ETHOSOMES: AN OVERVIEW

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Abstract
Ethosomes are soft, malleable vesicles and potential carrier for transportation of drugs. Ethosomes are characterized by simplicity in their preparation, safety and efficacy and can be tailored for enhanced skin permeation of active drugs. Ethosomes have been found to be much more efficient at delivering drug to the skin, than either liposomes or hydro alcoholic solution. Ethosomes have been tested to encapsulate hydrophilic drugs, cationic drugs, proteins and peptides. Ethosomal carrier opens new challenges and opportunities for the development of novel improved therapies.

Keywords: Ethosomes, Transdermal drug delivery, Ethosomal encapsulation, Ethosomes effect.

1. Introduction:
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 liposome. 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 (nm) to microns (µ) ethosomes permeate through the skin layers more rapidly and possess significantly higher transdermal flux. Ethosomes 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 delayed 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.\(^4\)

2. Advantages of Ethosomes\(^5\):

Although, the exact mechanism for comparison to other transdermal & dermal delivery systems:

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

3. Ethosomes Composition\(^6\):

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 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 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
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:

4. MECHANISM OF DRUG PENETRATION:

4.1. 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.

4.2. Ethosomes effect
Skin increased cell membrane lipid fluidity caused by the ethanol of ethosomes results increased permeability. So the ethosomes permeates very easily inside the deep skin layers, where it got fused with skin lipids.

5. Method for preparation of Ethosomes:

5.1. Cold Method
This is the most common method utilized for the preparation of ethosomal formulation. In this method phospholipids, 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.

5.2. 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 desire extent using probe sonication or extrusion method.

6. Characterizations of Ethosomes:

6.1. Visualization
Visualization of ethosomes can be done using transmission electron microscopy (TEM) and by scanning electron microscopy (SEM).
6.2. Vesicle size and Zeta potential\textsuperscript{12}

Particle size and zeta potential can be determined by dynamic light scattering (DLS) using a computerized inspection system and photon correlation spectroscopy (PCS).

6.3. Entrapment Efficiency\textsuperscript{13}

The entrapment efficiency of drug by ethosomes can be measured by the ultra centrifugation technique.

6.4. Transition Temperature \textsuperscript{14}

The transition temperature of the vesicular lipid systems can be determined by using differential scanning calorimetry.

6.5. Surface Tension Activity Measurement\textsuperscript{15}

The surface tension activity of drug in aqueous solution can be measured by the ring method in a Du Nouy ring tensiometer.

6.6. Vesicle Stability\textsuperscript{16}

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.

6.7. Penetration and Permeation Studies\textsuperscript{17}

Depth of penetration from ethosomes can be visualized by confocal laser scanning microscopy (CLSM).

7. Evaluation Tests:

7.1. Filter Membrane-Vesicle Interaction Study by Scanning Electron Microscopy\textsuperscript{19}

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).

7.2. 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 [30]. The effective permeation area of the diffusion cell and receptor cell volume was 1.0 cm\textsuperscript{2} 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.

7.3. 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.

7.4. 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.

7.5. 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, Japan) and examined under a fluorescence micro Cytotoxicity Assay

MT-2 cells (T-lymphoid cell lines) were propagated in Dulbecco’s modified Eagle medium (HIMEDIA, Mumbai, India) containing 10% fetal calf serum, 100 U/mL penicillin, 100 mg/mL streptomycin, and 2 mmol/L L-glutamine at 37°C under a 5% CO2 atmosphere. Cytotoxicity was expressed as the cytotoxic dose 50 (CD50) that induced a 50% reduction of absorbance at 540 nm.

7.6. 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.

7.7. HPLC Assay
The amount of drug permeated in the receptor compartment during in vitro skin permeation experiments and in MT-2 cell was determined by HPLC assay using methanol:distilled-water:acetonitrile (70:20:10 vol/vol) mixture as mobile phase delivered at 1 mL/min by LC 10-AT vp
pump (Shimadzu, Kyoto, Japan). A twenty-microliter injection was eluted in C-18 column (4.6×150 mm, Luna, 54, Shimadzu) at room temperature. The column eluent was monitored at 271 nm using SPD-M10A vp diode array UV detector[31]. The coefficient of variance (CV) for standard curve ranged from 1.0% to 2.3%, and the squared correlation coefficient was 0.9968.

7.8. Statistical Analysis
Statistical significance of all the data generated was tested by employing ANOVA followed by studentized range test. A confidence limit of $P < .05$ was fixed for interpretation of the results using the software PRISM (GraphPad, Version 2.01, San Diego, CA).

8. Applications of Ethosomes:

8.1. Pilosebaceous Targeting
Hair follicles and sebaceous glands are increasingly being recognized as potentially significant elements in the percutaneous drug delivery. Furthermore, considerable attention has also been focused on exploiting the follicles as transport shunts for systemic drug delivery. With the purpose of Pilosebaceous targeting, Maiden et al. prepared and evaluated minoxidil ethosomal formulation.

8.2. 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.

8.3. Delivery of anti-parkinsonism agent
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.

8.4. Transcellular Delivery
Ethosomes as compared to the marketed formulation suggested ethosomes to be an attractive clinical alternative for anti-HIV therapy.

8.5. 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.
Better skin permeation ability of ethosomes opens the possibility of using these dosage forms for delivery of immunizing agents.

8.6. 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.

8.7. 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.

9. Conclusion:

It can be easily concluded that ethosomes can provide better skin permeation than liposomes. The main limiting factor of transdermal drug delivery system i.e. epidermal barrier can be overcome by ethosomes to significant extent. Application of ethosomes provides the advantages such as improved permeation through skin and targeting to deeper skin layers for various skin diseases. Various hydrophilic drugs can be easily administered through transdermal route by ethosomal encapsulation. The ethosomal technology has a wide scope in drug delivery which is still to be explored.

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Table 1: Different Additives Employed In Formulation of Ethosomes

| Class       | Example                        | Uses                                      |
|-------------|--------------------------------|-------------------------------------------|
| Phospholipid| - Soya phosphatidyl choline    | Soya phosphatidyl choline                |
|             | - Egg phosphatidyl choline     | Egg phosphatidyl choline                 |
|             | - Dipalmityl phosphatidyl choline | Dipalmityl phosphatidyl choline |
| Polyglycol  | Polyglycol                     | As a enhancer                            |
| Alcohol     | Ethanol                        | For providing softness for vesicle membrane |
|             | Isopropyl alcohol              | As a penetration enhancer                |
| Cholesterol | Cholesterol                    | For providing the stability to vesicle membrane |
| Dye         | Carbopol D934                  | As a gel former                          |

Table 2: Ethosomes as a carrier of various drug molecules has been listed below-

| Drug                          | use              | Comments                                      |
|-------------------------------|------------------|-----------------------------------------------|
| Ammonium glycyrrhizinate      | Anti-inflammatory| Improved dermal deposition                    |
|                               |                  | Exhibiting sustained release                  |
|                               |                  | Improved biological anti-inflammatory activity |
| Compound         | Description                                      | Benefits                                                                 |
|------------------|--------------------------------------------------|--------------------------------------------------------------------------|
| Azelaic acid     | Azelaic acid                                    | Prolong drug release                                                     |
| Acyclovir        | Acyclovir                                        | Improved drug delivery, Increase skin permeation, Improved in biological activity, Improved in Pharmacodynamic profile |
| Bacitracin       | Treatment of dermal infections                   | Reduced drug toxicity, Improved dermal deposition, Improved dermal deposition, Improved intracellular delivery |
| Cannabidol       | Prevents inflammation and edema                  | Significant accumulation of the drug in the skin, Improved biological activity |
| DNA              | Better expression of genes, Selective targeting to dermal cells | Selective delivery of drug to desired side                               |
| Minoxidil        | Hair growth promotion effect                    | Higher skin retention                                                    |