer	1. Water 2. Ethanol 3. Charge inducer 4. Drug/agent 5. Phospholipids 6. Propylene glycol (PG) or other alcohol	1. Edge activator (surfactant) or penetration enhancer 2. Phospholipids 3. Water 4. Ethanol 5. Charge inducer 6. Drug/agent

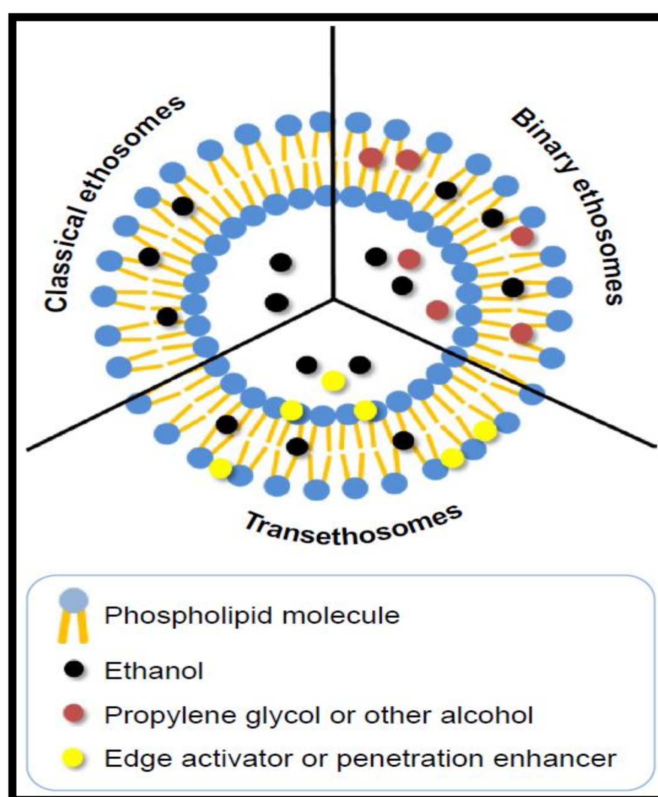

Figure 4: Diagrammatic Representation of Ethosomal types [66]

LIPOSOMES

Comparing the liposome system to alternative colloidal carrier systems, one can practically infinitely change structural and physicochemical properties. The preparation scientist can alter liposomal behaviour in vivo and modify liposome formulations to meet particular therapeutic needs thanks to this flexibility feature. It is feasible to distinguish between the several possible liposome variants in general terms based on structural characteristics as well as composition and application. Types of vesicular liposomes are shown in **Table 3**. Diagrammatic representation on Vesicular Types of liposomes is shown in **Figure 5**.

a) ***Based on Structural Parameters*** [67,68]

1) ***Uni-lamellar Vesicles***
    - Small Uni-lamellar Vesicles (SUV): 20 to 40 nm is the size range.
    - Medium Uni-lamellar Vesicles (MUV): 40 to 80 nm is the size range.
    - Large Uni-lamellar Vesicles (LUV): 100 to 1000 nm is the size range.

2) ***Oligo-lamellar Vesicles (OLV):*** These are composed of 2-10 bilayers enclosing a sizable interior volume.

3) ***Multi-lamellar Vesicles (MLV):*** They contain a number of bilayers. The aqueous volume can be divided in an endless number of ways. Depending on how they are prepared, they vary. The arrangements may resemble an onion, with concentric spherical LUV/MLV bilayers containing many SUVs, etc.

b) ***Based on Liposomal Formation Methods*** [69,70]

1) DRV: Dehydration-rehydration method.
2) MLV-REV: Multilamellar vesicles formed by Reverse-Phase Evaporation Method.
3) REV: Single or oligolamellar Vesicles formed by the above evaporation method.

c) ***Based upon Application & Composition*** [71,72]

1) Long-Circulatory (Stealth) Liposomes: They have derivatives of polyethylene glycol (PEG) bonded to their respective surfaces to reduce phagocyte system exposure (reticuloendothelial system; RES).
2) Conventional Liposomes (CL): Phospholipids that are negatively charged or neutral and cholesterol.

Table 3: Types of Vesicular Liposomes [73]

S.No.	Abbreviation	Type of Vesicle	Size	Number of lipid layers
1.	MUV	Medium-Sized Unilamellar Vesicles	Greater than 100 nm	1
2.	MVV	Multi-Vesicular Vesicles	Greater than 1.0 mm	Shape having multiple compartments
3.	OLV	Oligo Lamellar Vesicles	Ranges from 0.1 to 1.0 mm	Approx 0.5
4.	GUV	Giant Uni-lamellar Vesicles	Greater than 1.0 mm	1
5.	SUV	Small Uni-lamellar Vesicles	Extends from 20 to 100 nm	1

6.	LUV	Large Uni-lamellar Vesicles	Greater than 100 nm	1
7.	UV	Unilamellar Vesicles	Comes in all sizes	1
8.	MLV	Multi-Lamellar Large Vesicles	Greater than 0.5 mm	Ranges from 5 upto 25 layers

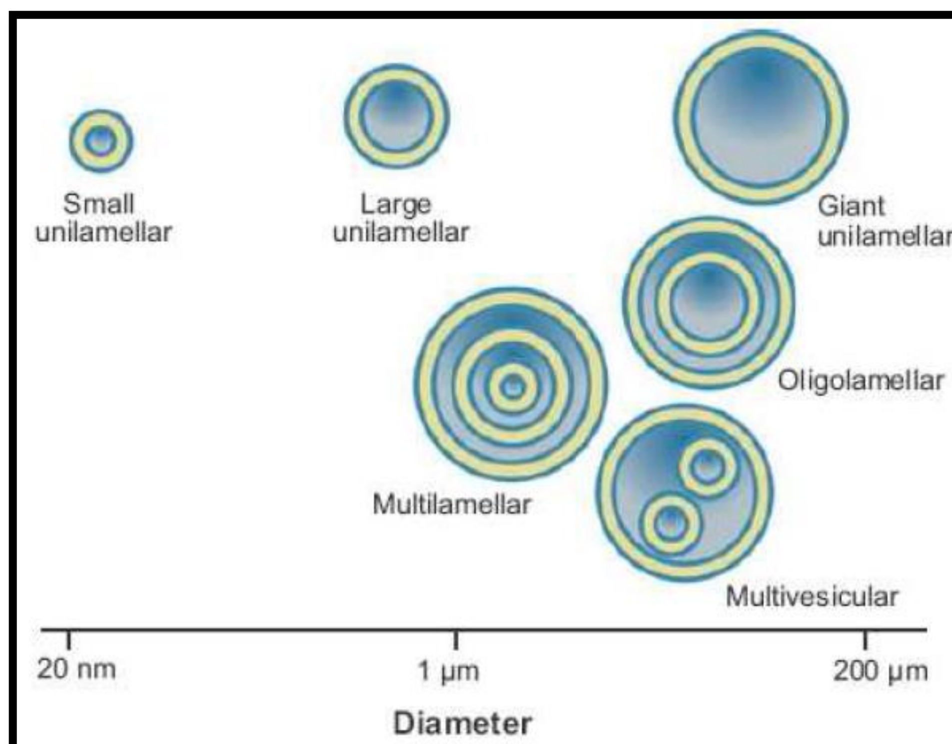

Figure 5: Diagrammatic Representation on Vesicular Type Liposomes [74]

COMPOSITION OF ETHOSOMES AND CONVENTIONAL LIPOSOMES

ETHOSOMES

Lipid bilayers are present in ethosomes, similar to those in liposomes, but their composition is different due to high ethanol content. The hydroalcoholic or hydro/glycolic phospholipids that make up the ethosomes have a comparatively high alcohol content. Phospholipids having different chemical structures, such as phosphatidyl ethanol amine, phosphatidyl choline, phosphatidic acid, phosphatidyl serine, phosphatidyl glycerol, and phosphatidyl inositol, as well as propylene glycol, alcohol (isopropyl alcohol or ethanol) and water, may be present in ethosomes (or other glycols). Several favoured phospholipids, including Phospholipon 90 (PL-90). It is often used between 0.5 and 10% weight per weight. The preparation may also contain 0.1–1% of cholesterol. In terms of glycols, Transcutol and propylene glycol are frequently utilised and can make up 20 to 50% of the finished product, respectively. Along with non-ionic surfactants like PEG-alkyl ethers, cationic lipids such as cocamide, cetrimide, dodecylamine, POE alkyl amines, can also be mixed with phospholipids in formulations. Alcohol and glycol together can have an aggregation of between 22 and 70% in the non-aqueous phase. Ethosomes are often made up of typically 2 to 5% phosphatidyl choline (PC), 20 to 45% ethanol, phospholipids and upto 100% water (w/w).[75] According to preliminary research from Touitou, high ethanol concentrations promoted the production of pliable, fluid

vesicles that are soft and flexible.[76] In addition to influencing ethosomes' stability, average size, zeta potential, entrapment effectiveness, ethanol is a common permeating enhancer which connects with the hydrophilic head group of SC lipid bilayer and improves lipid flexibility.[77-80] Subsequently it can affect vesicle-skin interaction and stability, the vesicular charge is further acknowledged as a crucial factor to consider while formulating ethosomes. The ethosomal negative charge grows proportionately with rising ethanol concentration because ethanol serves as a supply of negative charge for the ethosomal surface.[77] Previous ethosomal formulations have utilised a number of phospholipids, including Phospholipon®90, Phosphatidylethanolamine (PE), Dipalmitoyl phosphatidylcholine (DPPC), and Lipoid S100.[81] The chosen phospholipid will combine with the lipid bilayers of skin and enable the vesicle in forming tiny gaps in the SC, which will affect skin permeation.[82] As a result, choosing and concentrating the right phospholipid during the pharmaceutical development process is an essential step that will determine how successfully ethosomes will penetrate the skin. Ethosomes acquire a positive charge when cationic lipids are present, which increases their ability to interact with the negatively charged skin membrane. Since ethanol confers a negative charge, positively-charged vesicles can penetrate deeper into the SC and disrupt tight junctions despite this. This results in a reduction in the size of the SC. [83] When ethosomes are used instead of free ethanol, penetration seems to enhance, pointing to the possibility of a shared interaction between skin lipids, vesicles and ethanol. Different substances used in formation of ethosomes is shown in **Table 4.**

Table 4: Different Substances used in Formation of Ethosomes [84]

Substances	Examples	Uses
Cholesterol	Cholesterol	Stable vesicle membrane
Vehicles	Carbopol D-934	Gel formation in vesicles
Polyglycol	Propylene glycol	Skin permeable enhancer
Edge activator	Tween (22)	Improves permeation of skin
Phospholipids	Phosphatidyl choline from Egg Soya Phosphatidyl choline	Forming components of vesicles
Alcohol	Isopropyl alcohol Ethanol	Softening vesicle membrane Permeable enhancer
Dye	6 – Carboxy fluorescence Rhodamine – 123	For identifying purpose
Others	Dicetyl phosphate	Stops vesicle accumulation

LIPOSOMES

The components present in liposomes are given as follows: [85]

1) Phospholipids

The most often utilised component of liposome formulations, phospholipids that contain glycerol, account for greater than 50% of the lipid weight in biological membranes. They were produced using phosphatidic acid. The glycerol portion serves as the backbone of that molecule. A phosphoric acid ester was formed at a C_3 OH group. Long-chain esters are formed from the OH group at C_1 and C_2. This lipidic nature has been caused by a fatty acid. One of the residual OH groups of the phosphoric acid can also be esterified to create

a variety of natural alcohols, such as glycerol, inositol, choline, serine, and ethanol amine. Consequently, the phosphoric ester of glycerol is the parent component of the series.
Several phospholipids include:

- Phosphatidyl Glycerol (PG)
- Phosphatidyl Ethanolamine (Cephalin) – (PE)
- Phosphatidyl Choline (Lecithin) – PC
- Phosphatidyl Serine (PS)
- Phosphatidyl Inositol (PI)

Use of saturated fatty acids results in stable liposomes. It is uncommon to employ unsaturated fatty acids.

2) *Cholesterol*

In liposomes, cholesterol and its by-products are frequently used for

- Lowering the bilayer's elasticity or microviscocity,
- Lowering the membrane's permeability to water-soluble compounds,
- Causing the membrane to stabilise when exposed to biological fluids like plasma (This effect was employed in the preparation of intravenous liposomes).

It is well known that liposomes devoid of cholesterol interact quickly with plasma proteins like macroglobulin, transferrin, and albumin. These proteins are likely to remove large content of phospholipids from liposomes, depleting the outer mono-layer and causing the vesicles to become physically unstable. This kind of interaction appears to be significantly reduced by cholesterol. Because of its molecular structure and solubility, cholesterol has been specified as the "mortar of bilayers" because it fills in the gaps between phospholipid particles, supporting their bonds to the structure. These molecules transform the hydro-carbon chain on C_{17} group into a non-polar end, allowing the cholesterol to intercalate in the bilayers. The third position present inside the OH group supplies a small polar head group. Diagrammatic representation of composition of liposomes are shown in **Figure 6**. Diagrammatic representation of conventional liposomes entrapping both drugs is shown in **Figure 7**.

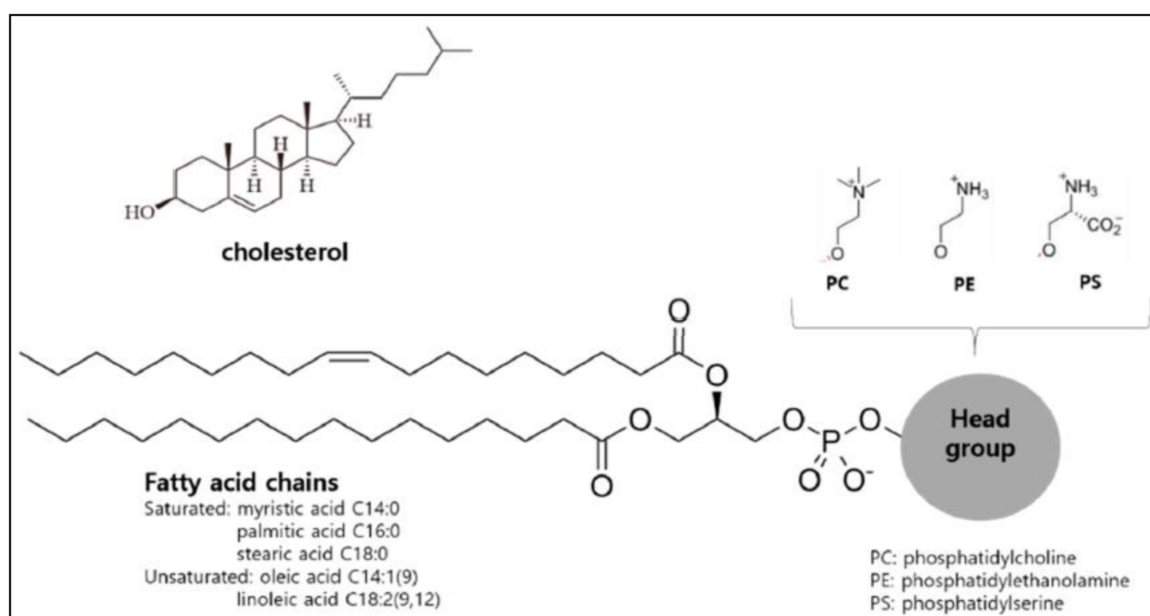

Figure 6: Diagrammatic Representation of Phospholipids with Cholesterol [86]

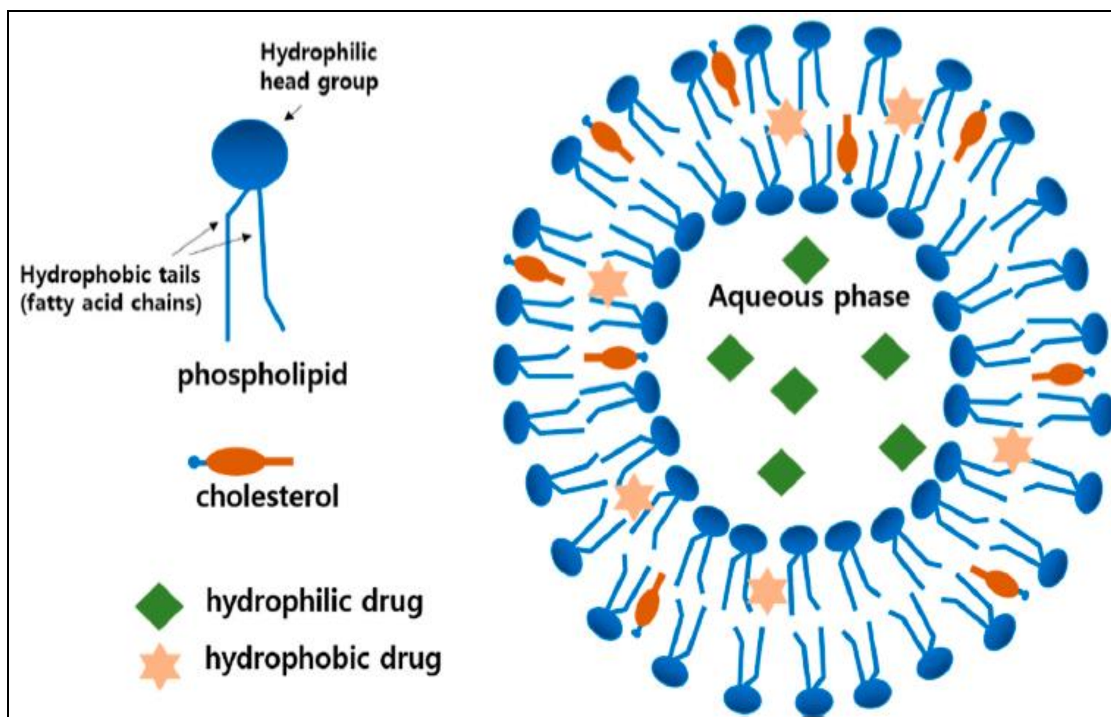

Figure 7: Diagrammatic Representation of Conventional Liposomes entrapping Hydrophobic and Hydrophilic Drugs [86]

MECHANISM OF ACTION

ETHOSOMES

Two concurrent mechanisms of ethanol and ethosomal effect on the SC lipid bilayer are involved in the ethosomes' ability to penetrate cells. The vesicle deformability is increased as a result of the ethanol usage in the formation of ethosomes. The SC lipids should be partially extracted due to the high alcohol concentration. Ethosomes' increased intercellular and intracellular permeability is caused by these processes.[87] Following this action is the "ethosome effect," which results in improved drug delivery by fusing the phospholipids within the SC with the ethosomal vesicle as shown in **Figure 8**. [88-90]

a) *Ethanol effect*

Through the skin, ethanol enhances permeation. Its permeation-amplifying effect has a common mechanism. Ethanol permeates into the intercellular lipids, increasing their fluidity and lowering the density of their multilayer cell membrane.[91]

b) *Ethosomal effect*

The ethanol from the ethosomes improves the elasticity of cell membrane lipids, improving skin permeability. Therefore, the ethosomes easily penetrate the deeper skin layers, resulting in the fusing with skin lipids and release the drugs.

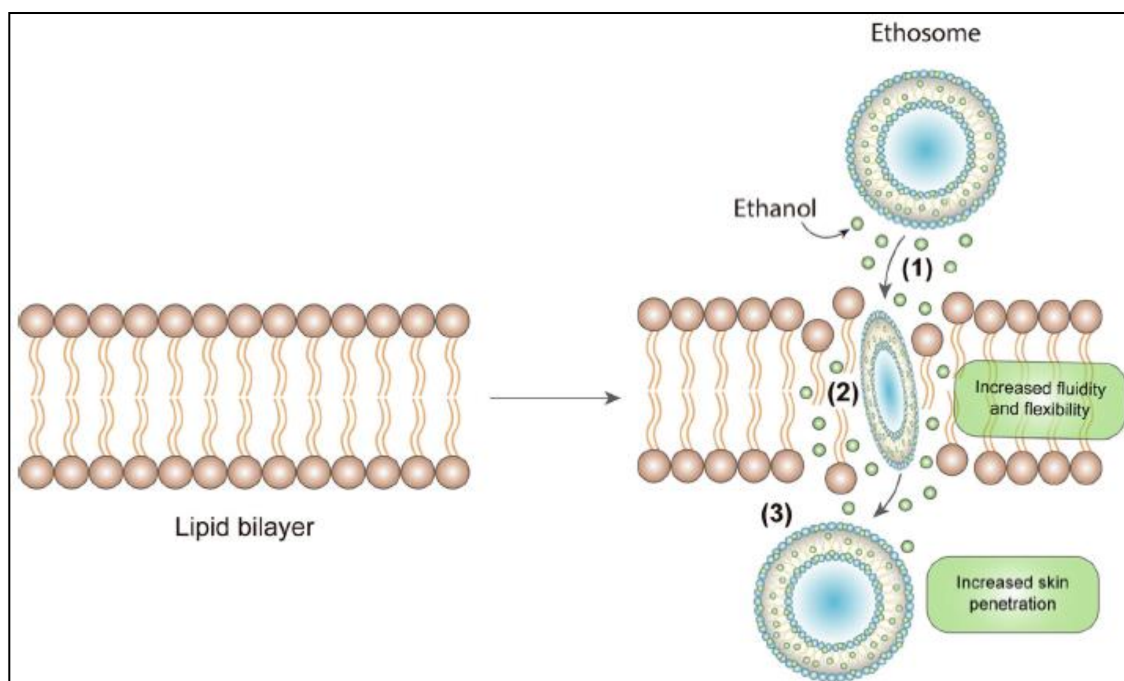

Figure 8: Diagrammatic Representation of Mechanism of Skin Permeation in Ethosomes [92]

LIPOSOMES

Liposome-forming lipids display a twin chemical nature. They have hydrophobic fatty-acyl chains and hydrophilic head groups.[93]

There are four different mechanisms by which liposomes work. They are given below:

1. Fusion – This happens when a bilayer from the liposome is infused into the plasma membrane and the liposomal component is endlessly discharged into the cytoplasm.[94]
2. Endocytosis – This is accomplished by phagocytic reticuloendothelial system cells, like neutrophils.[95]
3. Exchange of Lipids – Lipids from these liposomes are transferred to the cell membrane in this process except the associated liposomal contents.[96]
4. Adsorption – Non-specified electrostatic forces or interactions with components of cell surface cause it to affect the cell surface.[97]

ADVANTAGES AND DISADVANTAGES

S.NO.	ADVANTAGES OF ETHOSOMES [98]	DISADVANTAGES OF ETHOSOMES [99]
1.	Its formulation uses non-toxic raw materials.	Poorly shielded ethosomes may clump together, causing precipitation.
2.	Large particles, like peptides and protein particles, can be delivered.	Inadequate practical yield.
3.	Improved drug penetration over the skin for transdermal delivery.	Possibly not-cost effective.
4.	The semisolid form (gel or cream) in which the ethosomal drug is administered results in great patient compliance.	Not all skin types will cling to adhesive property.

5.	In comparison to Phonophoresis, Iontophoresis and other complex methods, this drug delivery method is very easy.	Product loss results from ethosome transfer from the organic to the aqueous layer.
6.	The pharmaceutical, veterinary, and cosmic industries can all benefit from ethosomal drug delivery systems.	Additives and enhancers used in drug delivery systems might lead to dermatitis or skin irritation.
7.	The ethosomal system can be instantly advertised which is passive and non-invasive.	The molecular size of the drug should be applicable for transcutaneous absorption.
8.	Under both occlusive and non-occlusive situations, ethosomes enhance skin delivery.	Drugs requiring extreme blood levels cannot be applied; only effective drugs can be given (daily dose having 10mg or less).

S.NO	ADVANTAGES OF LIPOSOMES [100,101]	DISADVANTAGES OF LIPOSOMES [102,103]
1.	Non-ionic	Short half-life period.
2.	Drug stability is increased by the liposome.	Lesser stability
3.	Increased therapeutic index and efficacy of drug.	Production is expensive.
4.	Biocompatible	Poor solubility.
5.	Liposomes aid in minimising toxic drug exposure to delicate tissues.	Encapsulated drug leakage and fusion are possible.
6.	Drug oxidation has been avoided.	It's possible for phospholipids to oxidise.

METHODS OF PREPARATION

ETHOSOMES

Two easy and practical methods, the hot method and the cold method, can be used to create ethosomes.

a) Cold Method

In this procedure, the phospholipids, such as cholesterol and soy lecithin, are dissolved in the organic phase and swirled continuously on a magnetic stirrer in a covered container at room temperature. While being stirred, propylene glycol is added, and the liquid solution is then boiled on a water bath upto 30°C. Drugs that are soluble in water are added to the water, whereas those that are soluble in organic phases are combined in ethanol along with phospholipids. The water is then introduced dropwise at 700 rpm to the organic phase and swirled for 15 minutes. Either extrusion or sonication can lower the size of the produced ethosomes' vesicles. The preparation is later placed in a refrigerator for storage.[104] This is the quickest and most popular method for creating ethosomal systems, and it can be carried out by undergoing nitrogen protection, if required.[105] It requires that the aqueous and organic phase should be prepared individually and was first introduced by Touitou in 1996.[106] According to its physicochemical characteristics, the drug that will be integrated into the ethosomal system would dissolve in either aqueous or organic phase. Preparation of ethosomes by utilizing this method is shown in **Figure 9.**

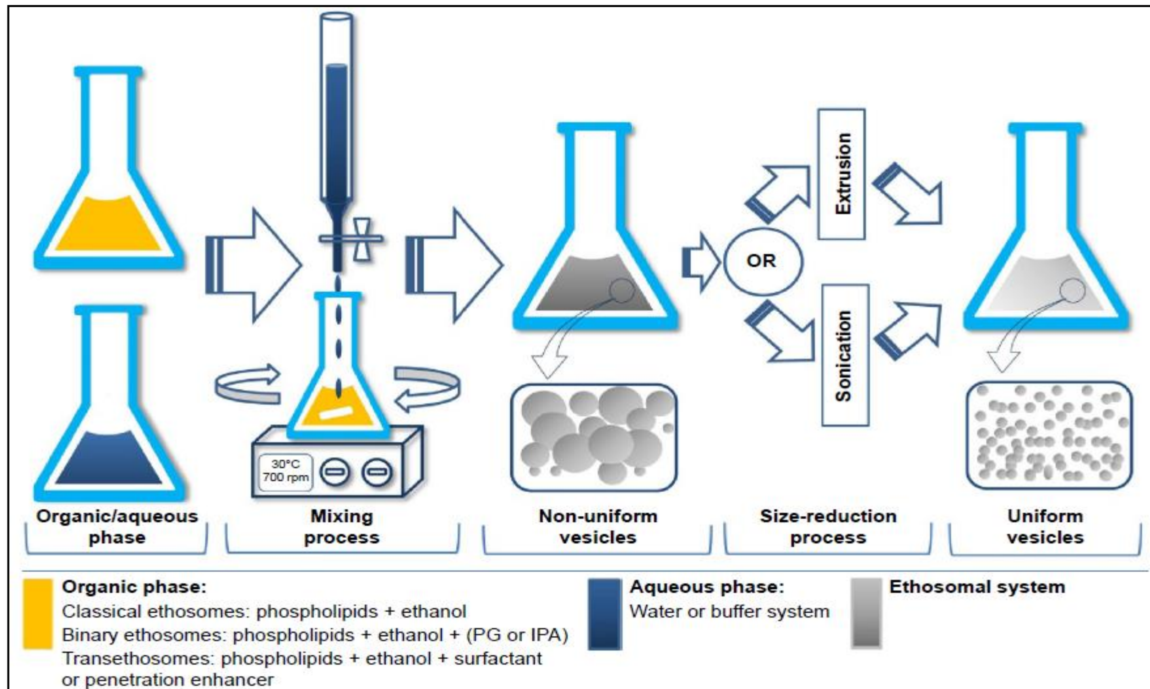

Figure 9: Diagrammatic Representation of Cold Method for Ethosomes[107]

b) Hot Method

Phospholipids are mixed with water and boiled on the water bath to 40°C in this method. Propylene glycol along with ethanol are combined and boiled in a different container. Depending upon the hydrophobic/hydrophilic characteristics, the drug liquifies in either the organic phase or aqueous phase. The organic phase is brought into the aqueous phase once both have reached 40°C. Additionally, extrusion or sonication can be used to reduce the ethosomes' vesicle size.[108] The creator of ethosomes initially explained this method in 1996.[109] Diagrammatic representation of this method is shown in **Figure 10**.

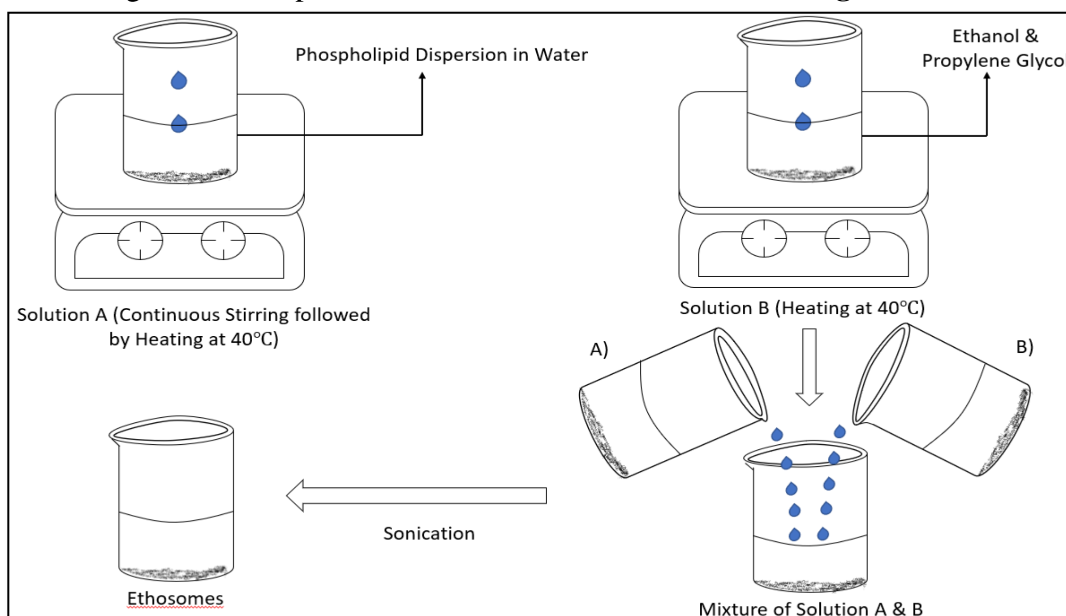

Figure 10: Diagrammatic Representation of Hot Method for Ethosomes [110]

c) Classic Mechanical-Dispersion Method

Inside the round bottom flask (RBF), the phospholipids are dissolved first in a combination of organic solvents. A thin lipid layer is then created on the RBF by employing a rotating vacuum evaporator to remove the organic solvent. By placing the contents under vacuum overnight, even tiny amounts of solvents can be eliminated. Water-ethanolic solution is utilised to further saturate the deposited lipid layer. The lipid film is heated in the RBF for 30 minutes while being rotated at a temperature that depends on the phospholipid's nature during hydration.[111] This is an improvement over the classic liposome-preparation method, however in this method, a hydroethanolic solution hydrates the lipid film. Diagrammatic representation of classic mechanical dispersion method is shown in **Figure 11**.

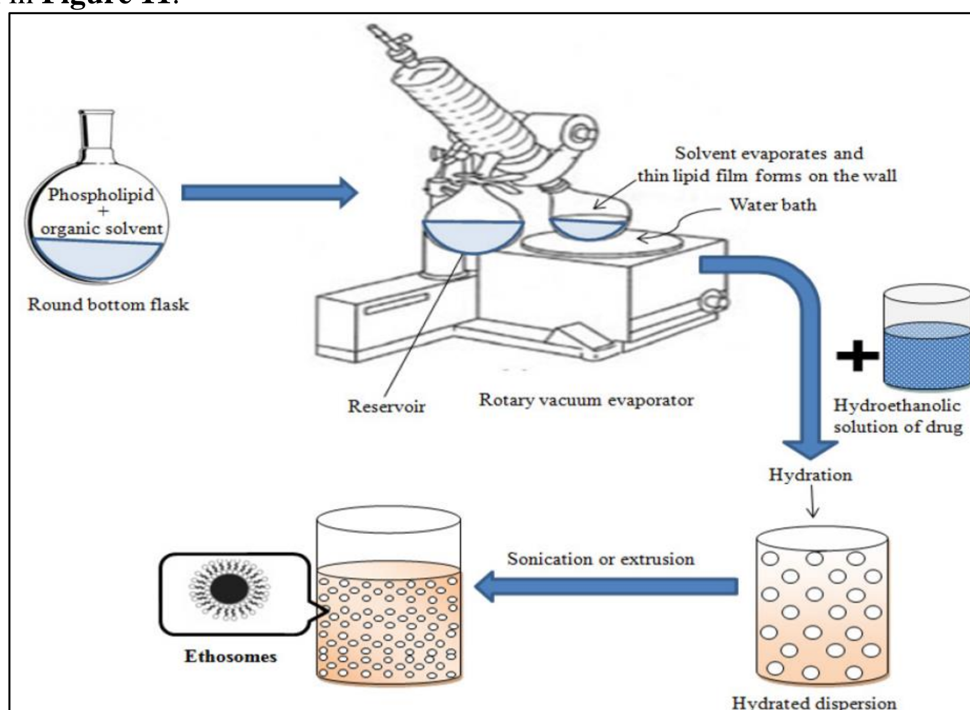

Figure 11: Diagrammatic Representation of Classic Mechanical Dispersion Method [112]

d) Ethanol Injection Sonication Method

In a glass bottle that is hermetically sealed and attached to a syringe so that ethanol can be added without evaporating, phospholipids are dissolved into ethanol. Separately, the drug has been liquified in double-refined water. Following the addition of the ethanolic lecithin solution, the aqueous drug solution is homogenised using the ultrasonic probe for 5 minutes duration at a flow rate of 200 μL/min. The drug-loaded ethosomes are then collected by filtering the ethosomal suspension afterwards using 0.45μm filters.[113] Diagrammatic representation of ethanol injection sonication method is shown in **Figure 12**.

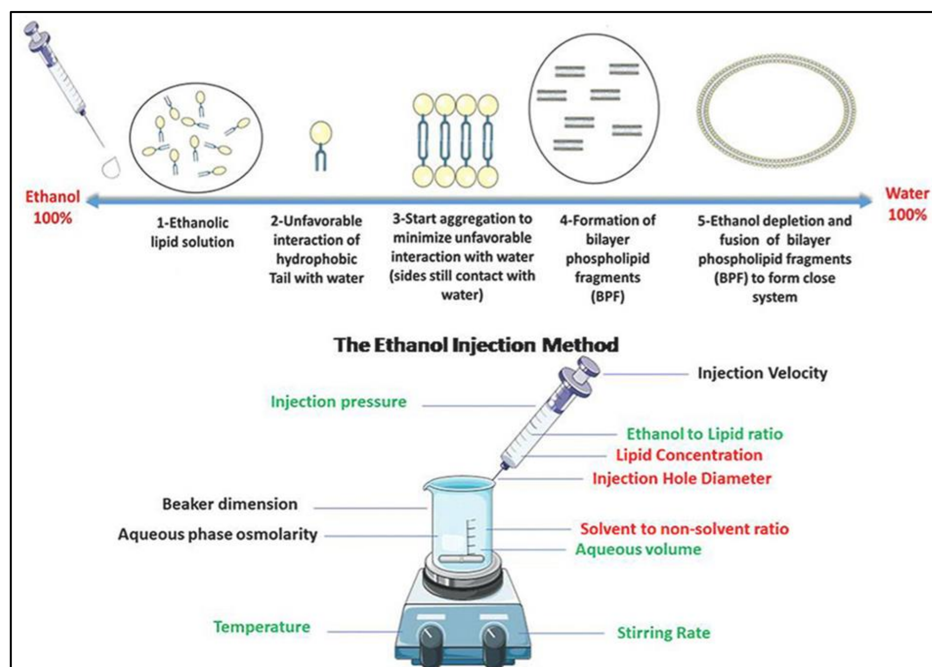

Figure 12: Diagrammatic Representation of Ethanol Injection Sonication Method[114]

e) Trans-membrane pH Gradient Method

These methods involve adding the drug to the aqueous or organic phase, where it is then "passively" or spontaneously packed into the ethosomal system. According to the pH gradient difference among the basic exterior of the external phase and the acidic interior of the internal phase of the ethosomal system, the drug is packed "actively" in the transmembrane pH Gradient method. [115-118] Three steps make up this method: ethosomal blank preparation, active drug loading, and finally incubation. Any of the aforementioned procedures are used in the initial stage to generate the empty ethosomal suspension, but an acidic buffer (like citrate buffer having pH 3) is used into the aqueous phase or through the hydration process. The drug is actively packed into the empty ethosomal suspension in the second stage, which is followed by continual stirring. After generating the pH gradient between the basic exterior phase of the ethosomal system and the acidic interior phase (pH 3), an antacid, typically a caustic soda solution of 0.5 M, was introduced to the exterior phase to raise its pH to 7.4. The final stage involves incubating the ethosomal system at a specific temperature of 30–60°C and time to allow the unionised drug to actively traverse the ethosomal vesicle bilayer and become entrapped.[119] The physicochemical characteristics of the agent or drug that will be included, the temperature the interior and exterior pH phases, and the length of the incubation period are some factors that need to be taken into account before ethosomal systems are created using this method. Preparation of ethosomes by utilizing this method is shown in **Figure 13.**

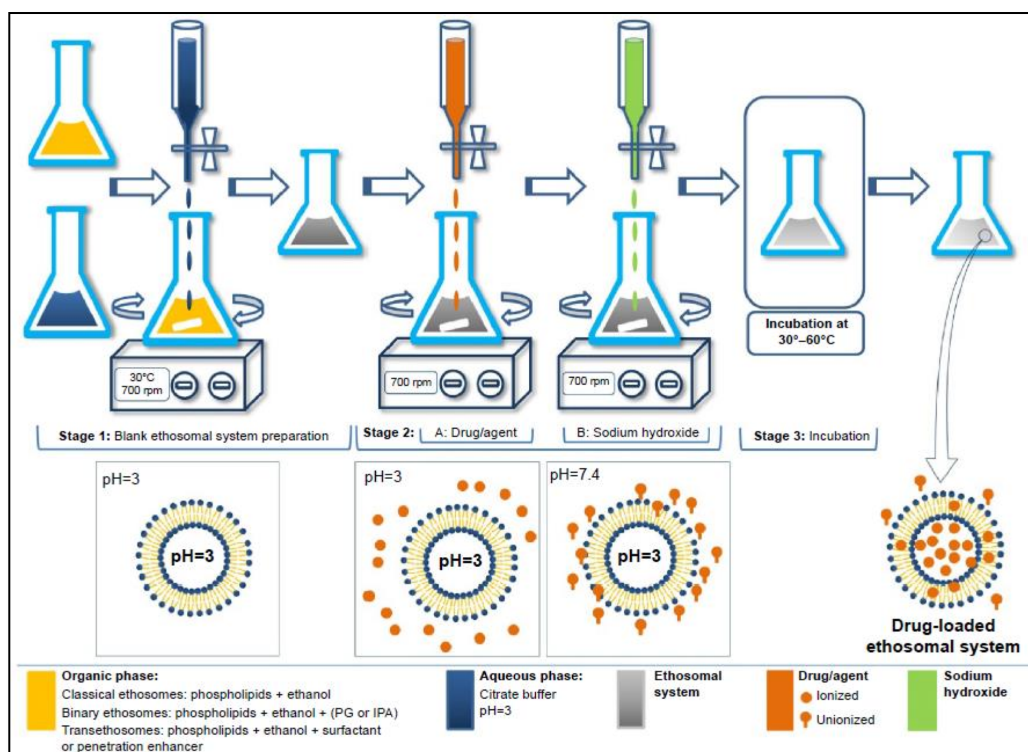

Figure 13: Ethosomal Formulation using Trans-membrane pH Gradient Method [120]

LIPOSOMES

There are two preparatory methods for liposomal formation that are as follows [121]:

A. General Methods
B. Specific Methods

1) General Methods

An organic solvent is used to dissolve the lipid. A narrow lipid layer is left on that container wall after the solvent evaporates. The drug is mixed into an aqueous solution. During the first procedure, the solution is mixed to create multi lamellar vesicles, which are subsequently extruded or sonicated to form SUVs. The next procedure involves sonicating the solution and evaporating the solvent to produce LUVs. If a drug is dissoluble in water, then it can be added to a buffer or an aqueous solution; if it is hydrophobic, then it can be added to a pure basic solvent. Gel chromatography can be used to separate liposomes and free drug. [122]

2) Specific Methods of Preparation

Depending on how they disperse, they are divided into two categories given below:

1. Physical Dispersion Method
2. Solvent Dispersion Method

1) Physical Dispersion Method

The aqueous volumes covered inside lipid membranes in these methods range from 5 to 10%, a tiny part of the overall volume utilised for the preparation. It wastes a huge amount of the water-soluble drug during preparation. However, a high percentage of

lipid-soluble drugs can be encapsulated. MLVs are created using these methods, and additional treatment is needed to create uni-lamellar vesicles.[123]

Hand-shaking Method

This is an easy and most popular method. The charged elements and lipid solution are liquified in a mixture of chloroform along with methanol (2:1) before being added to the 250 ml rounded bottom beaker. The beaker is mounted to a vacuum pump-fixed rotary evaporator and spun for 30 minutes time at a speed of 60 rpm. At a temperature of roughly 30 degrees, the organic solvents evaporate. After the flask's walls started to form a dry residue, spinning was kept going for another 15 minutes. After being separated from the vacuum pump, the evaporator is filled with nitrogen. After that, the flask is taken out of the evaporator and mounted on the lypholizer to discard any remaining solvent. 5 ml Disodium phosphate solution is added after the flask was once more nitrogen flushed. It eventually forms a milky white suspension. To finish the swelling process and provide MLVs, the suspension is left to stand for two hours.[124] Diagrammatic representation of hand-shaken method is shown in **Figure 14**.

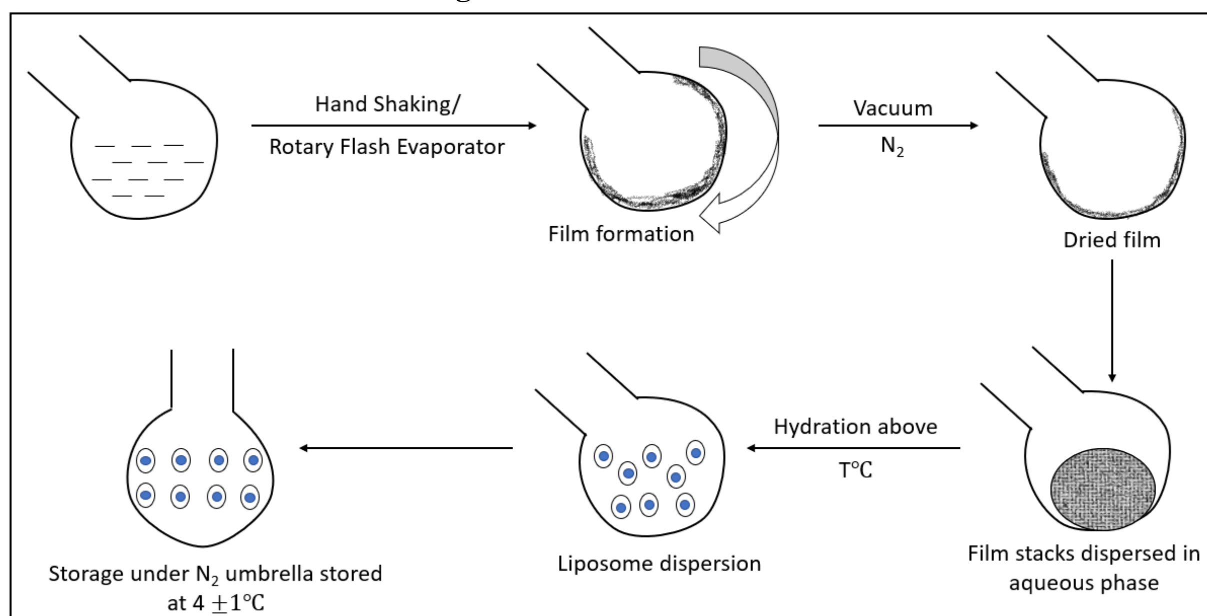

Figure 14: Diagrammatic Representation of Hand Shaking Method for Liposomes[125]

Freeze Drying

Freeze-drying the lipid after it has been dissolved in an appropriate organic solvent is another method to disperse the lipid in a finally separated form before adding hydrated media. Tertiary butanol is typically used as a solvent. [126] Diagrammatic representation of freeze-drying method is shown in **Figure 15**.

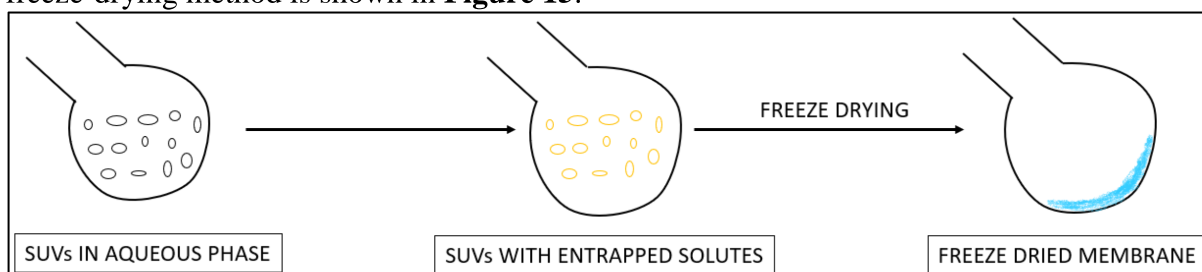

Figure 15: Diagrammatic Representation of Freeze-Drying Method for Liposomes[127]

2) *Solvent Dispersion Method*

The particular methods include liquifying lipids into an organic solution initially, followed by interaction with an aqueous phase that contains the materials that will be enclosed within the liposome. The formation of the monolayer of phospholipids at the boundary between organic and aqueous phases, being a critical step in creating the liposome's bilayer, is necessary.[128]

Ethanol Injection

It is an easier procedure. With the help of a small needle, an excess of saline or another hydrated media is rapidly injected with an ethanol-lipid solution in this method. Water is used to dilute the ethanol, and phospholipid molecules are evenly dispersed throughout the mixture. This procedure produces a significant quantity of SUVs having a diameter of about 25 nm.[129]

Ether Injection

This procedure is identical to the above one. It includes slowly infusing a non-miscible pure solution into the aqueous phase using a thin needle at an organic solvent's vaporisation temperature. Since the lipids are treated properly in this method, there is very little chance of oxidative degradation. The process takes a lot of time, and the introduction of the lipid solution requires careful handling.[130] Diagrammatic representation of ether injection method is shown in **Figure 16**.

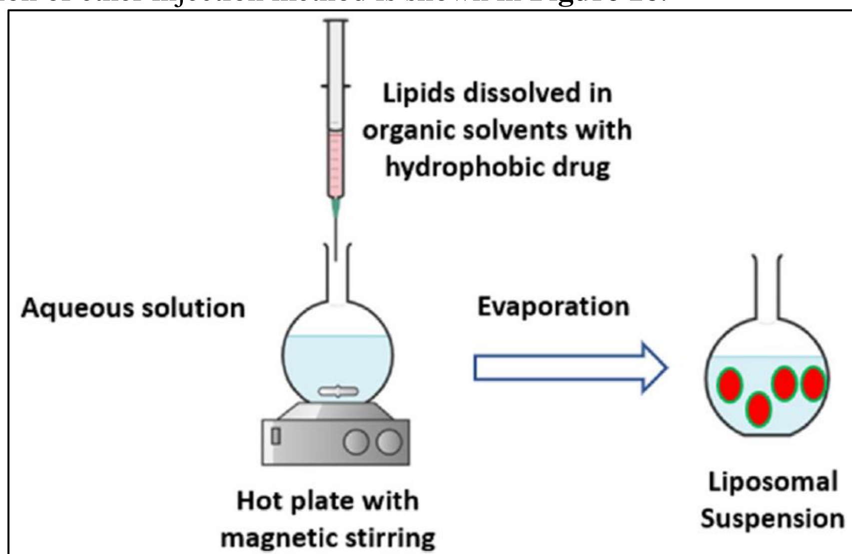

Figure 16: Diagrammatic Representation of Ether Injection Method for Liposomes[131]

CHARACTERIZATION

ETHOSOMES [132-138]

S.NO	PARAMETERS	PROCEDURE	EQUIPMENT USED
1.	Vesicle Shape	The ethosomes are placed onto double-sided tape covered with platinum, attached to copper stubs and exposed to various magnifications for analysis.	Transmission-Electron Microscopy (TEM) and Scanning-Electron Microscopy

			(SEM) are used.
2.	Zeta Potential and Vesicular Size	Two techniques are used to evaluate ethosomes with a computerised inspection system.	Dynamic-Light Scattering (DLS) and Photon-Correlation Spectroscopy (PCS) are employed.
3.	Entrapment Efficiency	The vesicles are dispersed in a max-speed cooling centrifuge for 90 minutes at 4°C while spinning at 20,000 rpm. Following equation is used: Entrapment efficiency = $\frac{DE}{DT} \times 100$ where, DE = Drug quantity in ethosomal sediment DT = Theoretical drug quantity used for preparing formulation	Ultracentrifugation method is used.
4.	Measuring Surface Tension	The ring method has been used for determining the drug's aqueous surface tension.	Du Nuoy ring tensiometer is used.
5.	Drug Content	Drug amount can be determined.	Improved high performance liquid chromatography technique is used.
6.	Transition Temperature	It is determined twice in the aluminium pan with the healing rate of 10°C per minute and a constant flow of nitrogen.	DSC is used.
7.	Stable Vesicles	Stability can be evaluated by storing the solutions at various temperatures, such as 25 $\pm$ 2°C (body temperature), 37.5 $\pm$ 2°C, and 45.5 $\pm$ 2°C for various amount of time (20, 40, 80 and 120 days).	TEM and DLS are used.

LIPOSOMES [139,140]

S.NO	PARAMETERS	PROCEDURE	EQUIPMENT USED
1.	Lamellarity and Vesicle Shape	It was used to examine vesicle shapes.	Electron microscope is used.
2.	In Vivo Release of Drug	It has a 22 ml reservoir compartment that was loaded with buffer that maintains the sink's condition by containing 20% v/v methanol.	25 mm-diameter Franz Diffusion Cell has been used.
3.	Distribution and Size of Particles	The size was measured with a minimum power of 5 MW.	Laser diffraction-based analyzer was used.
4.	Trapped Volume	It is a crucial liposome factor. It has the volume of aqueous trapped lipids per unit.	0.5 to 30 microliters/micromole is the range of the volume.

5.	Liposomal Percentage Yield	The drug amount as well as other ingredients used to prepare the liposomes was divided by measured weight.	Collection of prepared liposomes was used.
6.	Entrapment Efficiency	It establishes the quantity and rate of water-soluble agents' entrapment in the liposomes' aqueous compartment. Following equation is used: % Entrapment Efficiency = $\frac{Entrapped\ Drug \times 100}{Total\ Drug}$	Water-soluble agents are used.

APPLICATIONS

ETHOSOMES

S.NO.	DRUG USED	CLASS OF THE DRUG	APPLICATION	REFERENCE
1.	Acyclovir	Anti-Viral	Used to treat Herpes labialis. It has limited skin penetration, reducing its therapeutic efficacy.	141,142
2.	Erythromycin	Anti-Biotic	Used to treat severe allergic reactions that are caused by classic oral antibiotic therapy.	143
3.	Trihexyphenidyl hydrochloride (THP)	Anti-Parkinson	Developed as an ethosomal formulation having greater skin penetration than its liposomal formulation.	144
4.	Diclofenac	NSAIDS	Selective drug delivery for a prolonged time period.	145
5.	Cannabidol, Piroxicam	Anti-Arthritic	Cannabidol improved anti-inflammatory action. Piroxicam used to treat rheumatoid arthritis.	146

LIPOSOMES

a) Pulmonary Drug Application

Due to their ability to solubilize drugs, they serve as effective tools for pulmonary drug delivery.[147]

b) Genetic Therapy

In several gene therapy applications for treating diseases, liposomes are used.

c) Tumour Therapy

Macromolecules like cytokines and small cytotoxic molecules are transported by these molecules.

d) Respiratory Disorders

Since they have better stability, greater sustained release and lower toxicity than regular aerosols, liposomes have been discovered to have positive effects regarding the treatment of a number of respiratory disorders. For inhaling liposomes, dry or liquid forms can be used. It has been shown that drug release happens during nebulization.

e) Vaccine immunological adjuvants

Liposomes are employed in immunodiagnosis and immunoadjuvant.

f) *Cosmetic Use of Liposomes*

They are utilised in cosmetics because they release materials into the cells and their physiology is identical to that of the cell membrane.[148]

g) *Opthalmic Disorders*

Liposomes have been proven to be beneficial in treating a group of eye conditions, including proliferative vitreous retinopathy, keratitis, uveitis, corneal transplant rejection, and ondopthelmitis. Recently, a liposomal formulation of the drug verteprofin, which is useful against eye disorders, was approved.[149]

CONCLUSION: -

Ethosomes are effective in transdermal delivery compared to conventional liposomes due to having great deformability, skin penetration, fluidity & stability along with a small vesicular size. They have high ethanol content which is known to disrupt lipid bilayer structures in skin. Liposomes have numerous advantages such as non-toxicity, targetability & biocompatibility. Liposomal behaviour can be altered in vivo and modify liposomal formulation to meet therapeutic needs thanks to their flexibility. Ethosomes are basically composed of water, phospholipids and ethanol while liposomes are composed of cholesterol and phospholipids. Liposomes have better mechanism of action due to fusion, endocytosis, lipid exchange & adsorption, whereas Ethosomes have only two mechanisms that are ethosome effect & ethanol effect. Ethosomes have better preparation methods compared to liposomes like, Classic Dispersion Method, Cold Method, Ethanol Injection Method, Hot Method, Transmembrane pH-gradient Method. Liposomes also have their own preparatory methods like Hand-shaking Method, Freeze Drying Method, Ether Injection Method. They are applicable in anti-viral, anti-biotic, anti-parkinson, NSAIDS drug delivery. Liposomes are applicable in Genetic & Tumour Therapy, Cosmetics, Respiratory & Opthalmic Disorders, Vaccines along with Pulmonary Drugs. Previous research studies performed by researchers have classified ethosomes into three types: classic ethosomes, transethosomes & binary ethosomes. They also classified liposomes into many types like SUV, MUV, LUV, OLV, MLV, Stealth liposomes & Conventional liposomes. Ethosomes will become more preferrable for drug delivery due to their advantages, allowing more research on it than liposomes by many researchers.

ACKNOWLEDGEMENT

I would like to thank Professor Dr. S. Janaki Devi for her guidance in my article. She is Assistant Professor in GITAM Institute of Pharmacy.

Ethical Considerations

Ethics approval and consent to participate – Not applicable

Consent for publication – Other author has given consent for publication.

Availability of Data and Material – Not applicable.

Competing Interest – There was no competing interest found between the author.

Funding – Not applicable.

Author's contribution – Both the authors has given equivalent contribution in this article.

REFERENCES

1. Cevc G., Vierl U. Nanotechnology and the transdermal route: A state of the art review and critical appraisal. J Control Release. 2010; 141(3):277-299. https://doi.org/10.1016/j.jconrel.2009.10.016
2. Mitragotri S., Anissimov YG., Bunge AL., Frasch HF., Guy RH., Hadgraft J., Kasting GB., Lane ME., Roberts MS. Mathematical models of skin permeability: an overview. Int J Pharm. 2011; 418(1):115-129. https://doi.org/10.1016/j.ijpharm.2011.02.023
3. Scheuplein RJ. Permeability of the Skin- Comprehensive Physiology: Handbook of Physiology, Reactions to Environmental Agents.2011; 26: 299-322.
4. Negi L M., Garg A K., Chauhan M., Ultradeformable Vesicles: Concept and Execution Pharma Times. 2009; 41(9):11–14.
5. Sudhakar CK., Upadhyay N., Verma A., Jain A., Charyulu RN., Jain S., Nanomedicine and Tissue Engineering, In editors by Thomas S., Grohens Y., Ninan M., Nanotechnology Applications for Tissue Engineering. William Andrew Publishing, Oxford, 2015; 1-19.
6. Ita K. Current status of ethosomes and elastic liposomes in dermal and transdermal drug delivery. Curr Pharm Des. 2016 Press Print. https://doi.org/10.2174/1381612822666160511150228
7. Jain S., Tiwary AK, Sapra B., Jain NK. Formulation and evaluation of ethosomes for transdermal delivery of lamivudine. AAPS PharmSciTech. 2007; 8(4):E111. https://doi.org/10.1208/pt0804111
8. Sudhakar CK., Upadhyay N., Jain S., Charyulu RN., Ethosomes as Non-invasive Loom for Transdermal Drug Delivery In: Sebastian M., Ninan N., Haghi A.K. editors Nanomedicine and Drug Delivery. San Diego, Apple Academic Press.2012; 1:1-16.
9. Cevc G., Blume G. Lipid vesicles penetrate into intact skin owing to the transdermal osmotic gradients and hydration force. BiochimicaetBiophysica Acta, 1992:1104:226-232. https://doi.org/10.1016/0005-2736(92)90154-e
10. Cevc G., Schatzein A., Blume G. Transdermal drug carriers: Basic properties, optimization and transfer efficiency in the case of epicutaneously applied peptides. J Control Release. 1995:36:3-16 https://doi.org/10.1016/0168-3659(95)00056-e
11. Duangjit S., Obata Y., Sano H., Onuki Y., Opanasopit P., Ngawhirunpat T., Miyoshi T., Kato S., Takayama K. Comparative study of novel ultradeformable liposomes: ethosomes, transfersomes and liposomes for enhancing skin permeation of meloxicam. Biol Pharm Bull. 2014; 37(2):239-247. https://doi.org/10.1248/bpb.b13-00576
12. Cevc G., Blume G. New, highly efficient formulation of diclofenac for the topical, transdermal administration in ultradeformable drug carriers, Transfersomes. BiochimBiophys Acta 2001;1514:191-205 https://doi.org/10.1016/s0005-2736(01)00369-8
13. Zhang YT., Shen LN., Wu ZH., Zhao JH., Feng NP. Comparison of ethosomes and liposomes for skin delivery of psoralen for psoriasis therapy. Int J Pharm. 2014; 471(1-2):449-452.

https://doi.org/10.1016/j.ijpharm.2014.06.001
14. Goyal R, Macri LK, Kaplan HM, Kohn J. Nanoparticles andnanofibers for topical drug delivery. J Control Release. 2016;240:77–92.
15. Dragicevic N, Maibach H. Combined use of nanocarriers and physical methods for percutaneous penetration enhancement.Adv Drug Deliv Rev. 2018;127:58–84. https://doi.org/10.1016/j.addr.2018.02.003
16. Bellefroid C, Lechanteur A, Evrard B, Mottet D, Debacq-Chainiaux F, Piel G. In vitro skin penetration enhancement techniques: a combined approach of ethosomes and microneedles. Int J Pharm. 2019;572:118793. https://doi.org/10.1016/j.ijpharm.2019.118793
17. Elsayed MMA, Abdallah OY, Naggar VF, Khalafallah NM. Deformable liposomes and ethosomes: mechanism of enhancedskin delivery. Int J Pharm. 2006;322(1–2):60–6. https://doi.org/10.1016/j.ijpharm.2006.05.027
18. Verma P, Pathak K. Therapeutic and cosmeceutical potential of ethosomes: an overview. J Adv PharmTechnol Res. 2010;1(3):274–82. https://doi.org/10.4103/0110-5558.72415
19. Cristiano MC, Froiio F, Spaccapelo R, Mancuso A, Nistico SP, Udongo BP, et al. Sulforaphane-loaded Ultradeformable vesicles as a potential natural nanomedicine for the treatment of skinCancer diseases. Pharmaceutics. 2020;12(1):13. https://doi.org/10.3390/pharmaceutics12010006
20. Touitou E, Dayan N, Bergelson L, Godin B, Eliaz M. Ethosomes -novel vesicular carriers for enhanced delivery: characterization andskin penetration properties. J Control Release. 2000;65(3):403–18. https://doi.org/10.1016/s0168-3659(99)00222-9
21. Paolino D, Lucania G, Mardente D, Alhaique F, Fresta M. Ethosomes for skin delivery of ammonium glycyrrhizinate: in vitro percutaneous permeation through human skin and in vivo anti-inflammatory activity on human volunteers. J Control Release. 2005;106(1–2):99–110. https://doi.org/j.jconrel.2005.04.007
22. Bendas ER, Tadros MI. Enhanced transdermal delivery ofsulbutamolsulfate via ethosomes. AAPS Pharm Sci Tech 2007;8:1-7. https://doi.org/10.1208/pt0804107
23. Esposito, E.; Nastruzzi, C.; Sguizzato, M.; Cortesi, R. Nanomedicines to Treat Skin Pathologies with Natural Molecules. Curr.Pharm. Des. **2019**, 25, 2323–2337. https://doi.org/10.2174/1381612825666190709210703
24. Carter, P.; Narasimhan, B.; Wang, Q. Biocompatible nanoparticles and vesicular systems in transdermal drug delivery for variousskin diseases. Int. J. Pharm. **2019**, 555, 49–62. https://doi.org/10.1016/j.ijpharm.2018.11.032
25. Jain, S.; Patel, N.; Shah, M.K.; Khatri, P.; Vora, N. Recent Advances in Lipid-Based Vesicles and Particulate Carriers for Topicaland Transdermal Application. J. Pharm. Sci. **2017**, 106, 423–445. https://doi.org/10.1016/j.xphs.2016.10.001
26. Rakesh, R.; Anoop, K.R. Ethosomes for transdermal and topical drug delivery. Int. J. Pharm. Pharm. Sci. **2012**, 4, 17–24.
27. Godin, B.; Touitou, E. Ethosomes: New prospects in transdermal delivery. Crit. Rev. Ther. Drug Carrier Syst. **2003**, 20, 63–102. https://doi.org/10.1615/critrevthedrugcarriersyst.v20.i1.20

28. Esposito, E.; Menegatti, E.; Cortesi, R. Ethosomes and liposomes as topical vehicles for azelaic acid: A preformulation study. Int.J. Cosmet. Sci. **2004**, 26, 270–271. https://doi.org/10.1111/j.1467-2494.2004.00233_2.x
29. Ramkar, S.; Sah, A.K.; Bhuwane, N.; Choudhary, I.; Hemnani, N.; Suresh, P.K. Nano-Lipidic Carriers as a Tool for Drug Targetingto the Pilosebaceous Units. Curr. Pharm. Des. **2020**, 26, 3251–3268. https://doi.org/10.2174/1381612826666200515133142
30. Sala, M.; Diab, R.; Elaissari, A.; Fessi, H. Lipid nanocarriers as skin drug delivery systems: Properties, mechanisms of skininteractions and medical applications. Int. J. Pharm. **2018**, 535, 1–17. https://10.1016/j.ijpharm.2017.10.046
31. Carter, P.; Narasimhan, B.; Wang, Q. Biocompatible nanoparticles and vesicular systems in transdermal drug delivery for variousskin diseases. Int. J. Pharm. **2019**, 555, 49–62. https://doi.org/10.1016/j.ijpharm.2018.11.032
32. Rakesh, R.; Anoop, K.R. Ethosomes for transdermal and topical drug delivery. Int. J. Pharm. Pharm. Sci. **2012**, 4, 17–24.
33. Kapoor, B.; Gupta, R.; Gulati, M.; Singh, S.K.; Khursheed, R.; Gupta, M. The Why, Where, Who, How, and What of the vesiculardelivery systems. Adv. Colloid Interface Sci. **2019**, 271, 101985.
34. Jain, S.; Patel, N.; Shah, M.K.; Khatri, P.; Vora, N. Recent Advances in Lipid-Based Vesicles and Particulate Carriers for Topicaland Transdermal Application. J. Pharm. Sci. **2017**, 106, 423–445. https://doi.org/10.1016/j.xphs.2016.10.001
35. Hallan, S.S.; Sguizzato, M.; Drechsler, M.; Mariani, P.; Montesi, L.; Cortesi, R.; Björklund, S.; Ruzgas, T.; Esposito, E. The Potentialof Caffeic Acid Lipid Nanoparticulate Systems for Skin Application: In Vitro Assays to Assess Delivery and Antioxidant Effect. Nanomaterials **2021**, 11, 171. https://10.3390/nano11010171
36. Vikas Pandey, Dilip Golhani& Rajesh Shukla; Ethosomes: versatile vesicular carriers for efficient transdermal delivery of therapeutic agents; Drug Delivery, 22:8, 988-1002. https://doi.org/10.3109/10717544.2014.889777
37. Bangham AD. Liposomes. (Ed. 1), Marcel Dekker. NewYork. (1983) 1-26.
38. Bangham AD and Horne RW. Negative staining of phospholipids and their structural modification by surface-active agents as observed in the electron microscope. J Mol Biol (1964) 12: 660-668. https://doi.org/10.1016/s0022-2836(64)80115-7
39. Gregoriadis G. Engineering liposomes for drug delivery: progress and problems. Rev article. Trends Biotechnol. (1995) 13: 527–537. https://doi.org/10.1016/s0167-7799(00)89017-4
40. Gregoriadis G, Florence AT. Liposomes in drug delivery, clinical, diagnostic and ophthalmic potential. Drugs (1993) 45: 15–28. https://doi.org/10.2165/00003495-199345010-00003
41. Florence, 1993).
42. Mozafari, M.R., Reed, C.J., Rostron, C., Kocum, C. & Piskin, E. Construction of stable anionic liposome-plasmid particles using the heating method: a preliminary investigation. Cellular & Molecular Biology Letters, (2002) 7 (3): 923-927.
43. Dash Tapaswi Rani; LIPOSOME AS A POTENTIAL DRUG DELIVERY SYSTEM: A REVIEW; IRJP 2013, 4(1): 6-12.

44. Storm G, Crommelin DJA. Liposomes: quo vadis? Pharm Sci Techno Today (1998) 1: 19-31. https://doi.org/10.1016/s1461-5347(98)00007-8
45. Takeuchi H, Yamamoto H, Kawashima Y. Mucoadhesive nanoparticulate systems for peptide drug delivery. Adv Drug Deliv Rev. (2001) 47: 39-54. https://doi.org/10.1016/s0169-409x(00)00120-4
46. Gabizon A. Stealth Liposomes and Tumor Targeting: One Step Further in the Quest for the Magic Bullet. (2001) 7:223–225.
47. Ana Claudia Paiva-Santos, Ana Luisa Silva, Catarina Guerra, Diana Peixoto, Miguel Pereira-Silva, Mahdi Zeinali, Filipa Mascarenhas-Melo, Ricardo Castro, Francisco Veiga; Ethosomes as Nanocarriers for the Development of Skin Delivery Formulations; Pharm Res (2021); 38: 947-970. https://doi.org/10.1007/s11095-021-03053-5
48. Touitou E, Dayan N, Bergelson L, Godin B, Eliaz M. Ethosomes -novel vesicular carriers for enhanced delivery: characterization and skin penetration properties. J Control Release. 2000;65(3):403–18. https://doi.org/10.1016/s0168-3659(99)00222-9
49. Abdulbaqi IM, Darwis Y, Khan NAK, Abou Assi R, Khan AA.Ethosomal nanocarriers: the impact of constituents and formulationtechniques on ethosomal properties, in vivo studies, and clinical trials. Int J Nanomedicine. 2016;11:26. https://doi.org/10.2147/ijn.s105016
50. Tran VV, Moon JY, Lee YC. Liposomes for delivery of antioxidantsin cosmeceuticals: challenges and development strategies. J Control Release. 2019;300:114–40. https://doi.org/10.1016/j.jconrel.2019.03.003
51. Zhou Y, Wei YH, Liu HX, Zhang GQ, Wu XA. Preparation andin vitro evaluation of Ethosomal Total alkaloids of Sophora alopecuroides loaded by a transmembrane pH-gradient method. AAPS PharmSciTech. 2010;11(3):1350–8. https://doi.org/10.1208/s12249-010-9509-6
52. Zhang YT, Xia Q, Li YY, He ZH, Li Z, Guo T, et al. CD44 assiststhe topical anti-psoriatic efficacy of curcumin-loaded Hyaluronanmodified Ethosomes: a new strategy for clustering drug in inflammatory skin. Theranostics. 2019;9(1):48–64. https://doi.org/10.7150/thno.29715
53. Shen LN, Zhang YT, Wang Q, Xu L, Feng NP. Enhanced in vitroand in vivo skin deposition of apigenin delivered using ethosomes. Int J Pharm. 2014;460(1–2):280–8. https://doi.org/10.1016/j.ijpharm.2013.11.017
54. Song CK, Balakrishnan P, Shim CK, Chung SJ, Chong S, KimDD. A novel vesicular carrier, transethosome, for enhanced skin delivery of voriconazole: characterization and in vitro/in vivo evaluation. Colloid Surf B-Biointerfaces. 2012;92:299–304. https://10.1016/j.colsurfb.2011.12.004
55. Touitou E, Dayan N, Bergelson L, Godin B, Eliaz M. Ethosomes — novel vesicular carriers for enhanced delivery: characterization and skin penetration properties. *J Control Release*. 2000;65(3):403–418. https://doi.org/10.1016/s0168-3659(99)00222-9
56. Sarwa KK, Suresh PK, Rudrapal M, Verma VK. Penetration of tamoxifen citrate loaded ethosomes and liposomes across human skin: a comparative study with confocal laser scanning microscopy. *Curr Drug Deliv*. 2014;11(3):332–337. https://doi.org/10.2174/1567201811666140115113127

57. Jain S, Patel N, Madan P, Lin S. Quality by design approach for formulation, evaluation and statistical optimization of diclofenac-loaded ethosomes via transdermal route. *Pharm Dev Technol*. 2015;20(4):473–489.
58. Zhou Y, Wei Y, Liu H, Zhang G, Wu X. Preparation and in vitro evaluation of ethosomal total alkaloids of *Sophora alopecuroides*loaded by a transmembrane pH-gradient method. *AAPS PharmSciTech*. 2010;11(3):1350–1358. https://doi.org/10.1208/s12249-010-9509-6
59. Li G, Fan Y, Fan C, et al. Tacrolimus-loaded ethosomes: physicochemical characterization and in vivo evaluation. *Eur J Pharm Biopharm*. 2012;82(1):49–57. https://doi.org/10.1016/j.ejpb.2012.05.011
60. Zhang JP, Wei YH, Zhou Y, Li YQ, Wu XA. Ethosomes, binary ethosomes and transfersomes of terbinafine hydrochloride: a comparative study. *Arch Pharm Res*. 2012;35(1):109–117. https://doi.org/10.1007/s12272-012-0112-0
61. Akhtar N, Pathak K. Cavamax W7 composite ethosomal gel of clotrimazole for improved topical delivery: development and comparison with ethosomal gel. *AAPS PharmSciTech*. 2012;13(1):344–355. https://doi.org/10.1208/s12249-012-9754-y
62. Dave V, Kumar D, Lewis S, Paliwal S. Ethosome for enhanced transdermal drug delivery of aceclofenac. *Int J Drug Deliv*. 2010;2(1):81–92. https://doi.org/10.5138/ijdd.2010.0975.0215.02016
63. 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;460(1–2):280–288. https://doi.org/10.1016/j.ijpharm.2013.11.017
64. Song CK, Balakrishnan P, Shim CK, Chung SJ, Chong S, Kim DD. A novel vesicular carrier, transethosome, for enhanced skin delivery of voriconazole: characterization and in vitro/in vivo evaluation. *Colloids Surf B Biointerfaces*. 2012;92:299–304. https://10.1016/j.colsurfb.2011.12.004
65. Ibrahim M Abdulbaqi, Yusrida Darwis, Arshad A Khan; Ethosomal nanocarriers: the impact of constituents and formulation techniques on ethosomal properties, in vivo studies, and clinical trials; International Journal of Nanomedicine 2016:11 2279-2304. https://doi.org/10.2147/ijn.s105016
66. Amala Maxwell, Sneh Priya; Nanosized Ethosomes – A Promising Vesicular Drug Carrier for Transdermal Drug Delivery, Research J. Pharm. And Tech 2019; 12(2):876-880. https://doi.org/10.5958/0974-360x.2019.00150.1
67. Alving C.R.; Marcophages As Targets for Delivery of Liposome Encapsulated Antimicrobial Agents. Adv Drug Delivery Rev. (1998), 2. https://doi.org/10.1016/0169-409x(88)900007-5
68. C. J. Chapman Allison, A.C., Gregoriadis, G, 1974; Liposomes as Immunological Adjuvant. Nature 252, 252.
69. Deamer, D. and Uster, P.; Liposome Preparation Methods and Monitoring Liposome Fusion. In: Baserga, R., Croce, C, and Royeza, G. (Eds.); Introduction of Macromolecules into Viable Mammalian Cells; Alan R. Liss, New York, 1980, pp, 205-220.
70. de Marie, S., Janknegt, R., Bakker-Woudenberg, I.A.J.M., 1994. Clinical use of liposomal and lipid-complexed amphotericin B. J. Antimicrob. Chemother, 33, 907-916. https://doi.org/10.1093/jac/33.5.907

71. D.J.A Crommelin. Liposomes, Lasic, D.D., Papahadjopoulos, D., 1995, Liposomes revisited. Science 267, 1275-1276. https://doi.org/10.1126/science.7871422
72. Emanuel, N., Kedar, E., Bolotin, E.M, Smorodinsky, N.I., Barenholz, Y., 1996. Preparation and Characterization of doxorubicin-loaded sterically stabilized immunoliposomes. Pharm. Res. 13, 352-359.
73. Mishra H, Chauhan V, Kumar K, Teotia D, A comprehensive review on Liposomes: a novel drug delivery system, Journal of Drug Delivery and Therapeutics. 2018; 8(6):400-404 https://doi.org/10.22270/jddt.v8i6.2071
74. Dash Tapaswi Rani; LIPOSOME AS A POTENTIAL DRUG DELIVERY SYSTEM: A REVIEW; IRJP 2013, 4(1): 6-12.
75. Elsayed Mustafa MA et al. Deformable liposomes and ethosomes: mechanism of enhanced skin delivery. Int J pharm 2006; 322:60-66. https://doi.org/10.1016/j.ijpharm.2006.05.027
76. Romero EL, Morilla MJ. Highly deformable and highly fluidvesicles as potential drug delivery systems: theoretical and practical considerations. Int J Nanomedicine. 2013;8:3171–86. https://doi.org/10.2147/ijn.s33048
77. Touitou E, Dayan N, Bergelson L, Godin B, Eliaz M. Ethosomes -novel vesicular carriers for enhanced delivery: characterization and skin penetration properties. J Control Release. 2000;65(3):403–18. https://doi.org/10.1016/s0168-3659(99)00222-9
78. Abdulbaqi IM, Darwis Y, Khan NAK, Abou Assi R, Khan AA. Ethosomal nanocarriers: the impact of constituents and formulation techniques on ethosomal properties, in vivo studies, and clinical trials. Int J Nanomedicine. 2016;11:26. https://doi.org/10.2147/ijn.s105016
79. Xie J, Ji Y, Xue W, Ma D, Hu YF. Hyaluronic acid-containing ethosomes as a potential carrier for transdermal drug delivery. Colloid Surf B-Biointerfaces. 2018;172:323–9. https://doi.org/10.1016/j.colsurfb.2018.08.061
80. López-Pinto JM, González-Rodríguez ML, Rabasco AM. Effect ofcholesterol and ethanol on dermal delivery from DPPC liposomes. Int J Pharm. 2005;298(1):1–12. https://10.1016/j.pharm.2005.02.021
81. Ascenso A, Raposo S, Batista C, Cardoso P, Mendes T, Praca FG,et al. Development, characterization, and skin delivery studies of related ultradeformable vesicles: transfersomes, ethosomes, and transethosomes. Int J Nanomedicine. 2015;10:5837–51. https://doi.org/10.2147/ijn.s86186
82. Abdulbaqi IM, Darwis Y, Khan NAK, Abou Assi R, Khan AA. Ethosomal nanocarriers: the impact of constituents and formulation techniques on ethosomal properties, in vivo studies, and clinical trials. Int J Nanomedicine. 2016;11:26. https://doi.org/10.2147/ijn.s105016
83. Zhang YB,Ng W, Hu JG, MussaSS,GeYR,XuHX. Formulation and in vitro stability evaluation of ethosomal carbomer hydrogel fortransdermal vaccine delivery. Colloid Surf B-Biointerfaces. 2018;163:184–91. https://doi.org/10.1016/j.colsurfb.2017.12.031
84. Pandey V, Golhani D, Shukla R. Ethosomes: versatile vesicularcarriers for efficient transdermal delivery of therapeutic agents. Drug Deliv. 2015;22(8):988–1002. https://doi.org/10.3109/10717544.2014.889777

85. Kant Shashi, Kumar Satinder, Prashar Bharat; A COMPLETE REVIEW ON: LIPOSOMES; IRJP 2012, 3(7): 10-16, ISSN 2230-8407.
86. Mi-Kyung Lee; Liposomes for Enhanced Bioavailability of Water-Insoluble Drugs: In-Vivo Evidence and Recent Approaches; MDPI 2020, 12, 264. https://doi.org/10.3390/pharmaceutics12030264
87. Zylberberg C, Matosevic S. Pharmaceutical liposomal drug delivery: a review of new delivery systems and a look at the regulatorylandscape. Drug Deliv. 2016;23(9):3319–29. https://doi.org/10.1080/10717544.2016.1177136
88. Touitou E, Dayan N, Bergelson L, Godin B, Eliaz M. Ethosomes - novel vesicular carriers for enhanced delivery: characterization andskin penetration properties. J Control Release. 2000;65(3):403–18. https://doi.org/10.1016/s0168-3659(99)00222-9
89. Yang L,WuLF, WuDZ, ShiDS,Wang T, ZhuXL. Mechanism of transdermal permeation promotion of lipophilic drugs by ethosomes.Int J Nanomedicine. 2017;12:3357–64. https://doi.org/10.2147/ijn.s134708
90. Verma P, Pathak K. Therapeutic and cosmeceutical potential of ethosomes: an overview. J Adv PharmTechnol Res. 2010;1(3):274–82. https://doi.org/10.4103/0110-5558.72415
91. Kumar R, Aslam M.D, Tripathi A, Prasad D, Chaudhary V, Jain V, Mishra S K, Singh R. Ethosomes novel vesicular carriers in transdermal drug delivery. J Global Pharma Tech 2010; 2(6):1-7.
92. Ana Claudia Paiva-Santos, Ana Luisa Silva, Catarina Guerra, Diana Peixoto, Miguel Pereira-Silva, Mahdi Zeinali, Filipa Mascarenhas-Melo, Ricardo Castro, Francisco Veiga; Ethosomes as Nanocarriers for the Development of Skin Delivery Formulations; Pharm Res (2021); 38: 947-970. https://doi.org/10.1007/s11095-021-03053-5
93. Nasim Karami, Eskandar Moghimipour, Anayatollah Salimi; Liposomes as a Novel Drug Delivery System: Fundamental and Pharmaceutical Application.
94. Jr. F. Szoka and D. Papahadjopoulos. Proc. Natl. Acad. Sci. USA, (1978) 60:4194-4198.
95. Jesorka A, al., Liposomes: technologies and analytical applications, Annu. Rev. Anal. Chem. 1 (2008) 801–832 https://doi.org/101146/annurev.anchem.1.031207.112747
96. Kaur D., Kumar S., Niosomes: present scenario and future aspects. Journal of Drug Delivery and Therapeutics, 2018; 8(5):35-43. https://doi.org/10.22270/jddt.v8i5.1886
97. Vemuri S, et al., Preparation and characterization of liposomes as therapeutic delivery systems: a review, Pharm. Acta Helv. 1995; 70:95–111 https://doi.org/10.1016/0031-6865(95)00010-7
98. Gangwar S, Singh S, Garg G. Ethosomes: A Novel Tool for Drug Delivery Through the Skin, Journal of Pharmacy Research 2010; 3(4):688-691.
99. Zahid SR, Upmanyu N, Dangi S, Ray SK, Jain P, Parkhe G; Ethosome: a novel vesicular carrier for transdermal drug delivery, Journal of Drug Delivery and Therapeutics, 2018; 8(6): 318-326. https://10.22270/jddt.v8i6.2028
100. Rouf MA, Vural I, Renoir JM, Hincal AA. Development and characterization of liposomal formulations for rapamycin delivery and investigation of their antiproliferative effect on MCF7 cells. J Liposome Res 2009;19(4):322–31.

https://doi.org/10.3109/08982100902963043
101. Dayan N, Touitou E. Carriers for skin delivery of trihexyphenidyl HCl: ethosomes vs liposomes 2000;21:1879–85. https://doi.org/10.1016/s0142-9612(00)00063-6
102. Sujitha B, Krishnamoorthy B, Muthukumaran M. Formulation and Evaluation of Piroxicam Loaded Ethosomal Gel for Transdermal Delivery. Int J Adv Pharm Gen Res. 2014; 2(1): 34–45.
103. Ainbinder D, Touitou E. Testosterone ethosomes for enhanced transdermal delivery. Drug Deliv 2005;12:297–303. https://doi.org/10.1080/10717540500176910
104. Nagadevi B, Kumar KS, Venkanna P, Prabhakar D. Formulation and characterisation of Tizanidine hydrochloride loaded ethosomes patch. Int J Pharm Pharm Sci 2014;6(4):199–205.
105. Paolino D, Lucania G, Mardente D, Alhaique F, Fresta M. Ethosomesfor skin delivery of ammonium glycyrrhizinate: in vitro percutaneous permeation through human skin and in vivo anti-inflammatory activity on human volunteers. *J Control Release*. 2005;106(1–2):99–110. https://doi.org/10.1016/j.jconrel.2005.04.007
106. Touitou E. inventor and assignee. Compositions for applying active substances to or through the skin. United States patent US 5540934 A. 1996 Jul 30.
107. Abdulbaqi IM, Darwis Y, Khan NAK, Abou Assi R, Khan AA. Ethosomal nanocarriers: the impact of constituents and formulation techniques on ethosomal properties, in vivo studies, and clinical trials. Int J Nanomedicine. 2016;11:26. https://doi.org/10.2147/ijn.s105016
108. Shukla T, Verma A, Upmanyu N, Mishra SS, Shilpi S. Development and characterization of clopidogrel-loaded ethosomal transdermal patch for antiplatelet effect. Asian J Pharm 2016;10(4):S480–86.
109. Touitou E. inventor and assignee. Compositions for applying active substances to or through the skin. United States patent US 5540934 A. 1996 Jul 30.
110. Vikas Pandey, Dilip Golhani & Rajesh Shukla (2015) Ethosomes: versatile vesicular carriers for efficient transdermal delivery of therapeutic agents, Drug Delivery, 22:8, 988-1002. https://doi.org/10.3109/10717544.2014.889777
111. Vijayakumar KS, Parthiban S, Senthilkumar GP, Tamiz Mani T. Gliclazide loaded ethosomes as transdermal drug delivery carriers. Asian J Res Biol Pharm Sci 2014;2(2):89-98.
112. Dinesh Kumar Mishra, Neelam Balekar, Vinod Dhote, Pradyumna Kumar Mishra; Ethosomes: A Novel Carrier for Dermal or Transdermal Drug Delivery; Pan Stanford Publishing; ISBN 978-981-4745-59-8.
113. Liu X, Liu H, Liu J, He Z, Ding C, Huang G, *et al*. Preparation of a ligustrazine ethosome patch and its evaluation in vitro and in vivo. Int J Nanomedicine 2011;6:241–7. https://doi.org/10.2147/ijn.s16044
114. Ahmed Gouda, Omar S. Sakr, Maha Nasr, Omaima Sammour; Ethanol injection technique for liposomes formulation: An insight into development, influencing factors, challenges and applications, JDDST, Volume 61, 2021, 102174, ISSN 1773-2247. https://doi.org/10.1016/j.jddst.2020.102174

115. Zhou Y, Wei Y, Liu H, Zhang G, Wu X. Preparation and in vitro evaluation of ethosomal total alkaloids of *Sophora alopecuroides* loaded by a transmembrane pH-gradient method. *AAPS PharmSciTech*. 2010;11(3):1350–1358. https://doi.org/10.1208/s12249-010-9509-6
116. Nichols JW, Deamer DW. Catecholamine uptake and concentration by liposomes maintaining pH gradients. *Biochim Biophys Acta*. 1976;455(1):269–271. https://doi.org/101016/0005-2736(76)90169-3
117. Cramer JA, Prestegard JH. NMR studies of pH-induced transport of carboxylic acids across phospholipid vesicle membranes. *Biochem Biophys Res Commun*. 1977;75(2):295–301. https://doi.org/10.1016/0006-291x(77)91042-7
118. Fan C, Li X, Zhou Y, et al. Enhanced topical delivery of tetrandrine by ethosomes for treatment of arthritis. *Biomed Res Int*. 2013;2013:161943. https://doi.org/10.1155/2013/161943
119. Zhou Y, Wei Y, Liu H, Zhang G, Wu X. Preparation and in vitro evaluation of ethosomal total alkaloids of *Sophora alopecuroides* loaded by a transmembrane pH-gradient method. *AAPS PharmSciTech*. 2010;11(3):1350–1358. https://doi.org/10.1208/s12249-010-9509-6
120. Abdulbaqi IM, Darwis Y, Khan NAK, Abou Assi R, Khan AA. Ethosomal nanocarriers: the impact of constituents and formulation techniques on ethosomal properties, in vivo studies, and clinical trials. Int J Nanomedicine. 2016;11:26. https://doi.org/10.2147/ijn.s105016
121. Felgner, J.H., Kumar, R., Sridhar, C.N., Wheeler, C.J., Tsai,Y.J., Border, R., Ramsey, P., Martin, M., Feigner, P.L.,1994. Enhanced gene delivery and mechanism studies witha novel series of cationic lipid formulations. J. Biol. Chem.269, 2550-2561. https://doi.org/s0021-9258(17)41980-6
122. H.A.H Rongen, A. Bult and W.P van Bennekom. J. Immuno. Methods, (1997) 204:105-133.
123. New, R.R.C., Preparation of liposomes. In: New, R.R.C.(Ed.), Liposomes: a practical approach, IRL Press, Oxford,1990, pp. 33 104.
124. Jain N.K. Controlled and Novel Drug Delivery. CBS Publisher, Page no. 304-326.
125. Chandraprakash Dwivedi, Rajni Yadav, Sandip Prasad Tiwari, Trilochan Satapathy, Amit Roy; ROLE OF LIPOSOME IN NOVEL DRUG DELIVERY SYSTEM; JDDT, 2014, 4(2), 116-129. https://doi.org/10.2270/jddt.v4i2.768
126. Kersten, G.F.A., Crommelin, D.J.A., 1995. Liposomes and ISCOMS as vaccine formulations. Biochim. Biophys. Acta 1241, 117-138. https://doi.org/10.1016/0304-4157(95)00002-9
127. Chandraprakash Dwivedi, Rajni Yadav, Sandip Prasad Tiwari, Trilochan Satapathy, Amit Roy; ROLE OF LIPOSOME IN NOVEL DRUG DELIVERY SYSTEM; JDDT, 2014, 4(2), 116-129. https://doi.org/10.2270/jddt.v4i2.768
128. Sharma, A., Straubinger, R.M., 1994. Novel taxol formulations: preparation and characterization of taxol-containing liposomes Pharm. Res. 11, 889 896
129. Szoka, F.C., Liposomal drug delivery: current status and future prospects. In: Wilschut, J., Hoekstra, D. (Eds.), Membrane Fusion, Marcel Dekker, New York, 1991, pp.845-890.

130. Vingerhoeds, M.H., Storm, G. and Crommelin, D.J.A. Immunomethods (1994) 4:259–272.
131. Hamdi Nsairat, Dima Khater, Usama Sayed, Fadwa Odeh, Abeer Al Bawab, Walham Alshaer; Liposomes: structure, composition, types and clinical applications, Heliyon, 2022, 2405-8440. https://doi.org/10.1016/j.heliyon.2022.e09394
132. Akiladev D, Basak S, (2010) International Journal of Current pharmaceutical research. 2(4): 1-4.
133. Pravin P. Aute, Meghana S. Kamble, Pravin D. Chaudhari, Ashok V. Bhosale; A Comprehensive Review on Ethosomes, International Journal of Research & Development in Pharmacy & Life Sciences, December-January 2012-13; 2(1):218-224, ISSN: 2278-0238.
134. Godin B, Tauitou Elka. (2005) Current Drug Delivery. 2: 269-275.
135. Akiladev D, Basak S, (2010) International Journal of Current pharmaceutical research. 2(4): 1-4.
136. Michaels AS, Chandrasekaran SK, Shaw JW. (1975) Drug permeation through human skin: theory and in vitro ex-perimental measurement. AlChE 21: 985-96.
137. Horwitz E, Pisanty S, Czerninski R, Helser M, Eliav E, Touitou E. A clinical evaluation of a novel liposomal carrier for acyclovir in the topical treatment of recurrent herpes labialis. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 1999;87(6):700–5.
138. Touitou E, Godin B, Dayan N, Weiss C, Piliponsky A, Levi-Schaffer F. Intracellular delivery mediated by an ethosomal carrier. Biomaterials 2001;22:3053–9. https://doi.org/10.1016/s0142-9612(01)00052-7
139. Dwivedi C, Yadav R, Tiwari SP, Satapathy T, Roy A, Role of liposomes in novel drug delivery system, Journal of drug delivery and therapeutics 2014; 4(2):116-129. https://doi.org/10.2270/jddt.v4i2.768
140. Priyanka R Kulkarni, Jaydeep D Yadav, Kumar A Vaidya. Liposomes: A Novel Drug Delivery System. International journal of current Pharmaceutical and Research 2011; 3(2).
141. Amala Maxwell, Sneh Priya; Nanosized Ethosomes – A Promising Vesicular Drug Carrier for Transdermal Drug Delivery; Research Journal of Pharmacy and Technology 2019; 12(2):876-880.
142. Elsaied Hamada Elsaied, Hamdy Mohamed Dawaba, Elsherbini Ahmed Ibrahim, Mohsen Ibrahim Afouna . Investigation of proniosomes gel as a promising carrier for transdermal delivery of Glimepiride. Universal Journal of Pharmaceutical Research. 2016; 1(2): 1-18.
143. Sipai Altaf Bhai. M*, Vandana Yadav, Mamatha. Y, Prasanth V.V Department of pharmaceutics Gautam college of Pharmacy,Lipoosmes an Overview,Journal of pharmaceutical and Scientific innovation,accepted on 24/01/12.
144. Kant Shashi*, Kumar Satinder, Prashar Bharat; A complete review on liposomes; International Research Journal Of Pharmacy ISSN 2230-8407
145. Formulation and evaluation of liposomal drug delivery system of decitabine T. Veena* Dr. Manichandrik , Madav, Madhuri , Mounika , Bindu Rani , Ashwini Formulation and evaluation of liposomal drug delivery system of decitabine, Vol 6,Issue 3 , July -Sep 2017.
146. Hong MS et al., Prolonged blood circulation of methotrexate by modulation of liposomal composition, Drug Delivery 2001; 8:231–237

147. Li X, Chen D, Le C, Zhu C, Gan Y, Hovgaard L. Novel mucus-penetrating liposomes as a potential oral drug delivery system: preparation, in vitro characterization, and enhanced cellular uptake. Int J Nanomedicine, 2011; 6: 3151-3162.
https://doi.org/10.2147/ijn.s25741

148. Kant Shashi*, Kumar Satinder, Prashar Bharat; A complete review on liposomes; International Research Journal Of Pharmacy ISSN 2230-8407

149. Ugochukwu AE, Nnedimkpa OJ, Rita NO. Preparation and characterization of Tolterodine tartrate proniosomes, Universal Journal of Pharmaceutical Research. 2017; 2(2):22-25
https://doi.org/10.22270/ujpr.v2i2.r1