**Ethosomes - A noninvasive vesicular carrier for transdermal drug delivery**


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**ABSTRACT**

The transdermal route vied with oral treatment as the most successful innovative research area in drug delivery. The use of lipid vesicles in delivery systems for skin treatment has attracted increasing attention in recent years. Vesicles allow to control the release rate of drug over an extended time, keeping the drug shielded from immune response and would be able to release just the right amount of drug and keep that concentration constant for longer periods of time. One of the major advances in vesicle research was ethosomes. Ethosomes are noninvasive 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. Ethosomes are soft lipid vesicles containing phospholipids, alcohol in relatively high concentration and water. The size range of ethosomes may vary from tens of nanometers to microns (μ). Hot and cold methods are used for formulation of ethosomes. Evaluation parameters include size, shape, drug content, zeta potential, stability studies, skin permeation studies etc. These carriers open new challenges and opportunities for the development of novel improved therapies.

**Keywords:** Ethanol; Ethosomes; Phospholipids; Stratum corneum; Transdermal.

**INTRODUCTION**

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. Transdermal delivery route includes several advantages compared with oral route. This route has advantages of avoidance of first pass metabolism, predictable and extended duration of activity, minimizing under able side effects, utility of short half- life drugs, improving physiological and pharmacological response, avoiding the fluctuation in drug levels, inter and intra patient valuations, and most importantly, it provides patient compliance (Joke A. Bouwstra, 2005).

Despite the many advantages of the skin as a site of drug delivery, only few drugs are currently in the market for transdermal delivery system. The primary reason for this is the low permeability of drugs in the stratum corneum, as stratum corneum (outermost layer) acts as the main barrier in the skin (Blank IH, 1969). In general the highly organized crystalline lipid lamellae play an essential role in the barrier properties of the stratum corneum (Wertz PW, 2000; Williams ML, 1987; Bouwstra JA, 2003; Bouwstra JA, 2000; Pilgram GS, 1999). Many techniques have been aimed to disrupt and weaken the highly organized intercellular lipids in an attempt to enhance drug transport across the intact skin (Bouwstra JA, 1995; Barry BW, 2001) or to increase the driving force for permeation of drugs across this skin barrier. The vesicles have been well known for their important in cellular communication and particle transportation for many years. Researchers have understood the properties of vesicle structures for use in better drug delivery within their cavities that would allow for tagging the vesicle for cell specificity (Schreier H, 1994).

It is commonly agreed that classical liposomes were found to be effective in forming drug reservoir in only upper layer of the stratum corneum, for local skin therapy (Prausnitz MR, 2004, Bouwstra JA, 2003; Cevc G, 1996a; Cevc G, 1996b; Cevc G, 2001). One of the major advances in vesicle research was the finding a vesicle derivatives, known as an Ethosomes (Brau-Falco O, 1992). Ethosomes are noninvasive 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, phosphatidylserine, phosphatidic acid), high concentration of ethanol and water. It was found that ethosomes penetrate the skin and allow enhanced delivery of various compounds to the...
deep strata of the skin or to the systemic circulation (Touitou E, 2002). The high concentration of ethanol makes the ethosomes unique. The ethanol in ethosomes causes disturbance of skin lipid bilayer organization, hence when incorporated into a vesicle membrane, it enhances the vesicle’s 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 (Merdan VM, 1998).

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 (Gangwar Satyam, 2010).

This review describes the barrier properties of the skin, how drugs penetrate the skin by Ethosomal technique that has been used to enhance drug penetration across skin.

THE SKIN BARRIER: stratum corneum

The stratum corneum, or horny layer, is the outermost layer of the skin and has been identified as the principal barrier for penetration of most drugs (Touitou E, 1996). The horny layer represents the final stage of epidermal cell differentiation. The thickness of this layer is typically 10 µm, but a number of factors, including the degree of hydration and skin location, influence this. The stratum corneum consists of 10-25 rows of dead corneocytes embedded in a lipid matrix (Touitou E, 1996). The cells are joined together by desmosomes, maintaining the cohesiveness of this layer (Wiechers JW, 1989). The heterogeneous structure of the stratum corneum is composed of approximately 75-80% protein, 5-15% lipid and 5-10% unidentified on a dry weight basis (Michaels AS, 1975). The main lipids located in the stratum corneum are ceramides, fatty acids, cholesterol, cholesterol sulphate and sterol/wax esters (Michaels AS, 1975; Menon GK, 2002). These lipids are arranged in multiple bilayers called lamellae. Phospholipids are largely absent, a unique feature for a mammalian membrane. The ceramides are the largest group of lipids in the stratum corneum, accounting for a mammalian membrane. The ceramides are the largest group of lipids in the stratum corneum, accounting for approximately half of the total lipid mass (Williams AC, 2003), and are crucial to lipid organization of the stratum corneum (Wiechers JW, 1989). The brick and mortar model of the stratum corneum was first presented by Michaels et al. (Touitou E, 1998). The bricks correspond to parallel plates of dead keratinised corneocytes, and the mortar represents the continuous interstitial lipid matrix (Figure 1). The mortar is not a homogenous matrix, but rather lipids are arranged in the lamellar phase (alternating layers of water and lipid bilayers), with some of the lipid bilayers in the gel or crystalline state (Hadgraft J, 1989). The extracellular matrix is further complicated by the presence of intrinsic and extrinsic proteins such as enzymes. The barrier properties of the stratum corneum have been assigned to the multiple lipid bilayers residing in the intercellular space. This bilayer prevents desiccation of the underlying tissues by inhibiting water loss and limits the penetration of substances from the external environment (Menon GK, 2002).

![Figure 1: ‘Bricks and Mortar’ representation of the stratum corneum](image)

As stratum corneum is the principal barrier to drug permeation across the skin. Consequently, there has been a concerted effort to investigate and develop novel strategies of maximizing the amount of permeant crossing this barrier. Innovative approaches focus on altering the drug-vehicle interaction to enhance partitioning into the stratum corneum, or modifying the structure of the stratum corneum to make it less resistance to drug diffusion. Alternatively, energy-driven methods have been employed to propel drugs to deep into the skin.

ETHANOL- AS PENETRATION ENHANCER

Substances that reversibly reduce the barrier resistance of the stratum corneum are known as chemical penetration enhancers. Ethanol is one of the most commonly used permeation enhancers. A number of mechanisms have been proposed for permeation enhancing action of ethanol. As a solvent, ethanol can be included in the formulation to enhance the solubility of the drug. This is particularly important for poorly soluble permeants, as they are prone to depletion in the donor vehicle (Lodzki M, 2003). Ethanol is a relatively volatile solvent and will rapidly evaporate at skin temperature. Ethanol loss from a formulation may lead to the drug becoming supersaturated, which will influence drug flux across the membrane. In addition, etha-
nol is thought to alter the solubility properties of the stratum corneum, facilitating improved drug partitioning (Verma DD, 2004).

Ethanol has been employed in vitro to enhance transdermal delivery of levonorgestrol, hydrocortisone and 5-fluorouracil across rodent skin (Williams AC, 2003), and estradiol across human skin in vivo (Friend D, 1988). Megrab and collaborators (Megrab NA, 1995) noted that the enhancement effect of ethanol was concentration dependent. The authors investigated the effect of ethanol on skin water content and concluded that formulations containing high levels of alcohol were capable of dehydrating the skin, which may explain the concentration dependant action of ethanol.

**COMPOSITION AND VESICLE PREPARATION:**

**Composition**

Ethosomes are vesicular carriers composed of hydro alcoholic or hydro/alcoholic/glycolic phospholipid in which the concentration of alcohols or their combination is relatively high. (Table 1) (Friend D, 1988).

**VESICLE PREPARATION**

**Cold Method**

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 (Jain S, 2004). 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 (Touitou E, 2000a). The vesicle size of ethosomal formulation can be decreased to desire extent using sonicantion or extrusion method. Finally, the formulation is stored under refrigeration (Manosroi A, 2009).

**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 (Jain S 2004). In a separate vessel ethanol and propylene glycol are mixed and heated to 400°C (Bhalaria MK, 2009). 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 (Touitou E, 1996).

**MECHANISM OF PENETRATION**

The exact mode of action of ethosomes remains unclear (Touitou E, 2002). The high concentration of ethanol makes the ethosomes unique, as ethanol disturbs the skin lipid bilayer organization. High ethanol concentration in ethosomes, allowing a more malleable structure, giving it more freedom and ability to squeeze through small places such as the openings created in disturbing the stratum corneum lipid (Touitou E, 2001). Ethanol interacts with lipid molecules in the polar hard group region, resulting in reducing the rigidity of the stratum corneum lipids, increasing their fluidity. In the case of ethosomes encapsulating drugs, the higher positive zeta potential imparted by the drug can improve skin attachment of the vesicles.

Ethanol is a well known permeation enhancer (Friend D, 1988), and phospholipids can potentially cause disruption of the intercellular domains of the horny layer (Dayan, 2000). However, when compared; ethosomal preparations were found to be much more effective permeation enhancers than hydroethanolic solutions, ethanol or an ethanololic phospholipids solution (Patel S, 2007). An alternative theory that has been proposed is that ethanol initially acts to disrupt the lipid organization of the stratum corneum (Blume A, 1993). Subsequently, ethosomes, which are thought to be more flexible than liposomes due to their increased alcohol component, squeeze through the compromised horny layer. The efficient drug delivery shown together with

<table>
<thead>
<tr>
<th>Additives used in Ethosomal Preparation</th>
<th>Examples</th>
<th>Application</th>
</tr>
</thead>
<tbody>
<tr>
<td>Phospholipid</td>
<td>Soya phosphatidyl choline, Egg phosphatidyl choline, Dipalmityl phosphatidyl choline, Distearyl phosphatidyl choline</td>
<td>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>Alcohol</td>
<td>Ethanol, Isopropyl alcohol</td>
<td>For providing the softness for vesicle membrane</td>
</tr>
<tr>
<td>Cholesterol</td>
<td>Cholesterol</td>
<td>As a penetration enhancer</td>
</tr>
<tr>
<td>Dye</td>
<td>Rhodamine-123, Rhodamine red Fluorescence Isothiocyanate (FITC), 6- Carboxy fluorescence</td>
<td>For characterization study</td>
</tr>
<tr>
<td>Vehicle</td>
<td>Carbopol 934</td>
<td>As a gel former</td>
</tr>
</tbody>
</table>
the long-term stability of ethosomes makes this system a promising candidate for transdermal delivery of drug (Guo J, 2000; Maghraby GMM, 2000).

CHARACTERIZATION OF ETHOSOMAL SYSTEMS

Visualization of vesicles by TEM and by SEM

Vesicular shape of the ethosome preparations were assessed by using Transmission Electron Microscope (TEM). Samples were dried on carbon-coated grid and negatively stained with aqueous solution of phosphotungstic acid. After drying the specimen was viewed under the microscope at 10–100 k-fold enlargements at an accelerating voltage of 100 Kv (Vaibahav Dubey, 2007a).

The size and shape of the vesicles were observed in the Scanning Electron Microscopy (SEM). One drop of ethosomal suspension was mounted on a clear glass stub (Bouwstra JA, 1991; Asbill CS, 2000). It was then air dried and gold coated using sodium aurothiomalate to visualize under scanning electron microscope at 10,000 magnifications.

Size distribution and vesicular size

The size distribution of ethosomal preparation was measured in two sets of triplicates, in a multimodal mode, by Dynamic Light Scattering (DLS) technique using a computerized Malvern Autosizer 5002 inspection system (Malvern, UK) (Vaibahav Dubey, 2007b). For vesicle size measurement, ethosomal preparation was mixed with the appropriate medium, and the measurements were taken in triplicate (Kundalik Girhepunj, 2010).

Figure 2: Drug Penetration through Ethosomes

- Ethosomes
- Ethanol Cause Skin Disruption
- Increase Lipid Fluidity
- More Penetration Through Skin
- Ethosome Penetrates Inside
- Fuse with Skin Lipids
- Release Drug into Deep Skin Layers

Figure 3: Mechanism of action of ethosomes

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Entrapment efficiency

Entrapment efficiency of ethosomal vesicles was determined by centrifugation method. The vesicles were separated in a high speed cooling centrifuge at 20,000rpm for 90 minutes in the temperature maintained at 4°C (Lauer AC, 1996; Godin B, 1998; Zeng Zhaowu, 2009; Ehab R. Bendas, 2007). The sediment and supernatant liquids were separated amount of drug in the sediment was determined by lysing the vesicles using methanol. From this, the entrapment efficiency was determined by the following equation,

\[
\text{Entrapment efficiency} = \frac{D_E}{D_T} \times 100 \quad \text{Where,}
\]

\[D_E\] - Amount of drug in the ethosomal sediment
\[D_T\] - Theoretical amount of drug used to prepare the formulation (equal to amount of drug in supernatant liquid and in the sediment)

Transition Temperature

The Transition temperature (T) of vesicular lipids was measured in duplicate by DSC in an aluminum pan at a heating rate of 10°C per min, under a constant nitrogen stream (Biana Godin, 2005).

Confocal scanning laser microscopy (CSLM)

CLSM was used to investigate depth and mechanism of skin penetration of ethosomal preparation (Touitou E, 2000b). The skin thickness was optically scanned at different increments through the z-axis of a confocal laser scanning microscope.

Drug Content

Drug content of the ethosomes can be determined using UV spectrophotometer. This can also be quantified by a modified high performance liquid chromatographic method (New RRC, 1990).

Surface Tension Measurement

The surface tension activity of drug in aqueous solution can be measured by the ring method in a Du Nouy ring tensiometer (Verma DD, 2003).

Phospholipid-ethanol interaction

The Phospholipid-ethanol interaction was studied by using Proton decoupled 31P-NMR and Differential Scanning calorimetry (Jain S, 2005).

Degree of deformability and Turbidity

The Degree of deformability of the ethosomal Preparation was performed by Extrusion Method (Cevc G, 2004).

Table 2: Examples of Ethosomes as a Drug Carrier

<table>
<thead>
<tr>
<th>S.no.</th>
<th>Drug</th>
<th>Purpose of Ethosomal delivery</th>
<th>Application</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Azelaic acid</td>
<td>Improves the sustained release</td>
<td>Treatment of acne</td>
</tr>
<tr>
<td>2</td>
<td>Diclofenac</td>
<td>Selective targeting the cells</td>
<td>NSAIDS</td>
</tr>
<tr>
<td>3</td>
<td>Testosterone</td>
<td>Low oral bioavailability dose dependent side effects</td>
<td>Steroidal hormone</td>
</tr>
<tr>
<td>4</td>
<td>Trihexyphenidyl hydrochloride</td>
<td>4.5-times higher than that from liposome</td>
<td>Treatment of Parkinson’s disease</td>
</tr>
<tr>
<td>5</td>
<td>Zidovudine and lamivudine</td>
<td>Better cellular uptake</td>
<td>Anti-HIV</td>
</tr>
<tr>
<td>6</td>
<td>Bacitracin</td>
<td>Better cellular uptake</td>
<td>Antibacterial</td>
</tr>
<tr>
<td>7</td>
<td>Erythromycin</td>
<td>Better cellular uptake</td>
<td>Antimicrobial</td>
</tr>
<tr>
<td>8</td>
<td>DNA</td>
<td>Expression into skin cells</td>
<td>Treatment of genetic disorders</td>
</tr>
<tr>
<td>9</td>
<td>Cannabidol</td>
<td>Low bioavailability</td>
<td>Treatment of rheumatoid</td>
</tr>
<tr>
<td>10</td>
<td>Acyclovir</td>
<td>Poor skin permeation</td>
<td>Treatment of Herpes labialis</td>
</tr>
<tr>
<td>11</td>
<td>Insulin</td>
<td>GIT degradation</td>
<td>Treatment of diabetes</td>
</tr>
<tr>
<td>12</td>
<td>Cyclosporin</td>
<td>GIT degradation Poor oral</td>
<td>Treatment of Inflammatory skin disease</td>
</tr>
<tr>
<td>13</td>
<td>Ammonium glycyrrhizinate</td>
<td>Poor skin permeation</td>
<td>Treatment of inflammatory based skin diseases</td>
</tr>
<tr>
<td>14</td>
<td>Fluconazole</td>
<td>Poor skin permeation</td>
<td>Treatment of candidiasis</td>
</tr>
<tr>
<td>15</td>
<td>Methotrexate</td>
<td>Poor skin permeation</td>
<td>Treatment of psoriasis</td>
</tr>
<tr>
<td>16</td>
<td>Salbutamol</td>
<td>Enhanced drug delivery through skin with ethosomes</td>
<td>Anti-asthmatic</td>
</tr>
<tr>
<td>17</td>
<td>Minoxidil</td>
<td>Pilocebaceous targeting Accumulation in skin increased</td>
<td>Treatment of baldness</td>
</tr>
<tr>
<td>18</td>
<td>Proteins and Peptides</td>
<td>Large molecules</td>
<td>overcoming the problems associated with oral delivery</td>
</tr>
<tr>
<td>19</td>
<td>Enalapril maleate</td>
<td>Low oral bioavailability Major side effects in oral delivery</td>
<td>Treatment of Hypertension</td>
</tr>
</tbody>
</table>
2005; Berge V, 1997) and the turbidity of the preparation was performed by Using Nephelometer (El. Maghraby GMM, 2000).

In vitro drug release study and Drug Deposition study

In vitro drug release study and Drug Deposition of ethosomal preparation was performed by Franz diffusion cell with artificial or biological membrane, Dialysis bag diffusion (Jain S, 2003).

Storage-physical stability of ethosomes

The ability of ethosomal preparations to retain the drug (i.e., drug-retentive behavior) was checked by keeping the preparations at different temperatures, i.e., 25 ± 2°C (room temperature, RT), 37 ± 2°C and 45 ± 2°C for different periods of time (1, 20, 40, 60, 80 and 120 days). The ethosomal preparations were kept in sealed vials (10 ml capacity) after flushing with nitrogen (Patel S, 2007; Touitou E, 1998). The stability of ethosomes was also determined quantitatively by monitoring size and morphology of the vesicles using DLS and TEM (Vaibhav Dubey, 2007b).

THERAPEUTIC APPLICATIONS

Ethisomes, 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 (Persing LK, 1990; Megrab NA, 1995; Horwitz E, 1999; Touitou E, 1999; Lodzki M, 2003; Godin B, 2004). The uses of ethosomes as carrier system for transdermal/topical drug delivery are summarized below (Table 2) (Paolino D, 2005; Godin B, 2005; Touitou E, 2001; Kaplun-Frischhoff Y, 1997; Kim JC, 2003).

CONCLUSION

Transdermal route is promising alternative to drug delivery for systemic effect. Ethosomes has initiated a new area in vesicular research for transdermal drug delivery which 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. Ethosomes have been tested to encapsulate hydrophilic drugs, cationic drugs, proteins and peptides. Further, research in this area will allow better control over drug release in vivo and long-term safety data, allowing the therapy more effective. Thus, ethosomal formulations possess promising future in effective dermal/transdermal delivery of bioactive agents.

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