ETHOSOMES- A NEW TRENDS IN VESICULAR APPROACHES FOR TOPICAL DRUG DELIVERY

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ABSTRACT
Skin acts as a major target as well as a principal barrier for topical/transdermal drug delivery. Despite the many advantages of this system, the major obstacle is the low diffusion rate of drugs across the stratum corneum. One simple and convenient approach is application of drugs in formulation with elastic vesicles or skin enhancers. 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 method, Cold method and optimized method. The evaluation parameters of ethosomes include visualization, vesicle size and zeta potential, entrapment efficiency. Ethosomes have been found to be much more efficient at delivering drug to the skin than either liposomes or hydroalcoholic solution. Hence it can be a effective dermal/transdermal delivery of bioactive agents.

KEYWORD
Ethosomes, Transdermal, Vesicular Carrier, Skin Permeation and Topical Delivery.

INTRODUCTION
Transdermal administration of drugs is generally limited by the barrier function of the skin. Vesicular systems are one of the most advanced methods for transdermal delivery of active substances1. Skin forms a protecting covering layer against the external environment and prevents water loss from the underlying tissue. It is flexible enough to resist permanent distortion from movement and thin enough to allow the perception of stimuli. It also
performs many ancillary functions such as synthesis and metabolism and the production of sweat enables temperature control and excretion of waste products by means of sweating etc. It has been also reported that skin protects the body from antigenic stimuli by means of a part of the immune system known as skin associated lymphoid tissue. The skin can be considered to be composed of three layers: subcutaneous tissue, dermis and epidermis layer. 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. It has been shown that the stratum corneum is the main barrier to penetration through the skin. When a topical formulation is placed on the skin, the active drug is required to penetrate through the stratum corneum into the viable tissue. The limiting factor for these processes is the slow diffusion through the dead horny layer of skin. Stratum corneum behaves as a hydrophobic membrane. The rates of permeation of skin by low and high molecular weight organic non-electrolytes are mostly determined within the stratum corneum.

The molecular structures and appearance of the molecules can be examined using molecular modelling computer programs. There have been many discussions on the route of penetration. Under normal conditions, the main route is observed through the intercellular spaces or lipid bilayers. The diffusional path length is therefore much longer than simple thickness of the stratum corneum (20-30 mm). The penetration through skin is also affected by several biological factors such as skin age, body site, skin condition and diseases, water content of the skin or hydration. The intercellular spaces contain structured lipids/proteins and a diffusing molecule has to cross a variety of lipophilic and hydrophilic domains before reaching to the stratum corneum and viable epidermis junction. Although the nature of the barrier is very heterogeneous, the diffusion through the skin can be described by simple Flick’s laws.

To overcome the stratum corneum barrier, various mechanisms have been investigated, including use of chemical or physical enhancers such as iontophoresis, sonophoresis, etc. Liposomes, niosomes, transfersomes and ethosomes also have the potential of overcoming the skin barrier and have been reported to enhance permeability of drug through the stratum corneum barrier. The non-invasive approaches for providing transdermal drug delivery of various therapeutic substances are,

**Vesicular Approaches for Topical Drug Delivery**

Drug encapsulated in lipid vesicles prepared from phospholipids and nonionic surfactants is known to be transported into and across the skin. Lipids present in the skin contribute to the barrier properties of skin and prevent systemic absorption of drugs. Due to the amphiphilic nature, lipid vesicles may serve as non-toxic penetration enhancer for drugs. In addition, vesicles can be used for encapsulating hydrophilic and lipophilic as well as low and high molecular weight drugs. Therefore, these lipid rich vesicles are hypothesized to carry significant quantity of drugs across the skin thus, enhancing the systemic absorption of drugs.

Drug delivery from liposomes in transdermal formulation has been studied for many purposes but unstable nature and poor skin permeation limits their use for topical delivery. In order to increase the stability of liposomes, the concept of proliposomes was proposed. This approach was extended to niosomes, which exhibited superior stability as compared to liposomes. However, due to poor skin permeability, liposomes and noisomes could not be successfully used for systemic drug delivery and their use was limited for topical use. To overcome problems of poor skin permeability Cevc et al. and Touitou et al. recently introduced two new vesicular carrier systems transfersomes and ethosomes, respectively for non-invasive delivery of drugs into or across the skin. Transfersomes’ and ethosomes incorporated edge activators (surfactants) and penetration enhancers (alcohols and polyols), respectively, to influence the properties of vesicles and stratum corneum. The vesicles have been well known for their importance in cellular communication and particle transportation for many years. Researchers have understood the properties of vesicles structure for use in better drug delivery.

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within their cavities, which would tag the vesicle for cell specificity. One of the major advances in vesicle research was the finding of a vesicle derivatives, known as an Ethosomes.

**Ethosomes as a Novel Carrier**

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, phosph-aticylserine, phosphatitidic 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.

**Structure of Ethosomes**

Ethosomes (Figure No.1) are soft, malleable vesicles composed mainly of phospholipids, ethanol (relatively high concentration) and water (Table No.1). These “soft vesicles” represents novel vesicular carrier for enhanced delivery to/through skin. The size of Ethosomes vesicles can be modulated from tens of nanometres to microns².

**Mechanism of Drug Penetration**

The enhanced delivery of actives using ethosomes over liposomes can be ascribed to an interaction between ethosomes and skin lipids. A possible mechanism for this interaction has been proposed. It is thought that the first part of the mechanism is due to the ‘ethanol effect whereby intercalation of the ethanol into intercellular lipids increasing lipid fluidity and decreases the density of the lipid multilayer. This is followed by the ‘ethosome effect’, which includes inter lipid penetration and permeation by the opening of new pathways due to the malleability and fusion of ethosomes with skin lipids, resulting in the release of the drug in deep layers of the skin, shown in Figure No.2.

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:

- Ethanol effect
- Ethosomes effect

**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.

**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.

**Advantages of Ethosomal Drug Delivery**³⁵

- Ethosomes are enhanced permeation of drug through skin for transdermal and dermal delivery.
- Ethosomes are platform for the delivery of large and diverse group of drugs (peptides, protein molecules).
- Low risk profile- The technology has no large-scale drug development risk since the toxicological profiles.
- High patient compliance- The ethosomal drug is administrated in semisolid form (gel or cream) hence producing high patient compliance.
- It contains non-toxic raw material in formulation.
- Simple method for drug delivery in comparison to Iontophoresis and Phonophoresis and other complicated methods.
- The Ethosomal system is passive, non-invasive and is available for immediate commercialization.
• Ethosome composition is safe and the components are approved for pharmaceutical and cosmetic use.
• Ethosomal drug delivery system can be applied widely in Pharmaceutical, Veterinary, Cosmetic fields.
• Enhanced permeation of drug molecules through the skin to the systemic circulation.
• Contrary to deformation liposomes, ethosomes improve skin delivery of drugs both under occlusive and non-occlusive condition.
• Better stability and solubility of many drugs as compared to conventional vesicles.
• Relatively smaller size as compared to conventional vesicles.

Disadvantages of Ethosomes\(^{2-7}\)
• Drugs that require high blood levels cannot be administered – limited only to potent molecules, those requiring a daily dose of 10mg or less.
• Ethosomal administration is not a means to achieve rapid bolus type drug input, rather it is usually designed to offer slow, sustained drug delivery.
• Adequate solubility of the drug in both lipophilic and aqueous environments to reach dermal microcirculation and gain access to the systemic circulation.
• The molecular size of the drug should be reasonable that it should be absorbed percutaneously.
• Adhesive may not adhere well to all types of skin. Uncomfortable to wear.
• May not be economical. Poor yield.
• Skin irritation or dermatitis due to excipients and enhancers of drug delivery systems.
• In case if shell locking is ineffective then the ethosomes may coalesce and fall apart on transfer into water.
• Loss of product during transfer from organic to water media.
• The main advantage of ethosomes over liposomes is the increased permeation of the drug.

Applications
Ethosomes are used in pilosabceeous 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 (Table No.2)\(^{10}\).

CHARACTERIZATIONS OF ETHOSOMES
Visualization
Visualization of ethosomes can be done using transmission electron Microscopy (TEM) and by scanning electron microscopy (TEM)\(^{6}\).

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)\(^{11}\).

Entrapment Efficiency
The entrapment efficiency of drug by ethosomes can be measured by the ultra centrifugation technique\(^{12}\).

METHODS OF PREPARATION
Cold Method
This is the most common and widely used method for the ethosomal preparation. Phospholipid, drug and other lipid materials were dissolved in ethanol in a covered vessel at room temperature with vigorous stirring. The mixture was heated at 30\(^{\circ}\)C in a water bath. Water was heated up-to 30\(^{\circ}\)C in a separate vessel and was added to the mixture and then stirred for 5 min. The vesicle size of ethosomal formulation was decreased to desire extent using sonication. Finally, the formulation was properly stored.

Hot Method
According to this method, phospholipid was dispersed in water by heating in a water bath at 40\(^{\circ}\)C until a colloidal solution is obtained. Ethanol, propylene glycol and drug was mixed in a separate vessel and heated up-to 40\(^{\circ}\)C. Organic phase was added to aqueous phase and stirred for 5 min. The vesicle size of ethosomal formulation was decreased to desire extent using sonication. Finally, the formulation was properly stored\(^{8}\).
Optimized Method
The ethosomal formulations were prepared by classic mechanical dispersion containing phospholipon 90(1-4%) and ethanol (10 to 40%). The drug concentration was fixed as 10 mg/ml or 1% w/w. Accurately weighed quantity of phospholipon 90 was dissolved in chloroform: methanol (3:1) mixture and organic solvents were removed in the rotary flash evaporator above the lipid transition temperature (55°C) (at 60rpm) to form a thin lipid film in the flask. The traces of organic solvents mixture were further removed by maintaining the temperature under reduced pressure for additional 30 minutes after the thin film was formed. Then the lipid film was hydrated with different concentration of hydroethanolic mixture containing (1% w/v) in the rotary flash evaporator at 60 rpm for 1hour in the room temperature. The preparation was vortexed followed by sonication at 4°C in an ice bath using probe sonicator at 40W in three cycles of five minutes with five minutes rest in between the cycles. After sonication the ethosomal formulation was stored in refrigerator (4°C) for further studies.

Factors affecting physical nature of ethosomes
There are some factors such as hydration temperature, choice of surfactant, nature of membrane, nature of drug, etc., can affect significantly the physical nature of ethosomes.
- Hydration temperature
- Choice of main surfactant
- Nature of drug
- Nature of membrane additives
- Size reduction techniques
- Addition of kinetic energy

Table No.1: Composition of ethosomes

<table>
<thead>
<tr>
<th>S.No</th>
<th>Materials</th>
<th>Examples</th>
<th>Uses</th>
</tr>
</thead>
</table>
| 1    | Phospholipid | Soya phosphatidyl choline  
Egg phosphatidyl choline  
Dipalmityl phosphatidyl choline  
Distearyl phosphatidyl choline | Vesicles forming component |
| 2    | Polyglycol | Propylene glycol  
Transcutol RTM | As a skin penetration enhancer |
| 3    | Alcohol    | Ethanol  
Isopropyl alcohol | For providing the softness for vesicle membrane  
As a penetration enhancer |
| 4    | Cholesterol | Cholesterol | For providing the stability to vesicle membrane |
| 5    | Dye        | Rhodamine-123  
Rhodamine red  
Fluorescen Isothiocynate (FITC)  
6- Carboxy fluorescence | Rhodamine-123  
Rhodamine red  
Fluorescen Isothiocynate (FITC)  
6- Carboxy fluorescence |
| 6    | Vehicle    | Carbopol 934 | As a gel former |

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Table No.2: Therapeutic applications of ethosomes

<table>
<thead>
<tr>
<th>S.No</th>
<th>Drug</th>
<th>Applications</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Acyclovir</td>
<td>Treatment of Herpetic infection</td>
<td>Improved drug delivery</td>
</tr>
<tr>
<td>2</td>
<td>Zidovudine</td>
<td>Treatment of AIDS</td>
<td>Improved transdermal flux</td>
</tr>
<tr>
<td>3</td>
<td>Trihexyphenidyl HCl</td>
<td>Treatment of Parkinson an syndrome</td>
<td>Increased drug entrapment efficiency, reduced side effect and constant systemic levels</td>
</tr>
<tr>
<td>4</td>
<td>Erythromycin</td>
<td>Efficient healing of S. aureus - induced deep dermal infections</td>
<td>Improved drug penetration and systemic effect.</td>
</tr>
<tr>
<td>5</td>
<td>Insulin</td>
<td>Treatment of Diabetes</td>
<td>Improved therapeutic efficacy of drug</td>
</tr>
<tr>
<td>6</td>
<td>Testosterone</td>
<td>Treatment of male hypogonodism</td>
<td>Enhance skin permeation</td>
</tr>
<tr>
<td>7</td>
<td>Bacitracin</td>
<td>Treatment of dermal infections</td>
<td>Reduced drug toxicity</td>
</tr>
<tr>
<td>8</td>
<td>Minodixil</td>
<td>Hair growth promotion effect</td>
<td>Higher skin retention</td>
</tr>
</tbody>
</table>

Figure No.1: Structure of ethosomes
**CONCLUSION**

Ethosomal carrier opens new challenges and opportunities for the development of novel improved therapies. 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 hydroalcoholic solution. 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.

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**BIBLIOGRAPHY**


