

Ethosomes as Novel drug delivery system – An Overview

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## ABSTRACT

Ethosomes are novel, soft, malleable vesicles that contain relatively higher percentage of ethanol. Transdermal route is a promising method for the delivery of drugs. Ethosomes can effectively transport drugs across the skin and into deeper layers mainly due to its encapsulation efficiency and penetration into the skin. Due to the use of ethanol in the formulation of ethosomes, the permeability of drug enhances as the vesicular membrane becomes flexible. This article gives a review of numerous facts of ethosomes with their preparation, characterisation, advantages, disadvantages and their applications in drug delivery. Ethosomes as a novel drug delivery system can be considered as a potential carrier for the transportation of drugs.

**Keywords:** Ethosomes, transdermal, novel drug delivery, phospholipid, ethanol.

## INTRODUCTION

The application of medicinal substances to the skin is an old concept. In recent years innovative techniques have been developed for the delivery of drug through the skin. Human skin is an effective, selective barrier for chemical permeation. Transdermal and intranasal drug delivery system is a successful substitute dosage form for the delivery of drugs. Transdermal drug delivery is gaining importance due to its non-invasive procedure for administration. The transdermal drug delivery overcomes a number of limitations of oral drug delivery such as degradation of drugs by digestive enzymes, irritation of gastrointestinal mucosa and first pass effect. Also due to the pain on administration associated with parenteral route, patients highly prefer transdermal route. Hence transdermal dosage forms enjoy being the most patient compliant mode of drug delivery.

Delivery of drug through the skin is the most challenging area in research. In order to study the mechanism of ethosomal drug delivery system, the structure of skin and its layers must be considered. There are three structural layers the epidermis, dermis and subcutis. Epidermis is the outermost layer and is seen on the surface of the skin. The main cells of the epidermis are the keratinocytes, which synthesize the protein keratin. The keratinocytes develop at the bottom and rise to the top, where they are shed from the surface as dead cells. The outer most portion of the epidermis known as the stratum corneum. It consists of 10 to 25 layers of dead, extended, fully keratinized corneocytes, which are inserted in a matrix of lipid bilayers. It has been shown that the stratum corneum is the main barrier to penetration through the skin. It is relatively waterproof and, when undamaged, obstructs most bacteria, viruses, and

other foreign substances from entering the body. The keratinocytes in the stratum corneum are dead squamous cells that are no longer multiplying.

The dermis consists of dense irregular connective tissue and is much thicker than the epidermis. The dermis is responsible for the tensile strength of skin. Function is to regulate temperature and to supply the epidermis with nutrient-saturated blood. Much of the body's water supply is storage within the dermis. The subcutaneous layer lies below the dermis. It is primarily composed of fat and connective tissue. It performs as a protective cushion and helps to regulate body temperature.

Outermost layer of the skin, the stratum corneum, represents the most resistible barrier to drug permeation across the skin and reduce the bioavailability of the drugs. Therefore, special carriers are required to overcome the natural skin barrier to deliver drug molecules having different physicochemical properties to the systemic circulation. Advances in modern technologies are resulting in a larger number of drugs being delivered transdermally including conventional hydrophobic small molecule drugs, hydrophilic drugs and macromolecules.

Stratum corneum (SC) permits only the lipophilic drugs having molecular weight < 500 Daltons. Many approaches have been attempted to overcome this property of skin, includes the use of chemical enhancers like surfactants, organic solvents, physical enhancers such as iontophoresis, sonophoresis, micro needles, electroporation etc. and various methods have been assessed to increase permeation and amongst them the best is lipid vesicles can modulate barrier property of SC. Vesicles act as carrier systems, able to transport large molecular weight drugs into the skin or even into the systemic circulation.

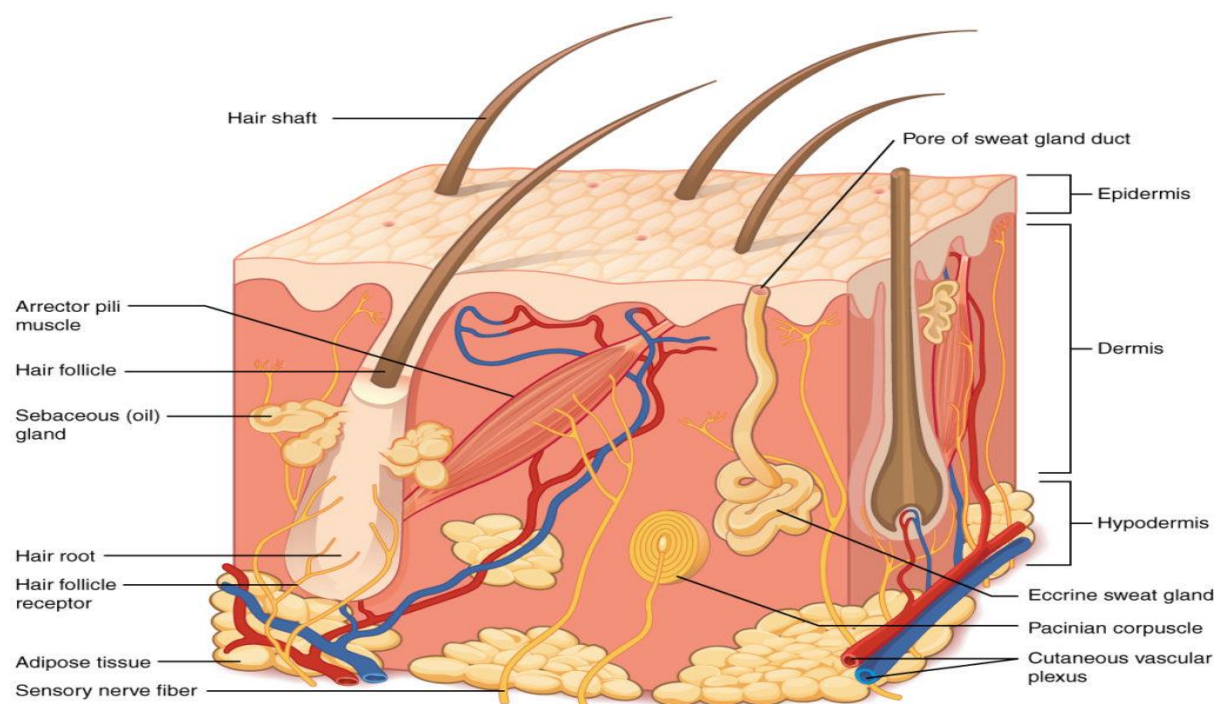


Fig 1: structure of the skin

## NOVEL DRUG DELIVERY SYSTEM

Novel Drug delivery System (NDDS) suggests to the approaches, formulations, technologies, and systems for delivering pharmaceutical compounds in the body for desired therapeutic effects.

A drug delivery system (DDS) is defined as a formulation or a device that enables the introduction of a therapeutic substance in the body and which improves its efficacy and safety by controlling the rate, time and place of release of drugs in the body. This process includes the administration of the therapeutic product, the release of the active ingredients by the product, and the subsequent transport of the active ingredients across the biological membranes to the site of action. Drug delivery system is an interface between the patient and the drug. It may be a formulation of a drug, to administer it for a therapeutic purpose or a device used to deliver the drug. India is progressing remarkably in DDS focusing mainly on Research and development activities and consequently various newer and controlled DDS like transdermal, ocular, aquasomes, ethosomes, liposomes, resealed erythrocytes, implants etc. These have a large number of advantages over conventional dosage forms like controlled and predictable release, lesser chance of dose dumping, reduction in the frequency of administration, minimization of side effects etc.

#### **IDEAL CHARACTERISTICS OF NDDS:**

- Convenient to administer.
- Should be Economical.
- Should not require special skill sets.
- Does not cause pain.
- Continuous absorption of drug.
- Minimal administration frequency.
- Is not embarrassing.

#### **ADVANTAGES OF NOVEL DRUG DELIVERY SYSTEM:**

- Solubilisation improvement.
- Increased bioavailability.
- Protection from toxicity.
- Improvement of stability.
- Provide sustained delivery.
- Improvement of pharmacological activity.
- Optimal dose at an accurate time and right location.
- Protection from chemical and physical degradation.

#### **MODES OF NOVEL DRUG DELIVERY SYSTEM:**

- Targeted drug delivery system
- Controlled drug delivery system

#### **TARGETED DRUG DELIVERY SYSTEM:**

Targeted drug delivery, also called smart drug delivery, is a method of treatment that involves the increase in medicament in one or more body parts in comparison to others. Therefore, medication is delivered only to the key areas of interest within the body. This offers an improved efficacy of treatment and also reduces side effects. There are four principle requirements for a successful targeted drug delivery system: retain, evade, target and release, i.e., there should be proper loading of the drug into an appropriate drug delivery vehicle, it must possess an ability to escape the body's secretions that may degrade it, leading to a long residence time in circulation and thereby reaching the site of interest and, should release the drug at the specific site within the span of effective drug function. Different sites of interest within the body necessitate the use of different drug delivery systems, depending upon the route to be followed.

#### **Advantages of targeted drug delivery system:**

- Increased treatment efficacy
- Increased specific localization
- Controlled bio distribution
- Modulated pharmacokinetics
- Decreased toxic side effect
- Reduced dose
- Improved patient compliance

#### **Strategies of Drug Targeting:**

Drug targeting to an area of interest within the body increases the therapeutic effectiveness as well as it reduces the toxicity that may arise otherwise. Two strategies are widely used for drug targeting to the desired organ/tissue.

##### **Passive targeting**

It is based on the accumulation of drug at areas around the site of interest, like tumour tissues. This is called Enhanced Permeability Retention (EPR) effect. Such types of targeting occurs with almost all types of drug delivery carriers. Passive targeting is a misnomer because it cannot really be described as a form of selective targeting. Although the EPR effect applies for nanoparticle administered, the majority (>95%) of these nanoparticles tend to accumulate in organs other than those of interest such as liver, lungs and spleen. Thus, it is the distribution of drug by blood circulation. Examples include the use of antimalarial drugs being targeted for treating microbial infections such as leishmaniasis, candidiasis and brucellosis.

##### **Active targeting**

Active targeting describes the drug targeting interactions with the use of ligand receptor interactions. However, interactions between a ligand and a receptor are possible only when the two are in close propinquity, (less than 0.5mm). The currently available drug delivery systems are able to reach the target by the virtue of blood circulation and extravasation. Active receptor targeting means ligand-receptor interaction but that takes place only after blood circulation and extravasation. Active targeting can further be divided into three different targeting levels:

**First order targeting:** This is the distribution of drug to capillary beds of target sites- organ or tissue, for example, in case of lymphatic tissue, peritoneal cavity, pleural cavity, cerebral ventricles, eyes, joints, etc.

**Second order targeting:** This is the targeting of drugs to specific sites such as the tumor cells for example, to kupffer cells in liver

**Third order targeting:** It is the type of drug targeting wherein the drug is intracellularly localized at the target site via endocytosis or through receptor-based ligand mediated entry.

### **CONTROLLED DRUG DELIVERY SYSTEM:**

An ideal dosage regimen of drug therapy is one which allows rapid attainment of the required plasma concentration that can be maintained for the entire period of treatment. The frequencies of drug administration primarily depend on the biological half-life of the drug and mean residential time (MRT). Conventional drug delivery system often produces over or under medication result in various adverse drug reactions (ADRs) due to unpredictable drug release pattern. The term controlled release (CR) implies the predictability and reproducibility in the drug release kinetics which means the drug release from the delivery system proceed at the rate profile not only expected kinetically but also reproducible from one division to another. CRDDS intended to exercise control drug release in the body; this may be temporal or spatial nature or both. The term sustained release also mentioned during the description of controlled release. Sustained release (SR) used to describe a pharmaceutical dosage form formulated to retard the release of API such a way that its appearance in the systemic circulation is delayed or prolonged and plasma concentration sustained in duration. The onset of drug action delayed and duration of therapeutic effect is maintained.

### **Advantages of controlled drug delivery system:**

- This delivery system improved the patient compliance especially with long-term treatments for chronic diseases.
- Conventional dosages form produce fluctuation in plasma drug concentration. These fluctuations depend on the drug kinetics within the body like absorption, distribution, metabolism and excretion. Controlled release eliminates this type of fluctuation in plasma drug concentration.
- Reduction in dose and dosing frequencies.
- Maintenance of required drug concentration in plasma thus eliminates the failure of drug therapy and improved the efficiency of treatments.
- A suitable delivery system for drugs which having a short biological half-life (3-4 hrs.) and drug rapidly eliminate from the body.

### **Disadvantages of controlled drug delivery system:**

- Dumping is a major disadvantage of CRDDS, which refers to the rapid release of a relatively large quantity of drug from a controlled release formulation. This phenomenon becomes hazardous with potent drugs.
- Poor in-vivo & in-vitro correlations
- Difficult to optimize the accurate dose and dosing interval
- Patient variability affects the release rate like GI emptying rate, residential time, fasting or non-fasting condition, etc.

**Characteristics of controlled drug delivery system:**

- Short elimination half-life
- Long elimination half-life
- Narrow therapeutic index
- Poor absorption
- Active absorption
- Low or slow absorption
- Extensive first pass effect

**RECENT CARRIERS OF NOVEL DRUG DELIVERY SYSTEM:**

Targeted drug delivery is performed through carrier system. The carrier is one of the special molecule or system essentially required for effective transportation of loaded drug up to the preselected sites. They are engineered vectors, which retain drug inside or onto them either via encapsulation or via spacer moiety and transport or deliver it into vicinity of target cell.<sup>13</sup>

Recent development in novel drug delivery system are:

1. Phytosome
2. Liposome
3. Nanoparticles
4. Emulsions
5. Microsphere
6. Ethosome
7. Solid lipid nanoparticles
8. Niosomes
9. Proniosomes
10. Transdermal Drug Delivery System
11. Dendrimers
12. Liquid Crystals
13. Hydrogels

## **ETHOSOMES**

Ethosomes are non-invasive delivery carriers that enable drugs to reach the deep skin layers and/or the systemic circulation. These are soft, malleable vesicles tailored for enhanced delivery of active agents. They are composed mainly of phospholipids, (phosphatidylcholine, phosphatidylserine, phosphatidic acid), high concentration of ethanol and water. The high concentration of ethanol makes the ethosomes unique, as ethanol is known for its disturbance of skin lipid bilayer organization; therefore, when integrated into a vesicle membrane, it gives that vesicle the ability to penetrate the stratum corneum. Also, because of their high ethanol concentration, the lipid membrane is packed less tightly than conventional vesicles but has equivalent stability, allowing a more malleable structure and improves drug distribution ability in stratum corneum lipids.

### **HISTORY OF ETHOSOMES:**

The topical delivery of drugs has been widely practiced in the treatment of majority of diseases for the past several years. The topical formulation commonly used are creams, ointments, and gellies to deliver the drugs to the affected parts. But the efficacy of the drugs were not felt at the desired level. There was tremendous change in the pharmaceutical sector with the arrival of vesicular delivery systems especially liposomes, where the drugs were encapsulated into liposomes and formulated as creams or ointments for the effective delivery. Confocal microscopy studies have shown that intact liposomes are not able to penetrate into the granular layers of the epidermis. Liposomal formulations mainly remain at the upper part of the stratum corneum, hence researches led to the development of novel vesicular delivery system known as “Ethosomes”.

Ethosomes were first developed by Touitou et al in 1996 which mainly comprises of phospholipids, propylene glycol, water and ethanol at the right concentrations. The ethanol is known as an efficient permeation enhancer. Even though the actual mechanism of ethosomes are not yet known and it act by affecting the intercellular region of the stratum corneum barrier and were reported to possess significantly higher transdermal flux in comparison to liposomes. The synergistic effects of combination of phospholipids and high concentration of ethanol in vesicular formulations have been suggested to be responsible for deeper distribution and penetration in the skin lipid bilayers. Ethosomes are shown to entrap drug molecule with various physicochemical characteristics of hydrophilic, lipophilic or amphiphilic than liposome.

### **STRUCTURE OF ETHOSOMES**

The main difference between ethosomes and liposomes is in their composition. Ethosome comprises of various types of phospholipid structures, water, and low molecular weight alcohol (ethanol or isopropyl alcohol) in high concentration that provide malleability to the vesicle membrane. The ethosomal lipids are in a more-fluid state than liposomes containing the same ingredients without ethanol. Thus the ethanol can act as a “mixing” agent for lipid vesicles and provide vesicles with softness characteristics, which allow them to increase their distribution in different skin layers. However, because of their high ethanol concentration, the lipid membrane is packed less firmly than conventional vesicles but has equivalent solidity, allowing

a more malleable structure and enhance drug distribution ability in stratum corneum lipids. In the cases of drugs with high solubility, the presence of ethanol in ethosomes can exhibit high encapsulation efficiency and improved drug loading. It has been reported that the decrease of ethanol concentration in the range of 20% to 45% can result in the increase in the size of ethosomes and makes the ethosomes unique. The no aqueous phase (alcohol and glycol combination) may range between 22 to 70%. And polyglycols like propylene glycol, transcuto RTM are used as skin penetration enhancer. Various phospholipids which are used as vesicle forming component are phosphatidylcholine (for instance: soya phosphatidylcholine, egg phosphatidylcholine, dipalmitoyl phosphatidylcholine, distearoyl phosphatidylcholine) phosphatidic acid, phosphatidylethanolamine, phosphatidylserine, phosphatidylglycerol, and phosphatidylinositol (PI). In addition, non-ionic surfactants (PEG alkyl ethers) can be combined with the phospholipids in these preparations. Cationic lipids like cocoamide, POE alkyl amines, dodecylamine, cetrimide etc. can be added too. Cholesterol used at a range of 0.1% - 1% provide stability to the vesicle membrane. Such a composition enables delivery of high concentration of active ingredients through skin. Drug delivery can be modulated by altering alcohol: water or alcohol-polyol: water ratio. In addition, soybean phosphatidylcholine (Phospholipon 90), ethanol, drug and distilled water can be served for production of Ethosomes.

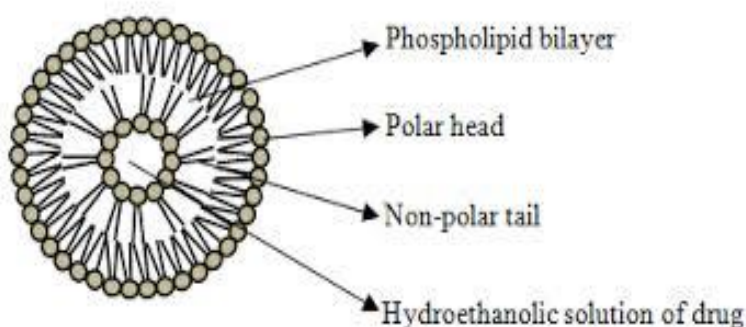


Figure: 2. structure of ethosome

Class	Examples	Uses
Phospholipid	Soya phosphatidyl choline, Egg phosphatidyl choline, Dipalmityl phosphatidyl choline, Distearyl phosphatidyl choline.	Vesicles forming components.
Polyglycol	Propylene glycol	As a skin penetration enhancer.
Alcohol	Ethanol, isopropyl alcohol	For providing the softness to vesicle membrane, as a penetration enhancer.
Cholesterol	Cholesterol	For providing the stability to vesicle membrane.
Dye	Rhodamine-123, rhodamine red, fluorescence isothiocyanate (FITC), 6-carboxy fluorescene	For characterization study.



Vehicle	Carbopol P934, HPMC	As a gel former.
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Table1: Different Additive Employed in preparation of Ethosomes

### ADVANTAGES OF ETHOSOMAL DRUG DELIVERY:

1. Ethosomes helps in enhanced permeation of drug through the skin for transdermal delivery.
2. Ethosomes are platform for the delivery of large and diverse group of drugs (peptides, protein molecules)
3. Ethosome composition is safe and the components are approved for pharmaceutical and cosmetic use.
4. Simple method for drug delivery in comparison to Iontophoresis and Phonophoresis and other complicated methods.
5. It contains non-toxic raw material in formulation.
6. High patient compliance-The ethosomal drug is administrated in semisolid form (gel or cream) hence producing high patient compliance.
7. The Ethosomal system is passive, non-invasive and is available for immediate commercialization.
8. Low risk profile- The technology has no large-scale drug development risk since the toxicological profiles of the ethosomal components are well documented in the scientific literature.

### DISADVANTAGES OF ETHOSOMAL DRUG DELIVERY:

1. Ethosomal administration is not a means to achieve rapid bolus type drug input, rather it usually designed to offer slow, sustained drug delivery.
2. The adequate solubility of the drug in both lipophilic and aqueous environments to reach dermal microcirculation and gain access to the systemic circulation.
3. The molecular size of the drug should be reasonable that it should be absorbed percutaneously.
4. An adhesive may not adhere well to all types of skin.
5. May not be economical.
6. Poor yield.
7. Skin irritation or dermatitis due to excipients and enhancers of drug delivery systems.

8. Loss of product during transfer from organic to water media.

### **TYPES OF ETHOSOMES:**

On the basis of their chemical composition ethosomes can be classified into three main types.

#### **Classical Ethosomes:**

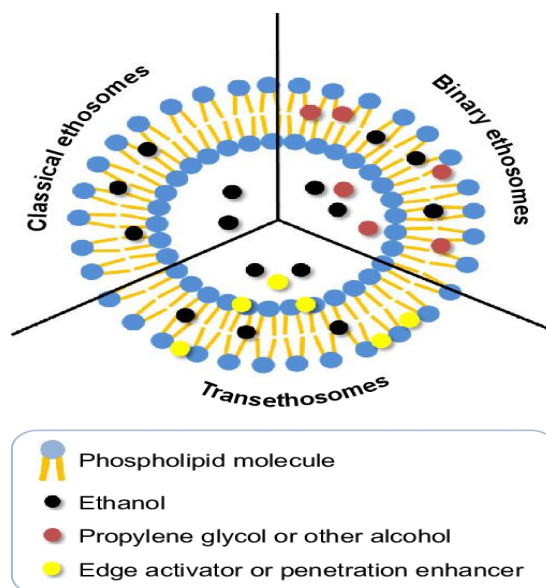
They are slight modifications of the conventional liposomes first prepared by Touitou et al. composed of Phospholipids and a relatively high concentration of ethanol (up to 45%). The ethosomes has been found to be superior to liposomes in possessing smaller size, more negative zeta potential, higher entrapment efficiency, better transdermal flux and higher stability.

#### **Binary ethosomes:**

They were first described by Zhou et al. They are just simple ethosomes the only difference between them and classical ethosomes is the type of alcohol, in classical ethosomes ethanol is the only alcohol used but in binary ethosomes alcohol used are propylene glycol (PG) and isopropyl alcohol (IPA). Zhou et al. observed that entrapment efficiency of sophoridine, matrine, sophocarpine, and lehmanine extracted from *Sophora alopecuroides* increased to a significant extent when PG was incorporated into the ethosomal system with a ratio of ethanol to PG of 1:1 (total alcohol up to 45%).

#### **Transethosomes:**

Transethosomes are a new generation of vesicular ethosomal systems first described by Song et al. in 2012 for efficient delivery of the drug across skin as they combine the advantages of classical ethosomes and deformable liposomes (transferosomes). They are ethosomes with an edge activator (surfactant) including tweens and span these activators. Therefore, in these systems the alcohol and edge activators act together to increase the vesicular malleability and lipid perturbation. Transethosomes has been proved to be better than other vesicular carriers in many studies. Garg et al. on comparison found that the optimized transethosomal formulation possessed highest entrapment, elasticity, improved stability and highest drug permeation as compared to other vesicular gel formulations.<sup>19</sup>



### ADVANTAGES OF HIGH ETHANOL CONTENT:

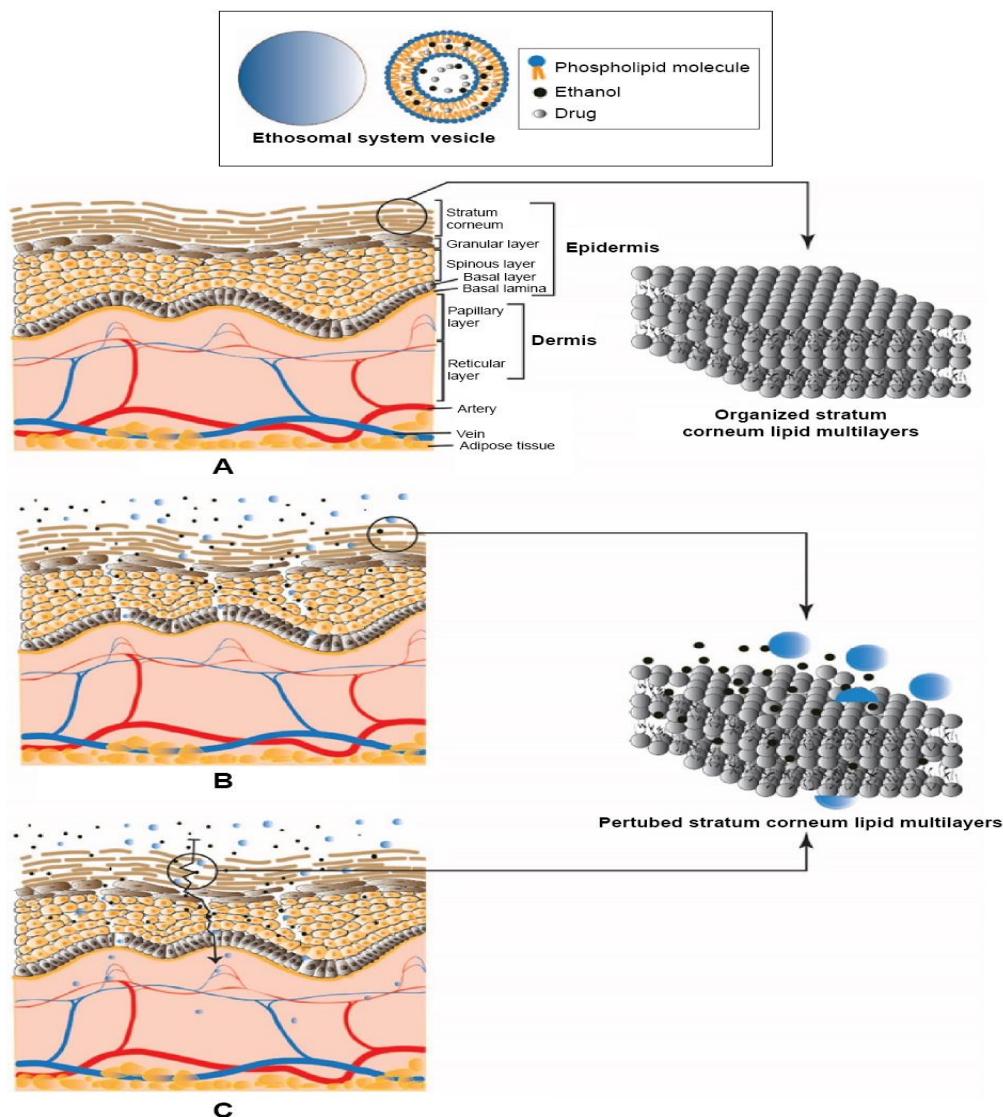
Ethosomes contain a very high concentration of ethanol (20-50%) which is an established permeation enhancer. However, due to disruption effect of ethanol on lipid bilayers, it was once thought that vesicles could not coexist with the high concentration of ethanol. Touitou discovered and investigated vesicular lipid systems embodying ethanol in relatively high concentration and named them ethosomes. The main difference between liposomes and ethosomes exists in their composition. The synergistic effect of the combination of the relatively high concentration of ethanol (20- 50%) in vesicular form in ethosomes was proposed to be the main reason for their better skin permeation ability. The high concentration of ethanol (20-50%) in ethosomal formulation could disturb the skin lipid bilayer organization. Therefore, when integrated into a vesicle membrane, it could give an ability to the vesicles to penetrate the SC. Furthermore, due to high ethanol concentration, the ethosomal lipid membrane was packed less tightly than conventional vesicles but possessed equivalent stability. Thus allowing it a softer and malleable structure giving more freedom and stability to its membrane. In addition, the vesicular nature of ethosomal formulations could be modified by varying the ratio of components and chemical structure of the phospholipids. The high concentration of ethanol imparts high entrapment efficiency. In terms of stability, the liposomes tends to fuse and grow into bigger vesicles due to the absence of electrostatic repulsion on neutral liposomes and this fusion and breakage cause drug leakage but in case of ethosomes ethanol causes a modification of net charge of the system and confers it some degree of steric stabilization leading to an increased stability against agglomeration that may lead to a decrease in mean vesicle size.

### MECHANISM OF DRUG PENETRATION:

The basic advantage of ethosomes over liposomes is the increase in permeation of drug. The mechanism of penetration of the ethosomes occurs in following two phases:

**1. Ethanol effect:** The first mechanism, ethosomal formulations contain ethanol in their composition that interacts with intercellular lipid molecules in the polar head group region, thereby increasing their fluidity and decreasing the density of the lipid multilayer, which results in an increase in membrane permeability.

**2. Ethosomes effect:** The high alcohol content is expected to result in increased skin permeability. So the ethosomes permeate very easily inside the deep skin layers, where they get combined with skin lipids and release the drugs into the deep layer of skin.



**Figure 5** Proposed mechanism for permeation of molecules from ethosomal system through the skin.  
**Notes:** (A) Normal skin; (B) Skin-lipid perturbation by ethanol effects; (C) Penetration of the soft malleable ethosomal system vesicles.

## METHODS OF PREPARATION OF ETHOSOMES:

Ethosomes can be prepared by either of the very simple and convenient methods:

- ❖ Cold method
- ❖ Hot method
- ❖ Ethanol injection method

- ❖ Mechanical Dispersion method
- ❖ Reverse phase evaporation method

### 1. Cold method:

This is the most common method employed for the preparation of ethosomal formulation. In this method phospholipid, drug and other lipid materials are dissolved in ethanol in a closed vessel at room temperature by vigorous stirring with the use of a mixer. Propylene glycol or another polyol is added during stirring. This mixture is heated to 30°C in a water bath. The water heated to 30°C in a separate vessel is added to the mixture in a fine stream with constant stirring in a covered vessel. The vesicle size of the ethosomal formulation can be reduced to the desired extent using sonication or extrusion method. Finally, the formulation is stored under refrigeration.

### 2. Hot method:

This method was first used by Touitou in 1996 in this method phospholipid is dispersed in water by heating it in a water bath at 40°C until a colloidal solution is obtained. In a separate vessel, ethanol and propylene glycol are mixed and heated to 40°C. Once both mixtures reach 40°C, the organic phase is added to the aqueous phase. The drug is dissolved in ethanol or water based on its hydrophilic/ hydrophobic properties. The vesicle size of the ethosomal formulation can be modified to the desired extent using probe sonication or extrusion method.

### 3. Ethanol Injection-sonication method:

This method is a slight alteration of the process described by Touitou et al.. In this method, lipids are dissolved in ethanol in a glass bottle, and the drug was independently dissolved in water and is filled in a syringe. The syringe is hermetically attached to the flask, and the aqueous phase was added to the organic phase in a fine stream with constant stirring.

### 4. Mechanical dispersion method:

In this method, the lipid is dissolved in a mixture of chloroform: ethanol (3:1) in round bottom flask. The organic solvents are extracted using rotary vacuum evaporator above the lipid transition temperature to form a thin lipid film on the wall of the flask. Finally, traces of solvent mixture are removed from the deposited lipid film by leaving the contents under vacuum overnight followed by hydration with different concentration of the hydroethanolic solution of the drug by rotation at the corresponding temperature.

### 5. Reverse phase evaporation method:

This is the least used method and especially used to produce large unilamellar vesicles. The organic is prepared by dissolving the phospholipid in diethyl ether and then mixing it with the aqueous phase at a ratio of 3:1 v/v in an ultrasonic bath at 0°C for 5 minutes to form water in oil emulsion. The organic solvent is removed under

reduced pressure to produce a gel, which turns into colloidal dispersion upon vigorous mechanical agitation.

## CHARACTERISATION OF ETHOSOMES

**1. Visualization or vehicle shape** - Visualization of ethosomes can be done using transmission electron microscopy (TEM) and by scanning electron microscopy (SEM).

**Scanning electron microscope (SEM)** uses a focused beam of high-energy electrons to generate a variety of signals at the surface of solid specimens. The signals that derive from electron sample interactions reveal information about the sample including external morphology (texture), chemical composition, and crystalline structure and orientation of materials making up the sample. Samples were prepared by mounting the dispersion to silver studs with carbon conductive double-sided tape. The samples were coated with a gold film under vacuum for 2 minute. The specimens were transferred to an ISI ABT SX40A scanning electron microscope and digital images were captured.

**High resolution – transmission electron microscopy** - TEM observations were performed to know the morphology of liquid crystals formulations following negative staining with sodium phosphotungstate solutio(0.2%w/v). A thin film was made on a carbon – coated copper grid by placing a drop of liquid crystals dispersion. Before the film was dried on the grid, it was negatively stained with phosphotungstic acid by adding a drop of the staining solution to the film, any excess solution was drained off with a filter paper. The grid was allowed to air dry, and samples can be viewd under transmission electron microscope.

**2. Vesicle size analysis** - The particle size of Ethosome was determined by dynamic light scattering technique using NanoPlus zeta/Nano particle analyser (Particulate system).

**3. Differential scanning calorimetry (DSC)** - Transition temperature (T<sub>m</sub>) of the vesicular lipid systems was determined by using the differential scanning calorimetry instrument.

**4. Surface Tension Activity Measurement** - The surface tension activity of drug in aqueous solution can be measured by the ring method with the help of tensiometer.

**5. Entrapment efficiency** - Eor determining entrapment efficiency, the ethosomes from the resulting dispersions were first separated by centrifuge. The separation of the free (non-entrapped) drug from the entrapped drug in the ethosome dispersion was achieved by centrifugation at 8000 rpm for 3 hours. The resulting solution was then separated and supernatant liquid was collected. The collected supernatant was then diluted appropriately and estimated using UV visible spectrophotometer at 362nm.

**6. Vesicle skin interaction study** - The vesicle skin interaction study can be found by using Confocal Laser scanning microscopy, Fluorescence microscopy, Transmission election microscopy and Eosin-Hematoxylin staining. Confocal laser scanning microscopy.

**7. Phospholipid-ethanol interaction** - This factor can be determined by 31P NMR Differential Scanning Calorimeter.

**8. Zeta potential** - Zeta potential was used to determine the surface charge which is important for predicting the long term stability of the colloidal dispersion. Zeta potential can be determined by dynamic light scattering technique using Malvern Instruments (Zetasizer).

**9. Turbidity** - To find out turbidity Nephelometer is used.

**10. In vitro drug release study** - To find out release study either Franz diffusion cell with artificial or biological membrane or Dialysis bag diffusion method can be used.

**11. Stability study** - Stability study can be carried out at two different temperature i.e. refrigeration temperature ( $4 \pm 2^{\circ}\text{C}$ ) and at room temperature ( $27 \pm 2^{\circ}\text{C}$ ) for 3 months. The formulation subjected for stability study was stored in borosilicate container to avoid any interaction between the ethosomal preparation and glass of container, which may affect the observations .

#### 1.2.9 THERAPEUTIC APPLICATIONS OF ETHOSOMES:

- 1. Pilosebaceous targeting:** Pilosebaceous units has been interestingly used particularly for the treatment of follicle-related disorders acne or alopecia.
- 2 Transdermal Delivery of Hormones:** Touitou et al (2000) investigated the efficiency of ethosome carriers for transdermal delivery of testosterone hormone comparing the skin permeation potential of ethosomal formulation of testosterone (Testosome) across rabbit pinna skin with marketed transdermal patch of testosterone (Testoderm patch). They observed nearly 30 times higher skin permeation of testosterone from ethosomal formulation as compared to that of marketed formulation.
- 3 Delivery of anti-parkinsonism agent:** Dayan and Touitou et al, 2002 prepared ethosomal formulation of psychoactive drug trihexyphenidyl·HCl (THP) used in treatment of Parkinson disease and compared its delivery with that from classical liposomal formulation. The value of transdermal flux quantity of drug, skin retention and stability was found to be superior to that of conventional liposomes.
- 4 Transcellular Delivery:** Touitou et al. in their study demonstrated better intracellular uptake of bacitracin, DNA and erythromycin using CLSM and FACS techniques in different cell lines. Better cellular uptake of anti-HIV drug zidovudine and lamivudine in MT-2 cell line from ethosomes as compared to the marketed formulation suggested ethosomes to be an attractive clinical alternative for anti-HIV therapy.
- 5 Topical Delivery of DNA:** Ethosomes is used for topical delivery of DNA molecules to express genes in skin cells. Touitou et al. in their study encapsulated the GFP-CMV-driven transfecting construct into ethosomal formulation and applied topically to the 5 week male mice.
- 6 Delivery of Anti-Arthritis Drug:** Cannabidiol (CBD) is a recently developed drug candidate for treating rheumatoid arthritis. Lodzki et al. prepared CBD ethosomal formulation for transdermal delivery. Results shows significantly increased in biological anti-inflammatory activity of CBD ethosomal formulation when tested by carrageenan induced rat paw oedema model.

- 7 **Delivery of Antibiotics:** Ethosomes penetrate rapidly through the epidermis and bring appreciable amount of drugs into the deeper layer of skin and suppress infection at their roots. Bacitracin and erythromycin loaded ethosomal formulation for dermal and intracellular delivery were developed and the studies indicated the penetration of ethosomes into the cellular membrane and released the entrapped drug molecules within the cells.
- 8 **Treatment of herpetic infection:** 5% acyclovir ethosomal preparation compared to the 5 % acyclovir cream showed significant improvements in treatment of herpetic infections.
- 9 **Delivery of Anti-Viral Drugs:** Jain et al., concluded that ethosomes could increase the transdermal flux, prolong the release and present an attractive route for sustained delivery of zidovudine.
- 10 **Used as bronchodilator:** Mina et al, formulated ethosomal formulation of salbutamol sulphate (SS); a hydrophilic drug used as bronchodilator, and compared its transdermal delivery potential with classic liposomes containing different cholesterol and dicetylphosphate concentrations. Study showed a significant decrease in vesicle size by decreasing cholesterol concentration and increasing dicetylphosphate and ethanol concentrations.
- 11 **Used in angina pectoris:** Ligustrazine plays a role in expanding blood vessels, increasing coronary and cerebral blood flow, preventing platelet aggregation, inhibiting thrombosis, and improving the microcirculation.
- 12 **Delivery of NSAIDs agent:** Vivek Dave et al formulated ethosomes of aceclofenac. The potential of ethosomes for delivering etodolace, a potent, water insoluble non-steroidal anti-inflammatory drug via skin to enhance skin permeation after topical application was developed by Bhale Shweta et al.

## CONCLUSION

Novel drug delivery system have revolutionised the methods of medication to provide better therapeutic efficacy. Transdermal drug delivery offers high patient compliance. Ethosomes is one of the better option for successful drug delivery to the affected areas of the skin that can be transported through channel like pores. Ethosomes help in prolonged release of medicament and enhancement in bioavailability and reduction in dose. Thus Ethosomal drug delivery method open new doors for the development of drug therapy.

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