ETHOSOMES: A NOVAL APPROACH FOR IMPROVED TRANSDERMAL DELIVERY AND AS A CARRIER SYSTEM FOR THE DELIVERY OF HIV DRUGS

Abhinav Shahi*

* Dept. of Pharmacy, Pranveer Singh Institute of Technology, Bhauti, Kanpur, UP, India.

ABSTRACT

The skin is one of the most extensive and readily accessible organs of the human body and the skin as a route of drug delivery can offer many advantages over traditional drug delivery systems. In transdermal drug delivery system the drug goes to the systemic circulation through the protective barrier i.e. Skin. Skin acts as a major target as well as a principal barrier for topical/transdermal drug delivery. One of the greatest disadvantages to transdermal drug delivery is the skin's low permeability that limits the number of drugs that can be delivered in this manner. Ethosomes as novel vesicles in transdermal drug delivery show significant effects on drug penetration through the biological membrane. This review article will focus on the various aspects of ethosomes including their mechanism of penetration, preparation, advantages, characterization, composition, application and marketed product of ethosomes.

Keywords: Ethosomes, Transdermal drug delivery, Ethanol, Vesicular Carriers, Phospholipid.

INTRODUCTION

About 74% of drugs are taken orally today and are found not to be as effective as desired, due to first pass metabolism of the drugs. To improve such characters transdermal drug delivery system (TDDS) was emerged. Drug delivery through the skin to achieve a systemic effect of a drug is commonly known as transdermal drug delivery and it differs from traditional topical drug delivery. Transdermal delivery of drugs offers many advantages as compared to
traditional drug delivery systems like patient acceptability, avoidance of gastrointestinal disturbances and first pass metabolism of the drug.

Other advantages of TDDS include, long-term duration (ranging from a few hours to one week), no interference with gastric and intestinal fluids, administration of drugs with a very short half-life, narrow therapeutic window, poor oral absorption, self administration is possible etc.\textsuperscript{1-2}

![Transdermal Patch](image1)

**Figure 1: Transdermal Patch**

The TDDS involves a patch, in which the drug permeates through various layers of skin via various pathways mainly through passive diffusion.

![Main routes of drug penetration](image2)

**Figure 2: Main routes of drug penetration.**

TDDS had various advantages over other drug delivery systems, but at the same time there were many problems that various researchers had to face while developing successful transdermal drug delivery system. The skin has three major tissue layers: the epidermis, the dermis and the hypodermis. Stratum corneum is the outermost layer of the epidermis. It consists of 10 to 25 layers of dead, elongated, fully keratinized corneocytes, which are embedded in a matrix of lipid bilayers. The stratum corneum plays a crucial role in barrier function for transdermal drug delivery. The stratum cornium is approximately 1000 times less permeable than other biological membranes & it is even more difficult for anything to penetrate to the deeper strata of skin.\textsuperscript{3-4}
To overcome this barrier, various methods have been investigated, including the use of chemical or physical enhancers such as sonophoresis, iontophoresis etc. Niosomes, liposomes and ethosomes also have the potential to overcome the skin barrier and thus have been reported to enhance permeability of drug through the stratum corneum barrier.

**Figure 3: Morphology of skin**

**ETHOSOMES AS A NOVEL CARRIER**
Ethosomes are noninvasive delivery carriers that enable drugs to reach the deep skin layers and/or the systemic circulation. Ethosome 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. These are soft, malleable vesicles tailored for enhanced delivery of active agents. The size range of ethosomes may vary from tens of nanometers to microns.\(^5\)-\(^6\)

**STRUCTURE OF ETHOSOMES\(^7\)**

**COMPOSITION OF ETHOSOMES**
Ethosomes are composed mainly of phospholipids, (phosphatidylcholine, phosph-
atidylserine, phosphatidic acid), high concentration of ethanol and water. The nonaqueous phase range between 22 % to 70 %. The alcohol may be ethanol or isopropyl alcohol. 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. 8-11

Table 1: Different Additives Employed In Formulation of Ethosomes

<table>
<thead>
<tr>
<th>Class</th>
<th>Example</th>
<th>Uses</th>
</tr>
</thead>
<tbody>
<tr>
<td>Phospholipid</td>
<td>Soya phosphatidyl choline</td>
<td>Vesicles forming component</td>
</tr>
<tr>
<td></td>
<td>Egg phosphatidyl choline</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Dipalmityl phosphatidyl choline</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Distearyl phosphatidyl choline</td>
<td></td>
</tr>
<tr>
<td>Polyglycol</td>
<td>Propylene glycol</td>
<td>As a skin penetration enhancer</td>
</tr>
<tr>
<td></td>
<td>Transcutol RTM</td>
<td></td>
</tr>
<tr>
<td>Alcohol</td>
<td>Ethanol</td>
<td>For providing the softness for</td>
</tr>
<tr>
<td></td>
<td>Isopropyl alcohol</td>
<td>vesicle membrane</td>
</tr>
<tr>
<td></td>
<td></td>
<td>As a penetration enhancer</td>
</tr>
<tr>
<td>Cholesterol</td>
<td>Cholesterol</td>
<td>For providing the stability to</td>
</tr>
<tr>
<td></td>
<td></td>
<td>vesicle membrane</td>
</tr>
<tr>
<td>Dye</td>
<td>Rhodamine-123</td>
<td>For characterization study</td>
</tr>
<tr>
<td></td>
<td>Rhodamine red</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Fluorescene Isothiocyanate (FITC)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>6-Carboxy fluorescence</td>
<td></td>
</tr>
<tr>
<td>Vehicle</td>
<td>Carbopol D934</td>
<td>As a gel former</td>
</tr>
</tbody>
</table>

ADVANTAGES OF ETHOSOMES

Ethosomes enhance the permeation of drug through skin for transdermal drug delivery. & delivery of large molecules (peptides, protein molecules) is possible. Ethosomal formulation generally contains non-toxic raw materials. Better stability and solubility of many drugs can be achieved in comparison to conventional vesicles. Ethosomes are relatively smaller in size as compared to conventional vesicles. High patient compliance can be achieved. The ethosomal drug is administrated in semisolid form (gel or cream) hence producing high patient compliance. Ethosomal drug delivery system can be applied widely in Pharmaceutical, Veterinary, Cosmetic fields. 12-13
LIMITATIONS OF ETHOSOMES
There are certain limitations to the use of ethosomes like poor yield, in case if shell locking is ineffective then the ethosomes may coalescence and fall apart on transfer into water & loss of product during transfer form organic to water media.\textsuperscript{14-15}

METHOD OF PREPARATION OF ETHOSOMES
Ethosomes can be prepared by the following two methods:\textsuperscript{16-18}
1. Hot method and
2. Cold method

1. HOT METHOD:
In this method disperse phospholipid in water by heating in a water bath at 40°C until a colloidal solution is obtained. In a separate vessel properly mix ethanol and propylene glycol and heat up to 40°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 30°C in a water bath. Heat the water up to 30°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.

![Flow chart representation of hot method and cold method](image-url)

Figure 5: Flow chart representation of hot method and cold method
CHARACTERIZATION OF ETHOSOMES\textsuperscript{19-25}

Table 2: Characterisation of ethosomes

<table>
<thead>
<tr>
<th>S.No</th>
<th>Parameter</th>
<th>Importance</th>
<th>Method</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Size and shape</td>
<td>Determine skin penetration</td>
<td>SEM, TEM, DLS</td>
</tr>
<tr>
<td>2</td>
<td>Zeta potential</td>
<td>Stability of vesicles</td>
<td>Zeta Meter</td>
</tr>
<tr>
<td>3</td>
<td>Entrapment efficiency</td>
<td>Important in deciding the amount of vesicle preparation to be used</td>
<td>Ultracentrifugation</td>
</tr>
<tr>
<td>4</td>
<td>Drug content</td>
<td>To determine the shelf life of vesicle formulation</td>
<td>UV, HPLC</td>
</tr>
<tr>
<td>5</td>
<td>Stability studies</td>
<td>Important in deciding the amount of vesicle formulation</td>
<td>SEM, TEM, HPLC</td>
</tr>
<tr>
<td>6</td>
<td>Invitro dissolution</td>
<td>Determine the drug release rate from vesicle</td>
<td>Franz diffusion cell</td>
</tr>
<tr>
<td>7</td>
<td>Skin permeation</td>
<td>Determines rate of drug transport through skin</td>
<td>CLSM</td>
</tr>
</tbody>
</table>

EVALUATION OF ETHOSOMES\textsuperscript{26-27}

1. Filter Membrane-Vesicle Interaction Study by Scanning Electron Microscopy

It involves application of vesicle suspension (0.2 mL) to filter membrane having a pore size of 50 nm and placing it in diffusion cells. The upper side of the filter was exposed to the air, whereas the lower side was in contact with phosphate buffer saline solution, (having pH 6.5). The filters were removed after 1 hour and were 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% v/v in water). Finally, filters were coated with gold and examined in SEM (Leica, Bensheim, Germany).

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. The effective permeation area of the diffusion cell and receptor cell volume was 1.0 cm\(^2\) and 10 mL, respectively. The temperature was maintained at 32°C ± 1°C. The receptor compartment contained phosphate buffer saline solution (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 & 24 hour time intervals and analyzed by high performance liquid chromatography assay.
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.

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.

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.

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 phosphate buffer saline solution (pH 7.4), ethosomal formulation, or marketed formulation, and then drug uptake was determined by analyzing the drug content by HPLC assay.

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 SPDM10A vp diode array UV detector. The coefficient of variance (CV) for standard curve ranged from 1.0% to 2.3%, and the squared correlation coefficient was 0.9968.

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).

MECHANISM OF DRUG PENETRATION
The main advantage of ethosomes over liposomes is the increased permeation of the drug. The drug absorption may occurs in following two phases:28-30

1. Ethanol effect
2. Ethosomes effect

1. Ethanol effect
Ethanol acts as a penetration enhancer through the skin. Ethanol penetrates into intercellular lipids and increases the fluidity of cell membrane lipids and decrease the density of lipid multilayer of cell membrane.

2. Ethosomes 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.

![Mechanism of penetration of ethosomal drug delivery system](image)

**Figure 6**: Mechanism of penetration of ethosomal drug delivery system
APPLICATION OF ETHOSOMES

The uses of ethosomes as carrier system for transdermal/topical drug delivery are summarized below.

In the treatment herpetic infection: 5% acyclovir ethosomal preparation when compared to the 5 % acyclovir cream showed significant improvements in the treatment of herpetic infections.

In pilosebaceous targeting: Ethosomes, the high ethanol containing vesicles are able to penetrate the deeper layers of the skin and hence appear to be vesicles of choice for transdermal drug delivery of hydrophilic and impermeable drugs through the skin. Pilosebaceous units have been use for localized therapy, particularly for the treatment of follicle related disorders such as acne or alopecia. Ethosomal formulation of minoxidil a lipid soluble drug used for baldness accumulate into nude mice skin two to seven fold higher and thus can be use for pilosebaceous targeting for better clinical efficacy.

In the 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

In the 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.

In the delivery of HIV drugs: An effective antiretroviral therapy is required on a long term basis and is associated with strong side effects. Adequate zero order delivery of zidovudine, Lamivudine a potent antiviral agent is required to maintain expected anti – AIDS effect.
Subheet Jain et al reported that ethosomal formulation of the above drugs prolong the release with increased transdermal flux. Conventional topical preparation acyclovir an topically used antiviral drug for treatment of herpes labials show low therapeutic efficiency due to poor permeation through skin as replication of virus take places at the basal dermis. Ethosomal formulation of acyclovir show high therapeutic efficiency with shorter healing time and higher percentage of abortive lesions.

**In Transdermal delivery:** As ethosomes enhance permeability of drug through stratum corneum barrier, it can be use for administration of drugs having poor skin permeation, low oral bioavability, first pass metabolism and dose skin and suppress infection at their root

**Table 3: Application of Ethosomes as a Drug Carrier**

<table>
<thead>
<tr>
<th>Drugs</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Anti-viral agents</strong></td>
<td></td>
</tr>
<tr>
<td>(Zidovudine)</td>
<td>Prolonged drug action, reduced drug toxicity.</td>
</tr>
<tr>
<td>(Lamivudine)</td>
<td>Control release for prolonged period of time.</td>
</tr>
<tr>
<td>(Stavudine)</td>
<td>Improved biological anti-inflammatory activity, sustained effect</td>
</tr>
<tr>
<td><strong>NSAIDS</strong></td>
<td></td>
</tr>
<tr>
<td>(Diclofenac)</td>
<td>Selective and prolong delivery of drug to desired site.</td>
</tr>
<tr>
<td>(Aceclofenac)</td>
<td>Superior to the marketed gel for the topical administration.</td>
</tr>
<tr>
<td><strong>Topical Photodynamic Therapy</strong></td>
<td></td>
</tr>
<tr>
<td>(PDT)</td>
<td>Greater penetration ability than that of liposomes, More entrapment efficiency</td>
</tr>
<tr>
<td>(5-aminolevulinic acid)</td>
<td></td>
</tr>
<tr>
<td><strong>Acyclovir</strong></td>
<td>Increased skin permeation and biological activity two to three times.</td>
</tr>
<tr>
<td><strong>Trihexyphenidyl Hydrochloride</strong></td>
<td>Higher entrapment capacity, improved transdermal flux, improved patient compliance.</td>
</tr>
<tr>
<td><strong>Insulin</strong></td>
<td>Significant decrease in blood glucose level.</td>
</tr>
<tr>
<td><strong>Antibiotic</strong></td>
<td></td>
</tr>
<tr>
<td>(Erythromycin)</td>
<td>Complete inhibition of infection, prolonged drug action.</td>
</tr>
<tr>
<td>(Cannabidol)</td>
<td>Improved skin deposition and biological activity.</td>
</tr>
<tr>
<td><strong>Ammonium Glycerrhizinate</strong></td>
<td>Improved biological anti-inflammatory activity, sustained effect.</td>
</tr>
<tr>
<td><strong>Pilosebaceous</strong></td>
<td></td>
</tr>
<tr>
<td>(Minoxidil)</td>
<td>High penetration into deep layers of the skin.</td>
</tr>
<tr>
<td><strong>Salbutamol sulfate</strong></td>
<td>Targeting</td>
</tr>
<tr>
<td><strong>Gold Nanopartical</strong></td>
<td>Controlled release rate, enhanced skin permeation.</td>
</tr>
<tr>
<td><strong>Propranolol</strong></td>
<td>Gold nanopartical in ethosomes shows enhancement of pharmacological efficacy in transdermal and dermal delivery systems.</td>
</tr>
<tr>
<td><strong>Finasteride</strong></td>
<td>Better skin permeation.</td>
</tr>
<tr>
<td><strong>Testosterone</strong></td>
<td>Enhanced percutaneous absorption.</td>
</tr>
<tr>
<td><strong>Bacitracin</strong></td>
<td>Significantly higher permeation into the skin increased systemically delivery</td>
</tr>
<tr>
<td><strong>Zinc</strong></td>
<td>Reduced drug toxicity.</td>
</tr>
</tbody>
</table>
PATENTED AND MARKETED FORMULATION OF ETHOSOME

Ethosome was invented and patented by Prof. Elka Touitou along with her students of department of Pharmaceutics at the Hebrew University School of Pharmacy. Novel Therapeutic Technologies Inc (NTT) of Hebrew University have been succeeded in bringing a number of products to the market based on ethosome delivery system. Noicellex TM an anti – cellulite formulation of ethosome is currently marketed in Japan. Lipoduction TM another formulation is currently used in treatment of cellulite containing pure grape seed extracts (antioxidant) is marketed in USA. Similarly Physonics is marketing anti – cellulite gel Skin Genuity in London. Nanominox© containing monoxidil is used as hair tonic to promote hair growth is marketed by Sinere.

CONCLUSION

Ethosomes are the non invasive drug delivery carriers that enable drugs to reach the deep skin layers finally delivering to the systemic circulation. Ethosomes are characterized by simplicity in their preparation, safety and efficacy and can be tailored for enhanced skin permeation of active drugs. It delivers large molecules such as peptides, protein molecules. Ethosomal drug delivery is simple in comparison to Iontophoresis and Phonophoresis and other complicated methods. High patient compliance is achieved as it is administrated in semisolid form (gel or cream). Ethosomal carrier have opened new challenges and opportunities for the development of novel improved therapies.

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34. Touitou E. Composition of applying active substance to or through the skin. US Patent: 5716638, 1996.