Ethosomes are the ethanolic phospholipid vesicles which are used mainly for transdermal delivery of drugs. Ethosomes are soft, malleable vesicles composed mainly of phospholipids, ethanol (relatively high concentration) and water. These “soft vesicles” represents novel vesicular carrier for enhanced delivery to/through skin. The size of Ethosomes vesicles can be modulated from tens of nanometers to microns. Ethosomes have higher penetration rate through the skin as compared to liposomes hence these can be used widely in place of liposomes. Ethosomes have become an area of research interest, because of its enhanced skin permeation, improved drug delivery, increased drug entrapment efficiency etc. Ethosomes are provides a number of important benefits including improving the drug's efficacy, enhancing patient compliance and comfort and reducing the total cost of treatment. This review mainly focus on the various aspects of ethosomes including their mechanism of penetration, preparation, composition, characterization, advantages, application and marketed product of ethosomes. The Ethosomes were found to be suitable for various applications within the pharmaceutical, biotechnology, veterinary, cosmetic, and nutraceutical markets.

**Key words:** Ethosomes, Phospholipid Vesicles, Penetration, Ethanol, Transdermal drug delivery

**Introduction**

Transdermal drug delivery offers many advantages as compared to traditional drug delivery systems, including oral and parenteral drug delivery system. Transdermal drug delivery uses the skin as an alternative route for the delivery of systemically acting drugs. This has the advantage that high concentrations of drugs can be localized at the site of action, reducing the systemic drug levels and therefore also reducing the systemic side effects. The ethosomes more advantages when compared to transdermal and dermal delivery. It delivers large molecules such as peptides, protein molecules. Simple method for drug delivery in comparison to Iontophoresis and Phonophoresis and other complicated methods. 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. High patient compliance as it is administrated in semisolid form (gel or cream) and various application in Pharmaceutical, Veterinary, Cosmetic field.

To improve the permeation of drugs through the skin various mechanisms have been investigated, including use of chemical or physical enhancers, such as iontophoresis, sonophoresis, etc. Liposomes, niosomes, transferosomes and ethosomes also have been reported to enhance permeability of drug through the stratum corneum barrier. Permeation enhancers increase the permeability of the skin, so that the drugs can cross through the skin easily. Unlike classic liposomes, that are known mainly to deliver drugs to the outer layers of skin, ethosomes can enhance permeation through the stratum corneum barrier. Ethosomes can entrap drug molecule with various physicochemical characteristics i.e. of hydrophilic, lipophilic, or amphiphilic.

Ethosomes are lipid vesicles containing phospholipids, alcohol (ethanol and isopropyl alcohol) in relatively high concentration and water. 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. Ethosomes permeate through the skin layers more rapidly and possess significantly higher transdermal flux in comparison to conventional liposomes. 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 bilayers.
Advantages of Ethosomal Drug Delivery

1. The Ethosomal system is passive, non-invasive and is available for immediate commercialization.
2. Delivery of large molecules (peptides, protein molecules) is possible.
4. Enhanced permeation of drug through skin for transdermal drug delivery.
5. It contains non-toxic raw material in formulation.
6. Ethosomal drug delivery system can be applied widely in Pharmaceutical, Veterinary, Cosmetic fields.
7. High patient compliance: The ethosomal drug is administrated in semisolid form (gel or cream) hence producing high patient compliance.

Ethosomes Composition and Preparation

Ethosomes may contain phospholipids with various chemical structures like phosphatidylcholine (PC), hydrogenated PC, phosphatidic acid (PA), phosphatidyl serine (PS), phosphatidyl ethanolamine (PE), phosphatidyl glycerol (PPG), phosphatidylinositol (PI), hydrogenated PC, alcohol (ethanol or isopropyl alcohol), water and propylene glycol (or other glycols). 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% can also be added to the preparation. 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% (Table 1). Ethosomes can be prepared from soybean phosphatidylcholine (Phospholipon 90), ethanol, drug and distilled water. Phospholipon 90 and drug should be dissolved in ethanol. Water has to be added in small quantities and the preparation mixed by mechanical stirring under controlled conditions.

<table>
<thead>
<tr>
<th>Class</th>
<th>Example</th>
<th>Uses</th>
</tr>
</thead>
<tbody>
<tr>
<td>Phospholipid</td>
<td>Soya phosphatidyl choline, Dipalmityl phosphatidyl choline, Egg phosphatidyl choline, Distearyl phosphatidyl choline</td>
<td>As Vesicles forming component</td>
</tr>
<tr>
<td>Polyglycol</td>
<td>Propylene glycol, Transcutol RTM</td>
<td>As a skin penetration enhancer</td>
</tr>
<tr>
<td>Cholesterol</td>
<td>Cholesterol</td>
<td>For providing the stability to vesicle membrane</td>
</tr>
<tr>
<td>Alcohol</td>
<td>Isopropyl alcohol, Ethanol</td>
<td>For providing the softness for vesicle membrane As a penetration enhancer</td>
</tr>
<tr>
<td>Vehicle</td>
<td>Carbopol D-934</td>
<td>As a gel former</td>
</tr>
<tr>
<td>Dye</td>
<td>Rhodamine red, Rhodamine-123, 6-Carboxy fluorescence, Fluorescence Isothiocynate (FITC)</td>
<td>For characterization study</td>
</tr>
</tbody>
</table>

Methods of Preparation Ethosomes

Ethosomes can be prepared by two very simple and convenient methods that is hot method and cold method

1. **Cold Method:**
   This is the most common method utilized for the preparation of ethosomal formulation. In this method phospholipid, drug and other lipid materials are dissolved in ethanol in a covered vessel at room temperature by vigorous stirring with the use of mixer. Propylene glycol or other polyol is added during stirring. This mixture is heated to 300°C in a water bath. The water heated to 300°C in a separate vessel is added to the mixture, which is then stirred 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 is stored under refrigeration.

2. **Hot method:**
   In this method phospholipid is dispersed in water by heating in a water bath at 400°C until a colloidal solution is obtained. In a separate vessel ethanol and propylene glycol are mixed and heated to 400°C. Once
both mixtures reach 400°C, the organic phase is added to the aqueous one. The drug is dissolved in water or ethanol depending on its hydrophilic/hydrophobic properties. The vesicle size of ethosomal formulation can be decreased to the desired extent using probe sonication or extrusion method\textsuperscript{10,11}.

**Characterizations of Ethosomes**

1. **Visualization**
   Visualization of ethosomes can be done using transmission electron microscopy (TEM) and by scanning electron microscopy (SEM)\textsuperscript{11}.

2. **Entrapment Efficiency**
   The entrapment efficiency of drug by ethosomes can be measured by the ultra centrifugation technique\textsuperscript{12}.

3. **Differential scanning calorimetry (DSC)**
   Transition temperature (Tm) of the vesicular lipid systems was determined by using the Mettler DSC 60 computerized with Mettler Toledo star software system (Mettler, Switzerland)\textsuperscript{12,13}. The transition temperature was measured by using the aluminium crucibles at a heating rate 10 degree/minute, within a temperature range from 20°C–300°C.

4. **Vesicle size and Zeta potential**
   Particle size and zeta potential can be determined by dynamic light scattering (DLS) using a computerized inspection system and photon correlation spectroscopy (PCS)\textsuperscript{14}.

5. **Surface Tension Activity Measurement**
   The surface tension activity of drug in aqueous solution can be measured by the ring method in a Du Nouy ring tensiometer\textsuperscript{11}.

6. **Penetration and Permeation Studies**
   Depth of penetration from ethosomes can be visualized by confocal laser scanning\textsuperscript{11}.

7. **Vesicle Stability**
   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\textsuperscript{11,13}.

8. **Drug Content**
   Drug can be quantified by a modified high performance liquid chromatographic method\textsuperscript{12,14}.

**Evaluation Tests**

1. **Skin Permeation Studies**
   The hair of test animals (rats) were carefully trimmed short (<2 mm) with a pair of scissors, and the abdominal skin was separated from the underlying connective tissue with a scalpel. The excised skin was placed on aluminium foil, and the dermal side of the skin was gently teased off for any adhering fat and/or subcutaneous tissue. The effective permeation area of the diffusion cell and receptor cell volume was 1.0 cm² and 10 mL, respectively. The temperature was maintained at 32°C ± 1°C. The receptor compartment contained PBS (10 mL of pH 6.5). Excised skin was mounted between the donor and the receptor compartment. Ethosomal formulation (1.0 mL) was applied to the epidermal surface of skin. Samples (0.5 mL) were withdrawn through the sampling port of the diffusion cell at 1-, 2-, 4-, 8-, 12-, 16-, 20-, and 24-hour time intervals and analyzed by high performance liquid chromatography (HPLC) assay\textsuperscript{11,15}.

2. **Stability Study**
   Stability of the vesicles was determined by storing the vesicles at 4°C ± 0.5°C. Vesicle size, zeta potential, and entrapment efficiency of the vesicles was measured after 180 days using the method described earlier\textsuperscript{15}.

3. **Filter Membrane-Vesicle Interaction Study by Scanning Electron Microscopy**
   Vesicle suspension (0.2 mL) was applied to filter membrane having a pore size of 50 nm and placed in diffusion cells. The upper side of the filter was exposed to the air, whereas the lower side was in contact with PBS (phosphate buffer saline solution), (pH 6.5). The filters were removed after 1 hour and prepared for SEM studies by fixation at 4°C in Karnovsky’s fixative overnight followed by dehydration with graded ethanol solutions (30%, 50%, 70%, 90%, 95%, and 100% vol/vol in water). Finally, filters were coated with gold and examined in SEM (Leica, Bensheim, Germany)\textsuperscript{11,15}.

4. **Drug Uptake Studies**
   The uptake of drug into MT-2 cells (1×10⁶ cells/mL) was performed in 24-well plates (Corning Inc) in which 100 µL RPMI medium was added. Cells were incubated with 100 µL of the drug solution in PBS (pH 7.4), ethosomal formulation, or marketed formulation, and then drug uptake was determined by analyzing the drug content by HPLC assay\textsuperscript{11,15}.

5. **Vesicle-Skin Interaction Study by TEM and SEM**
   From animals ultra thin sections were cut (Ultracut, Vienna, Austria), collected on formvar-coated grids and examined under transmission electron microscope. For SEM analysis, the sections of skin after dehydration were mounted on stubs using an adhesive tape and were coated with gold palladium alloy using a fine coat ion sputter coater. The sections were examined under scanning electron microscope\textsuperscript{11,16}.

6. **Vesicle-Skin Interaction Study by Fluorescence Microscopy**
   Fluorescence microscopy was carried according to the protocol used for TEM and SEM study. Paraffin blocks are used, were made, 5-µm thick sections were cut using microtome (Erma optical works, Tokyo,
Applications of Ethosomes

1. **Topical Delivery of DNA**

   Many environmental pathogens attempt to enter the body through the skin. Skin therefore, has evolved into an excellent protective barrier, which is also immunologically active and able to express the gene. On the basis of above facts another important application of ethosomes is to use them 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. They applied this formulation to the dorsal skin of 5-week male CD-1 nude mice for 48 hr. After 48 hr, treated skin was removed and penetration of green fluorescent protein (GFP) formulation was observed by CLSM. It was observed that topically applied ethosomes-GFP-CMV-driven transfecting construct enabled efficient delivery and expression of genes in skin cells. It was suggested that ethosomes could be used as carriers for gene therapy applications that require transient expression of genes. These results also showed the possibility of using ethosomes for effective transdermal immunization. Gupta et al. recently reported immunization potential using transfersomal formulation. Hence, better skin permeation ability of ethosomes opens the possibility of using these dosage forms for delivery of immunizing agents.

2. **Delivery of anti-parkinsonism agent**

   Dayan and Touitou prepared ethosomal formulation of psychoactive drug trihexyphenidyl hydrochloride (THP) and compared its delivery with that from classical liposomal formulation. THP is a M1 muscarinic receptors antagonist and used in the treatment of Parkinson disease. The results indicated better skin permeation potential of ethosomal-THP formulation and its use for better management of Parkinson disease.

3. **Transdermal Delivery of Hormones**

   Oral administration of hormones is associated with problems like high first pass metabolism, low oral bioavailability and several dose dependent side effects. The risk of failure of treatment is known to increase with each pill missed. Touitou et al. compared the skin permeation potential of testosterone Ethosomes (Testosome) across rabbit pinna skin with marketed transdermal patch of testosterone (Testoderm patch, Alza). They observed nearly 30-times higher skin permeation of testosterone from ethosomal formulation as compared to that marketed formulation.

4. **Delivery of Anti-Arthritis Drug**

   Topical delivery of anti-arthritis drug is a better option for its site-specific delivery and overcomes the problem associated with conventional oral therapy. Cannabidol (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 was observed when tested by carrageenan induced rat paw edema model. It was concluded encapsulation of CBD in ethosomes significantly increased its skin permeation, accumulation and hence it’s biological activity.

5. **Delivery of Anti-Viral Drugs**

   Zidovudine is a potent antiviral agent acting on acquired immunodeficiency virus. Oral administration of zidovudine is associated with strong side effects. Therefore, an adequate zero order delivery of zidovudine is desired to maintain expected anti-AIDS effect. Jain et al. concluded that ethosomes could increase the transdermal flux, prolong the release and present an attractive route for sustained delivery of zidovudine. Acyclovir is another anti-viral drug that widely used topically for treatment of Herpes labialis. The conventional marketed acyclovir external formulation is associated with poor skin penetration of hydrophilic acyclovir to dermal layer resulting in weak therapeutic efficiency. It is reported that the replication of virus takes place at the basal dermis. To overcome the problem associated with conventional topical preparation of acyclovir. Horwitz et al. formulated the acyclovir ethosomal formulation for dermal delivery. The results showed that shorter healing time and higher percentage of abortive lesions were observed when acyclovir was loaded into ethosomes.
6. Delivery of Antibiotics

Topical delivery of antibiotics is a better choice for increasing the therapeutic efficacy of these agents. Conventional oral therapy causes several allergic reactions along with several side effects. Conventional external preparations possess low permeability to deep skin layers and subdermal tissues [23]. Ethosomes can circumvent this problem by delivering sufficient quantity of antibiotic into deeper layers of skin. Ethosomes penetrate rapidly through the epidermis and bring appreciable amount of drugs into the deeper layer of skin and suppress infection at their root. With this purpose in mind Godin and Touitou prepared bacitracin and erythromycin loaded ethosomal formulation for dermal and intracellular delivery. The results of this study showed that the ethosomal formulation of antibiotic could be highly efficient and would overcome the problems associated with conventional therapy.

7. 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.

Ethosomes as a Carrier of Various Drug Molecules

<table>
<thead>
<tr>
<th>Drug</th>
<th>Applications</th>
<th>Remark</th>
</tr>
</thead>
<tbody>
<tr>
<td>Testosterone</td>
<td>Treatment of male hypogonodism</td>
<td>Enhance skin permeation</td>
</tr>
<tr>
<td>Zidovudine</td>
<td>Treatment of AIDS</td>
<td>Improved transdermal flux</td>
</tr>
<tr>
<td>Trihexyphenidyl</td>
<td>Treatment of Parkinsonian syndrome</td>
<td>Increased drug entrapment efficiency, reduced side effect &amp; constant systemic levels</td>
</tr>
<tr>
<td>HCl</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Acyclovir</td>
<td>Treatment of Herpetic infection</td>
<td>Improved drug delivery</td>
</tr>
<tr>
<td>Insulin</td>
<td>Treatment of Diabetes</td>
<td>Improved therapeutic efficacy of drug</td>
</tr>
<tr>
<td>Bacitracin</td>
<td>Treatment of dermal infections</td>
<td>Reduced drug toxicity</td>
</tr>
<tr>
<td>Cannabidol</td>
<td>Prevents inflammation and edema</td>
<td>Significant accumulation of the drug in the skin</td>
</tr>
<tr>
<td>Minodixil</td>
<td>Hair growth promotion effect</td>
<td>Higher skin retention</td>
</tr>
</tbody>
</table>

Discussion and Conclusion

Ethosomes provides a number of important benefits including improving the drug’s efficacy, enhancing patient compliance and comfort and reducing the total cost of treatment. Ethosomes are the non invasive drug delivery carriers that enable drugs to reach the deep skin layers finally delivering to the systemic circulation. Enhanced delivery of bioactive molecules through the skin and cellular membranes by means of an ethosomal carrier opens numerous challenges and opportunities for the research and future development of novel improved therapies.

References