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ETHOSOMES AS ELASTIC VESICLES IN TRANSDERMAL DRUG DELIVERY: AN OVERVIEW

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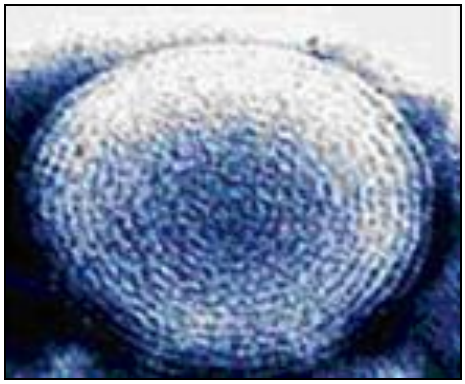
ABSTRACT

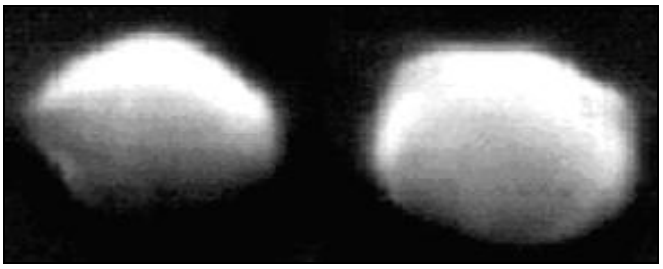
Ethosomes are as novel vesicles in transdermal drug delivery show significant effects of drug penetration through the biological membrane with slight modification of well established drug carrier liposomes. Ethosomes are soft, malleable vesicles composed mainly of phospholipids, ethanol and water. The size of ethosome vesicles can be modulated from tens of nanometer to microns. The ethosomes can be prepared by Hot as well as Cold method. The evaluation parameters of ethosomes include visualization, vesicle size and zeta potential, transition temperature, surface tension activity measurement, vesicle stability, drug content, penetration and permeation studies. Ethosomes have been found to be much more efficient at delivering drug to the skin than either liposomes or hydroalcoholic solution. Thus, it can be a logical conclusion that ethosomal formulation possesses promising future in effective dermal/transdermal delivery of bioactive agents.

INTRODUCTION: Transdermal administration of drugs is generally limited by the barrier function of the skin. Vesicular systems are one of the most controversial methods for transdermal delivery of active substances [1]. The interest in designing transdermal delivery systems was relaunched after the discovery of elastic vesicles: Ethosomes. Ethosomes are novel carrier system used for delivery of drugs having low penetration through the biological membrane mainly skin. Ethosomes are the slight modification of well established drug carrier liposomes [2].

Ethosomes: Ethosomes are lipid vesicles containing phospholipids, alcohol (ethanol and isopropyl alcohol) in relatively high concentration and water[3]. Ethosomes are soft vesicles made of phospholipids and ethanol (in higher quantity) and water. The size range of ethosomes may vary from tens of nanometers to microns (μ) [4]. Ethosomes permeate through the skin layers more rapidly and possess significantly higher transdermal flux in comparison to conventional liposomes [4, 5]. Visualization of ethosomes is shown in **Figure 1**. Although, the exact mechanism for better permeation into deeper skin layers from ethosomes is still not clear. 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 bi-layers.



VISUALIAZATION OF ETHOSOME (TEM MAGNIFICATION: 315000)



VISUALIAZATION OF ETHOSOME (SEM X 100, 000)

FIGURE 1: VISUALIZATION OF ETHOSOMAL VESICLES

Composition: The ethosomes are vesicular carrier comprise of hydroalcoholic or hydro/alcoholic/glycolic phospholipid in which the concentration of alcohols or their combination is relatively high. Typically, ethosomes may contain phospholipids with various chemical structures like phosphatidylcholine (PC), hydrogenated PC, phosphatidic acid (PA), phosphatidylserine (PS), phosphatidylethanolamine (PE), phosphatidylglycerol (PPG), phosphatidylinositol (PI), hydrogenated PC, alcohol (ethanol or isopropyl alcohol), water and and propylene glycol (or other glycols) [6, 7].

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. Some preferred phospholipids are soya phospholipids such as Phospholipon 90 (PL-90). It is usually employed in a range of 0.5-10% w/w. Cholesterol at concentrations ranging between 0.1-1 percent can also be added to the preparation[1]. Examples of alcohols, which can be used, include ethanol and isopropyl alcohol. Among glycols, propylene glycol and Transcutol are generally used.

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. The concentration of alcohol in the final product may range from 20 to 50%. The concentration of the non-aqueous phase (alcohol and glycol combination) may range between 22 to 70% [8, 9]. Different additives used in the ethosomal formulation are presented in the **table 1**.

TABLE 1: TABULAR FORM REPRESENTS DIFFERENT ADDITIVES USED IN THE ETHOSOMAL FORMULATION

Class	Examples	Uses
Phospholopids	Soya phosphatidyl choline; Egg phosphatidyl choline; Diestearyl phopshatidyl choline	Vesicle forming components
Polyglcerol	Propylene glycol; Transcutol RTM	As a skin penetration enhancer
Alcohol	Ethanol; Isopropyl alcohol	For providing the softness for vesicle membrane; As a skin penetration enhancer
Cholesterol	Cholesterol	For providing the stability for vesicle membrane
Dyes	Rhodamine 123; Rhodamine red	For characterization studies
Vehicles	Carbopol D934	As a gel former

Advantages of Ethosomal Drug Delivery [3, 10, 11]: In comparison to other transdermal & dermal delivery system;

- Ethosomes have enhanced permeation of drug through skin for transdermal drug delivery.
- The delivery of large molecules (peptides, protein molecule) is possible.
- It contains non-toxic raw material in formulation.
- High patient compliance- The ethosomal drug is administrated in semisolid form (gel or cream) hence producing high patient compliance.
- The Ethosomal system is passive, non-invasive and is available for immediate commercialization.
- Ethosomal drug delivery system can be applied widely in Pharmaceutical, Veterinary, Cosmetic fields.
- Simple method for drug delivery in comparison to Iontophoresis and Phonophoresis and other complicated methods.

Mechanism of Drug Penetration [12]: The main advantage of ethosomes over liposomes is the increased permeation of the drug. The mechanism of the drug absorption from ethosomes is not clear. The drug absorption probably occurs in following two phases [13, 14] (**Figure 2**):

1. **Ethanol Effect:** Ethanol acts as a penetration enhancer through the skin. The mechanism of its penetration enhancing effect is well known. Ethanol penetrates into intercellular lipids and increases the fluidity of cell membrane lipids and decrease the density of lipid multilayer of cell membrane.

2. **Ethosomal Effect:** Increased cell membrane lipid fluidity caused by the ethanol of ethosomes results increased skin permeability. So the ethosomes permeates very easily inside the deep skin layers, where it got fused with skin lipids and releases the drugs into deep layer of skin.

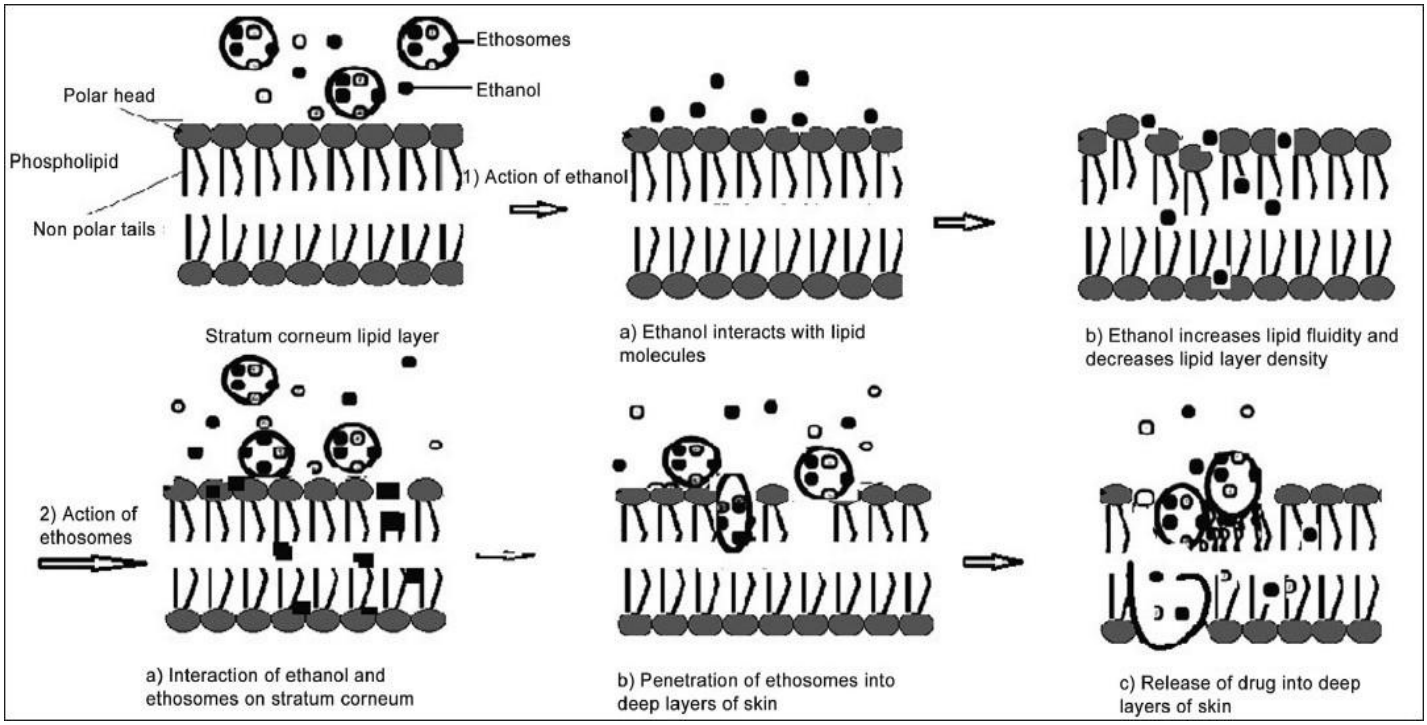


FIGURE 2: DIAGRAMMATICALLY REPRESENTATION OF MECHANISM OF ACTION OF ETHOSOMES

Method of Preparation [4, 8, 15]: There are two methods which can be used for the formulation and preparation of ethosomes. Both of the methods are very simple and convenient and do not involve any sophisticated instrument or complicated process.

Ethosomes can be formulated by following two methods: The formulation of ethosomes involves hot and cold method (**see table 2**)

1. **Hot Method:** In this method disperse phospholipid in water by heating in a water bath at 400°C until a colloidal solution is obtained. In a separate vessel properly mix ethanol and propylene glycol and heat up to 400°C. Add the organic phase into the aqueous phase. Dissolve the drug in water or ethanol depending on its solubility. The vesicle size of ethosomal formulation can be decreased to the desire extent using probe sonication or extrusion method.

2. **Cold Method:** This is the most common and widely used method for the ethosomal preparation. Dissolve phospholipids, drug and other lipid materials in ethanol in a covered vessel at room temperature with vigorous stirring. Add propylene glycol or other polyglycol during stirring. Heat the mixture up to 300°C in a water bath. Heat the water up to 300°C in a separate vessel and add to the mixture and then stir it for 5 min in a covered vessel. The vesicle size of ethosomal formulation can be decreased to desire extend using sonication or extrusion method. Finally, the formulation should be properly stored under refrigeration.

TABLE 2: FLOW CHART REPRESENTATION OF HOT METHOD AND COLD METHOD

HOT METHOD	COLD METHOD
Phospholipids is dispersed in water by heating in water bath at 40C	Phospholipids + Drug
⇩	⇩
Colloidal solution is obtained	Dissolve in ethanol
⇩	⇩
In a separate vessel ethanol and propylene glycol are mixed and heated to 40°C	Add propylene glycol
⇩	⇩
Organic phase is added to aqueous phase	Mixture is heated to 30°C± 1°C
⇩	⇩
Drug is dissolved in water or ethanol based on hydrophilic properties	Double distilled water is added with constant stirring for 5 minutes
⇩	⇩
Vesicle size is controlled by sonication or extrusion method	Vesicle size is controlled by using sonication and extrusion method
	⇩
	Formation is stored in refrigerator

Evaluation: The methods of evaluation for ethosomes are discussed below:

- **Vesicle Shape** [16]**:** Ethosomes can be easily visualized by using transmission electron microscopy (TEM) and by scanning electron microscopy (SEM).
- **Vesicle Size and Zeta Potential** [15]**:** Particle size of the ethosomes can be determined by dynamic light scattering (DLS) and photon correlation spectroscopy (PCS). Zeta potential of the formulation can be measured by Zeta meter.
- **Transition Temperature** [14]**:** The transition temperature of the vesicular lipid systems can be determined by using differential scanning calorimetry (DSC).
- **Drug Entrapment** [17]**:** The entrapment efficiency of ethosomes can be measured by the ultracentrifugation technique.
- **Drug Content** [18]**:** Drug content of the ethosomes can be determined using UV spectrophotometer. This can also be quantified by a modified high performance liquid chromatographic method.
- **Surface Tension Measurement** [19]**:** The surface tension activity of drug in aqueous solution can be measured by the ring method in a Du Nouy ring tensiometer.
- **Stability Studies** [20]**:** The stability of vesicles can be determined by assessing the size and structure of the vesicles over time. Mean size is measured by DLS and structure changes are observed by TEM.
- **Skin Permeation Studies** [13]**:** The ability of the ethosomal preparation to penetrate into the skin layers can be determined by using confocal laser scanning microscopy (CLSM).

Evaluation parameters and instrument/methods used in Ethosomes are shown in **table 3**.

TABLE 3: EVALUATION PARAMETERS AND INSTRUMENT/METHODS USED IN ETHOSOMES

PARAMETERS	INSTRUMENTS/METHODS USED	IMPORTANCE
Vesicle Shape	Transmission Electron Microscopy (TEM) Scanning Electron Microscopy (SEM)	Determines skin penetration
Vesicle Size and Zeta Potential	Dynamic Light Scattering (DLS), Photon Correlation Spectroscopy (PCS) and Zeta Meter	Determines skin penetration and stability of vesicles
Transition Temperature	Differential Scanning Calorimetry (DSC)	Determines transition temperature of lipid vesicles
Drug Entrapment	Ultracentrifugation Technique	Suitability of method
Drug Content	UV Spectrophotometer, High Performance Liquid Chromatographic Method (HPLC)	Important in deciding the amount of vesicle preparation to be used
Surface Tension Measurement	Ring Method in a Du Nouy ring tensiometer	Determines surface tension activity of drug in aqueous solution
Stability Studies	Dynamic Light Scattering (DLS), Transmission Electron Microscopy (TEM)	To determine the shelf-life of vesicle formulation
Skin Permeation Studies	Confocal Laser Scanning Microscopy (CLSM)	Determines rate of drug transport through skin
In-vitro dissolution	Franz diffusion cell	Determines the drug release rate from vesicle

Ethosomes as a Drug Carrier: Ethosomes can be used for many purposes in drug delivery. Ethosomes are mainly used as replacement of liposomes. Ethosomes can be used for transdermal delivery of hydrophilic and impermeable drugs through the skin. Following drugs have been used with ethosomal carrier (**see Table 4**).

TABLE 4: DRUG INCORPORATED IN ETHOSOMAL CARRIER

NAME OF DRUG	DRUG INCORPORATED IN ETHOSOMAL CARRIER	USES
Acyclovir[21]	Improved skin permeation. Improved in pharmacodynamics profile. Improved in biological activity two to three times.	Treatment of Herpes labialis
Anti-HIV agents[22] (Zidovudine, Lamivudine)	Reduced drug toxicity. Prolonging drug action. Improved transdermal flux. Affected the normal histology of skin. Improved in biological activity two to three times.	Anti-HIV
Azelaic acid[23] **Ammonium glycyrrhizinate**[24]	Prolong drug release. Improved in biological anti-inflammatory activity. Improved dermal deposition exhibiting sustained release.	Treatment of various inflammatory based skin diseases
Bacitracin[25]	Increased bioavailability. Improved dermal deposition. Improved intracellular delivery.	Anti-bacterial
Cannabidol[26]	Improve bioavailability. Increased skin permeation. Improved GIT degradation.	Treatment of Rhematoid arthritis
Cyclosporin A[6]	Prolong drug action. Improved bioavailability. Improved skin deposition.	Treatment of inflammatory skin diseases
DNA[9]	Better expression of genes. Selective targeting to dermal cells.	Treatment of genetic disorders
Erythromycin[27]	Better cellular uptake	Anti-microbial
Fluconazole[7]	Better skin permeation	Treatment of Candidiasis
Insulin[8]	Provide control release. Significant decrease in blood glucose level.	Treatment of diabetes
Minoxidol[28]	Pilocebaceous targeting. Accumulation in skin increased significantly.	Treatment of baldness
Methotrexate[9]	Better skin permeation	Treatment of Proriasis
NSAIDs[9] (Diclofenac)	Selective delivery of drug to described side for prolong period of time	Analgesic and anti-inflammatory
Testosterone[29]	Improved oral bioavailability. Reduced side effects.	Steroidal hormone
Trihexyphenidyl hydrochloride[18]	Provide control release. Improved transdermal flux. Improved patient compliance.	Treatment of Parkineonis disease
Salbutamol Sulfate[5]	Enhanced drug delivery through skin	Anti-asthmatic

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