Chapter - I

INTRODUCTION

India is a country with vast diversity in the climatic and weather conditions. The climate is generally hot, humid and tropical type. This type of climate tends to favour the growth of variety of micro organisms and disease causing pathogens. Majority of studies showed that occurrence of various diseases and infections caused by such organisms in our country is at very high percentage. About 10% of annual funding for health research is spent on health problems that account for 90% of global disease burden. However only a fraction of new chemical entities is specifically indicated for tropical diseases. It is observed further that less studies have been undertaken with regards to fungal infections and therefore the treatment for various dermatological infections due to fungus is minimal. It has been observed that people tend to neglect the fungal infections greatly and majority doesn’t consider it as a disease and prefer to go for unconventional treatments.

Around the world fungal infections have recently emerged as a growing threat to human health, especially in persons whose immune systems are compromised in some way. For example, fungi are associated with complex disease entities in complex medical patients (e.g., cryptococcosis in AIDS patients or aspergillosis in bone marrow or organ transplant patients). Fungi usually make their homes in moist areas of the body where skin surfaces meet between the toes, in the genital area, and under the breasts. Many fungi that infect the skin (dermatophytes) live only in the topmost layer of the epidermis (stratum corneum) and do not penetrate deeper. Obese people are more likely to get these infections because they have excessive skin folds. People with diabetes tend to be more susceptible to fungal infections as
well. Strangely, fungal infections on one part of the body can cause rashes on other parts of the body that are not infected. For example, a fungal infection on the foot may cause an itchy, bumpy rash on the fingers. These eruptions (dermatophytids or id reactions) are allergic reactions to the fungus. They do not result from touching the infected area.

Research on fungal diseases focuses on three goals: providing better means of diagnosis, treatment, and prevention of the most important human fungal infections. Objectives leading to the achievement of these goals are grouped in the following five research areas:

- Molecular biology—transferring the technology developed in model systems to the medically important fungi in order to address topics of clinical relevance, including vaccine candidates and new drug and diagnostic targets.
- Immunobiology—identifying immunologically protective antigens, antibodies, and pathways in order to plan vaccine approaches and improve therapy.
- Pathogenesis—identifying mechanisms of pathogenesis in order to interrupt or prevent the infectious process.
- Therapy—facilitating improvements in available treatment through study, including clinical trials, of new treatments and comparative treatments of the systemic fungal diseases.
- Genome sequencing and genomics/proteomics—providing complete genomic sequences for the community and facilitating genomics/proteomics approaches that address the key fungal pathogens of humans.

Even though there are several treatment methods against fungal infections, clinicians are particularly concerned that the increasing use of antifungal drugs which may lead to drug-resistant fungi, especially in settings such as hospitals where nosocomial (hospital-acquired) infections are a growing problem. Recent studies have documented resistance of _Candida_ species to fluconazole and other
azole and triazole drugs, which are used widely to treat patients with systemic fungal diseases.

One of the major areas of interest is in the field of therapy. The interest in this has led to work on the developments of novel molecules, delivery systems as well as formulations which can control these fungal infections to a greater extent than before. Most of the fungal infections are appear over the skin, the treatment regimen of these infections always comprises of external application formulations such as creams, ointments, lotions etc. It’s a fact that delivery of drugs to the skin is an effective and targeted therapy for local dermatological disorders caused by fungi. But apart from that, dermal drug delivery has gained popularity because of its advantages over other routes of administrations such as, it avoids first-pass effects, gastrointestinal irritation, and metabolic degradation associated with oral administration. The topical route of administration has been utilized to produce local effects for treating skin disorders or to produce systemic drug effects. With topical dosage forms, great attention has been devoted towards newer formulations which can ensure maximum localization of drug within the affected area to enhance the local effect or increase the penetration through the stratum corneum and viable epidermis for systemic effects. To design a better dermal delivery system, it is very much important to understand the structure of skin.

The human skin is the largest organ of the body, providing a protective coverage for the internal structure and organs. Skin comprises an area of between 16.1 ft$^2$ and 21.6 ft$^2$ (1.5 m$^2$ and 2.0 m$^2$), at an average thickness of 0.00394 in (0.1 mm), accounting for between 15% and 18% of the total body weight. The skin has three layers—the epidermis, dermis, and fat layer (also called the subcutaneous layer). Each layer performs specific tasks. Given its role as the protective shell for the internal organs and structure, the skin sustains constant contact and consequently, it is the most injured human organ.
Epidermis: The epidermis is the relatively thin, tough, outer layer of the skin. Most of the cells in the epidermis are keratinocytes. They originate from cells in the deepest layer of the epidermis called the basal layer. New keratinocytes slowly migrate up toward the surface of the epidermis. Once the keratinocytes reach the skin surface, they are gradually shed and are replaced by younger cells pushed up from below.

The outermost portion of the epidermis, known as the stratum corneum, is relatively waterproof and, when undamaged, prevents most bacteria, viruses, and other foreign substances from entering the body.

The epidermis (along with other layers of the skin) also protects the internal organs, muscles, nerves, and blood vessels against trauma. In certain areas of the body that require greater protection (such as the palms of the hands and the soles of the feet), the outer keratin layer of the epidermis (stratum corneum) is much thicker. Scattered throughout the basal layer of the epidermis are cells called melanocytes, which produce the pigment melanin, one of the main contributors to...
skin color. Melanin's primary function, however, is to filter out ultraviolet radiation from sunlight which can damage DNA, resulting in numerous harmful effects, including skin cancer. The epidermis also contains Langerhans cells, which are part of the skin's immune system. Although these cells help detect foreign substances and defend the body against infection, they also play a role in the development of skin allergies.

Dermis: The dermis, the skin's next layer, is a thick layer of fibrous and elastic tissue (made mostly of collagen, elastin, and fibrillin) that gives the skin its flexibility and strength. The dermis contains nerve endings, sweat glands and oil glands, hair follicles, and blood vessels. The nerve endings sense pain, touch, pressure, and temperature. Some areas of the skin contain more nerve endings than others. For example, the fingertips and toes contain many nerves and are extremely sensitive to touch.

The sweat glands produce sweat in response to heat and stress. Sweat is composed of water, salt, and other chemicals. As sweat evaporates off the skin, it helps cool the body. Specialized sweat glands in the armpits and the genital region (apocrine sweat glands) secrete a thick, oily sweat that produces a characteristic body odor when the sweat is digested by the skin bacteria in those areas. The sebaceous glands secrete sebum into hair follicles. Sebum is an oil, that keeps the skin moist and soft and acts as a barrier against foreign substances.

The hair follicles produce the various types of hair found throughout the body. Hair not only contributes to a person's appearance but has a number of important physical roles including regulating body temperature, providing protection from injury, and enhancing sensation. A portion of the follicle also contains stem cells capable of regrowing damaged epidermis. The blood vessels of the dermis provide nutrients to the skin and help regulate body temperature. Heat makes the blood vessels enlarge (dilate), allowing large amounts of blood to circulate near the skin surface, where the heat can be released. Cold makes the blood vessels narrow (constrict), retaining the body's heat. Over different parts of the body, the number of nerve endings, sweat glands and sebaceous glands, hair...
follicles, and blood vessels varies. The top of the head, for example, has many hair
follicles, whereas the soles of the feet have none.

Fat Layer: Below the dermis lies a layer of fat that helps insulate the body
from heat and cold, provides protective padding, and serves as an energy storage
area. The fat is contained in living cells, called fat cells, held together by fibrous
tissue. The fat layer varies in thickness, from a fraction of an inch on the eyelids to
several inches on the abdomen and buttocks in some people.4

Skin being a large part of the human body with a huge surface area as well
as the properties which promote drug delivery through it, this route has received
wide attention for many decades. However, skin tends to be the strongest barrier
for the entry of drug entities and hence it is essential to design the drug delivery
system in the most appropriate manner which includes selection of a vehicle to
deliver the medicament into the skin layers (cutaneous delivery), or through the
skin and into the systemic circulation (percutaneous absorption). Different types of
drug delivery systems have been designed for application on to the skin. In
dermatopharmacotherapy, the primary purpose is to apply drugs to the skin for
inducing local effects at the site of application. To achieve this target in
dermatopharmacotherapy, selective delivery systems are developed to enhance
penetration of active ingredients with localization at the site of action. But the poor
skin penetration by most of the drugs is one of the major problems in
dermatopharmacotherapy, since only a small portion of dose finally reaches the
sites of action producing limited local activity. Moreover, a few drugs which can
penetrate the skin easily are quickly removed by blood circulation, thus producing
systemic effect rather than local effects. This has been a complicated task due to
the highly effective barrier properties of the skin. In order to deliver drugs through
the skin, most compounds require various degrees of permeation enhancement.
Classic enhancement methods focus primarily on chemical enhancement or
modulation of interactions between the drug and the vehicle. More recent literature
indicates the use of innovative vesicular earners, electrically assisted delivery and
various microinvasive methods, some incorporating technologies from other fields.
The best avenue to improve drug penetration and/or localization is obviously to manipulate the vehicle or to utilize a drug carrier concept. Dermatological and cosmetic preparations frequently contain active principles, which can only act when they penetrate at least the outermost layer of the skin. However, the efficacy of topically applied actives is often suboptimal because the transport into the skin is slow due to the resistance of the outermost layer of the skin, the stratum corneum\textsuperscript{4,5}.

Despite major research and development efforts in topical and transdermal systems and the advantages of these routes, low stratum corneum permeability limits the usefulness of topical drug delivery. To overcome these limitations, methods have been assessed to increase permeation. Among many such methods, the use of vesicular systems, such as liposomes, niosomes, ethosomes etc has great importance. The effectiveness of these vesicular systems depends on their physicochemical properties. However, of late there has been a surge in these vesicular delivery systems with wide variation and flexibility in their interior designs and structures. Most of these vesicular systems comprise of lipids or its derivatives. The importance of lipids has especially increased after realizing the utility of phospholipids, the natural bio-friendly molecules which in collaboration with water and other solvents can form diverse types of supramolecular structures. Vesicular systems are drug encapsulated in lipid vesicles prepared from phospholipids and non-ionic surfactants used to transport the drug 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.

At present, no available drug delivery system behaves ideally achieving all the lofty goals, but sincere attempts have been made to achieve them through novel
approaches in drug delivery. A number of novel drug delivery systems have emerged encompassing various routes of administration, to achieve controlled and targeted drug delivery. Encapsulation of the drug in vesicular structures is one such system, which can be predicted to prolong the existence of the drug in systemic circulation, and reduce the toxicity, if selective uptake can be achieved. Consequently a number of vesicular drug delivery systems such as liposomes, niosomes and ethosomes were developed. Advances have since been made in the area of vesicular drug delivery, leading to the development of systems that allow drug targeting, and the sustained or controlled release of conventional medicines\textsuperscript{6}.

**LIPOSOMES**

Liposomes were discovered in the early 1960's by Bangham and colleagues (Bangham et al., 1965) and subsequently became the most extensively explored drug delivery system\textsuperscript{6,7}. Liposomes are small, spherical vesicles which consist of amphiphilic lipids, enclosing an aqueous core. The lipids are predominantly phospholipids which form bilayers similar to those found in biomembranes. In most cases the major component is phospholipids. Depending on the processing conditions and the chemical composition, liposomes are formed with one or several concentric bilayers. In early 1960's a great knowledge of vesicle derivatives has been tested for their abilities. Most experiments, however, have centered on liposomes since derivations only add to their basic properties. Vesicles are closed, spherical membrane that separates a solvent from the surrounding solvent. Possible use of liposomes in topical drug delivery vehicles for both water and lipid soluble drug has been investigated. While it has been suggested that the external envelop of a liposomes would allow it to pass through lipophilic skin, most researches show that liposomal vesicles become trapped within the top layer of the stratum corneum cells. Generally liposomes are not expected to penetrate into viable skin, although occasional transport processes were reported\textsuperscript{8,9}. This behavior is useful both for local treatment of skin disorders and for cosmetic formulations. Specific drug accumulation at the site of action and decreased systemic drug absorption can
impart increased efficiency as well as decreased side effect to a compound applied topically.

**NIOSOMES**

Niosomes are non-ionic surfactant based liposomes. They are mostly formed by incorporation of cholesterol as an excipient. Other excipients can also be used. Niosomes have more penetrating capability than the liposomes. They are structurally similar to liposomes in having a bilayer, however, the materials used to prepare niosomes makes them more stable and thus niosomes offer many more advantages over liposomes\(^\text{10}\).

**ETHOSOMES**

The vesicles have been well known for their importance in cellular communication and particle transportation for many years. Researchers have been understanding the properties of vesicle structures for use in better drug delivery within their cavities, that would allow to tag the vesicle for cell specificity. Vesicles would also allow to control the release rate of drug over an extended time, keeping the drug shielded from immune response or other removal systems 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 the finding a vesicle derivative, known as an ethosomes\(^ \text{8,11}\).

The main advantages of Ethosome technology\(^ {12}\) are

- Enhanced permeation
- Passive and non-invasive
- Suitable for a wide range of drugs with various chemical properties
- Suitable for a wide range of applications
- Made from safe and approved materials
- Protected by international patents
- Proven feasibility - scientifically and commercially
- Well established and published technology
Ethosomal carriers are systems containing soft vesicles and are composed mainly of phospholipids (Phosphatidyl choline; PC), ethanol at relatively high concentration 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\(^1\) (Table No 1).

**Table No 1: Composition 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 vesicle</td>
</tr>
<tr>
<td></td>
<td>Isopropyl alcohol</td>
<td>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 vesicle</td>
</tr>
<tr>
<td></td>
<td></td>
<td>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 Isothiocynate</td>
<td></td>
</tr>
<tr>
<td></td>
<td>(FITC)</td>
<td></td>
</tr>
<tr>
<td>Vehicle</td>
<td>Carbopol O934</td>
<td>As a gel former</td>
</tr>
</tbody>
</table>
MECHANISM OF PENETRATION

Although the exact process of drug delivery by ethosomes remains a matter of speculation, the enhanced delivery of drugs using ethosomes can be ascribed to an interaction between ethosomes and skin lipids. The stratum corneum lipid multilayers at physiological temperature are densely packed and are in high conformational order. It is thought that there are two sets of possible mechanism behind the efficient drug delivery by the ethosomes. First part of the mechanism is due to the ‘ethanol effect’, 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 vesicles have 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, giving it more freedom and ability to squeeze through small places such as the openings created in disturbing the stratum corneum lipid.

Ethanol interacts with lipid molecules in the polar hard group region, resulting in reducing the rigidity of the stratum corneum lipids, increasing their fluidity. The intercalation of ethanol into the polar head group environment can result in an increase in the membrane permeability. In addition to the effect of ethanol on stratum corneum structure, the ethosome itself may interact with the stratum corneum barrier.

This ‘ethanol effect’ 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. The interdigitated, malleable ethosome vesicle can forge paths in the disordered stratum corneum. In the case of ethosomes encapsulating drugs, the higher positive zeta potential imparted by the drug can improve skin attachment of the vesicles. While encapsulated drug in classic
liposomes remained primarily at the surface of the skin, the ethosomal system was showed to be highly efficient carrier for enhanced drug delivery through the skin. The efficient drug delivery shown together with the long-term stability of ethosomes makes this system a promising candidate for transdermal delivery of drug\textsuperscript{14}.

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{figure2.png}
\caption{Ethosomal delivery of drugs across the skin}
\end{figure}

**Method for Preparing Ethosomes**

Ethosomal formulation may be prepared by hot or cold method as described below. Both the methods are convenient, economical and do not require any sophisticated equipment. The method for preparations are easy to scale up at industrial level\textsuperscript{11}.

**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 30°C in a water bath. The water heated to 30°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 desired extent using sonication or extrusion method. Finally, the formulation is stored under refrigeration\textsuperscript{12}.
Hot method

In this method phospholipid is dispersed in water by heating in a water bath at 40°C until a colloidal solution is obtained. In a separate vessel ethanol and propylene glycol are mixed and heated to 40°C. Once both mixtures reach 40°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\(^1\).

FACTOR AFFECTING CHARACTERS OF ETHOSOMES

Ethosomes consist of ethanol (10 -50 %), phosphatidyl choline (0.5 - 4 %) and drug. Concentration of ethanol and phospholipid are the factors which affect the characters of ethosome e.g. vesicular size, entrapment efficiency and dermal delivery. Their effects are as follows:

Ethanol

The effect of ethanol concentration on size distribution of ethosomal vesicles can be investigated using dynamic light scattering (DLS). In the ethanol concentration range of 10 - 50%, the size of the vesicles was found to be decreasing with increasing ethanol concentration. The largest vesicles are found in the preparation containing 10% ethanol and the smallest in the preparation containing 50% ethanol\(^1\). The data indicates entrapment efficiency also depends on the ethanol concentration. Increasing the ethanol from 10% to 30% w/w, increases the entrapment efficiency and with further increase in the ethanol concentration (>30% w/w) the entrapment efficiency of ethosomal formulation decreases, the reason may be vesicle membrane becoming more permeable\(^3\). The value of transdermal flux may be also depend upon ethanol concentration. As the concentration of ethanol increases, transdermal flux of entrapped drug increases up to 30% w/w and further increase in the ethanol concentration significantly decreases the transdermal flux. At higher concentration of ethanol there may be
deteriorating effect on lipid bilayers which may lead to decrease in transdermal flux.

**Phospholipid**

Jain et al, reported that ethosomal size exhibited limited dependence on the phospholipid concentration. An eight fold increase in phospholipids concentration (from 0.5 to 4.0 %) resulted in insignificant increase in ethosomal size.

**CHARACTERIZATION**

There are various methods applied for characterization of ethosomes and they are as follows:-

1. **Visualization**

   For the initial characterization of the vesicles, ethosomal preparation can be examined by negative stain electron microscopy (TEM). It also visualizes the lamellar character of ethosomes. The three dimensional nature of phospholipid vesicle can be confirmed by further analysis by scanning electron microscopy (SEM)\(^{13}\).

2. **Vesicle size and zeta potential**

   Particle size of vesicle can be determined by dynamic light scattering (DLS). The charge of the ethosomal vesicle is an important parameter that can influence both vesicular properties such as stability as well as skin-vesicle interactions and its zeta potential can also be determined using a computerized inspection system\(^{6,13}\).

   The size of the vesicles can be characterized by light microscopy with an eyepiece micrometer which is calibrated with a stage micrometer.

3. **Differential scanning calorimetry (DSC)**

   DSC is used to determine the transition temperature of vesicular lipid system. Lipid bilayers exhibit various phase transitions that are studied for their
roles in triggered drug release. Lipid bilayers can exist in a low-temperature solid ordered phase and above a certain temperature in a fluid-disordered phase, the temperature of this phase transition can be tailored by selecting the proper lipids.\(^{15}\)

4. **Entrapment efficiency**

Separation of untrapped drug and evaluation of entrapment efficiency can be measured by ultracentrifugation.\(^{11}\)

**Ultracentrifugation:** Procedure was reported by Touitou et al. where ethosomal preparation was centrifuged at 4°C 40,000 rpm for 3 hours. The supernatant layer was removed and drug quantity was determined in both the sediment and the supernatant. The entrapment efficiency was calculated as follows.\(^{11}\)

\[
\text{Entrapment efficiency} = \frac{(T - C)}{T} \times 100
\]

Where 'T' is total amount of drug that is detected both in the supernatant layer and resident layer.

'C' is the amount of drug detected only in the supernatant.

5. **Assay**

5.1 HPLC method

Drug can be quantified by a modified HPLC method using a UV detector, column oven, auto-sample, pump and computerized analysis program.\(^{16}\)

5. 2 Ethanol quantification

Touitou et al. reported quantitative determination of ethanol using enzymatic diagnostic kit based on the oxidation of alcohol to acetaldehyde. The reduction of nicotinamide adenine dinucleotide (NAD) was followed in a UV spectrophotometer as an increase in absorbance at 340 nm. The increase in absorbance is directly proportional to alcohol concentration in the sample.\(^{13}\)
5. Phospholipid quantification

The Barlett assay can be used for quantitative analysis of phospholipid. Color intensity is to be measured spectroscopically at 830 nm

6. Vesicle stability

The stability of vesicles can be determined by assessing the size and structure of vesicles over time. Mean size is measured by DLS and structural changes are observed by TEM. Touitou et al. performed stability study by DLS measurement with ethosomes of three batches, one after two years and the other two during a period of 105 days storage.

7. Solubility measurement

The solubility of drug in ethosomal medium can influence its entrapment efficiency, vesicular structure and consequently permeation of drug through the skin. Dayan and Touitou found the solubility of drug in water, phosphate buffer and 30% hydro-ethanolic solution at 22°C and 37°C by solubility method.

8. Penetration and permeation studies

Depth of penetration of ethosomes can be visualized by confocal laser scanning microscopy (CLSM). The ability of ethosomes to deliver lipophilic molecules to the deep layers of the skin was investigated using a lipophilic fluorescent probe, Rhodamine red and confocal laser scanning microscopy.

Advantages of Ethosomal Drug delivery

In comparison to other transdermal & dermal delivery systems ethosomal formulation has following advantages,

1. Ethosomes has enhanced permeation of drug through skin for transdermal and dermal delivery.
2. Ethosomes are platform for the delivery of large and diverse group of drugs (peptides, protein molecules etc)

3. Ethosome composition is safe and the components are approved for pharmaceutical and cosmetic use.

4. 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.

5. High patient compliance- The Ethosomal drug is administered in semisolid form (gel or cream), producing high patient compliance. In contrast, Iontophoresis and Phonophoresis are relatively complicated to use which will affect patient compliance.

6. High market attractiveness for products with proprietary technology.

7. Relatively simple to manufacture with no complicated technical investments.

8. The Ethosomal system is passive, non-invasive and is available for immediate commercialization.


**Therapeutic Application of Ethosomes**

**Delivery of Anti-Viral drugs**

Horwitz et al. reported that a 5 % acyclovir ethosomal preparation compared to the 5 % acyclovir cream showed significant improvements in treatment of herpetic infections\(^{19}\).

Jain et al. prepared Zidovudine ethosomes and characterized them in vitro and in vivo. The effect of different formulation variables on skin permeation of Zidovudine was studied using locally fabricated Keshry-Chien type of diffusion
To understand the mechanism of better skin permeation of ethosomes, vesicle skin interaction study was carried out. To confirm the better skin permeability of ethosomes, fluorescence microscopy using Rhodamine-123 as fluorescence probe was performed. The optimized ethosomes showed transdermal flux of $78.5 \pm 2.5 \mu g/cm^2/hr$ across the rat skin. Vesicle skin interaction study showed that ethosomes affected the ultra-structure of the stratum corneum as distinct regions with lamellar stacks derived from the vesicles were observed in the intercellular spaces of the stratum corneum. Thus ethosomes can increase the transdermal flux, prolong the release and present an attractive route for sustained delivery of Zidovudine.

**Treatment of Parkinsonian syndrome**

Dayan et al. investigated the delivery of Trihexyphenidyl HCl (THP) from ethosomes versus classic liposomes. As the THP concentration was increased from 0 to 3%, the size of the vesicles decreased from 154 to 90 nm. This is most likely due to the surface activity of THP (critical micelle concentration of 5.9 mg/ml), as measured in this work. In addition, the ethosome zeta potential value increased as a function of THP concentration, from -4.5 to +10.4 when the THP concentration was increased from 0 to 3%. In contrast, THP liposomes were much larger and their charge was not affected by THP. When compared with standard liposomes, ethosomes had a higher entrapment capacity and a greater ability to deliver entrapped fluorescent probe to the deeper layers of skin. The flux of THP through nude mouse skin from THP ethosomes (0.21 mg/cm2 h) was 87, 51 and 4.5 times higher than from liposomes.

**Delivery of Antibiotics**

Godin et al. investigated a new approach to treat deep skin and soft tissue bacterial infections by dermal application of Erythromycin in an ethosomal carrier. The efficiency of ethosomal Erythromycin applied to the skin-infected site was compared with intraperitoneal Erythromycin administration and with local application of hydroethanolic Erythromycin solution. Bacterial counts and
histological evaluation of the skin treated with ethosomal antibiotic revealed no bacterial growth and normal skin structure. On the contrary, no sub dermal healing was observed in infected animals treated with topical hydroethanolic Erythromycin solution.

Godin et al. studied the dermal and intracellular delivery of Bacitracin from ethosomes. Efficient delivery of antibiotics to deep skin from ethosomal application was reported to be highly beneficial, reducing possible side effects.

**Improved Anti-Inflammatory activity**

Donatella et al. studied in-vitro percutaneous permeation of Ammonium glycyrrhizinate ethosomes through human stratum corneum and epidermis membranes by using Franz's cells and compared with the permeation profiles of drug solutions either in water or in a water–ethanol mixture. The ethosomal suspension showed very good skin tolerability in human volunteers, also when applied for a long period (48 h). Ethosomes elicited an increase of the in vitro percutaneous permeation of both Methyl nicotinate and Ammonium glycyrrhizinate. Ethosomes were able to significantly enhance the anti-inflammatory activity of Ammonium glycyrrhizinate compared to the ethanolic or aqueous solutions of this drug.

Lodzki et al. designed a transdermal delivery system for Cannabidol by using ethosomal carriers. Transdermal application of ethosomal Cannabidol prevented the inflammation and edema induced by sub-plantar injection of Carrageenan in the same animal model. Thus, ethosomes enabled Cannabidol skin permeation and its accumulation in a depot at levels that demonstrated.

**Transdermal delivery of Insulin**

Touitou et al. experimentally tested the effect of an ethosomal Insulin formulation that was applied to the skin on blood glucose level. The ethosomal formulation caused much as a 60% decrease in blood glucose levels in both normal and diabetic rats and kept the level constant for at least 8 hours.
Transdermal delivery of Hormonal agents

Kaplun and Touitou et al. have demonstrated in-vitro and in-vivo delivery of Testosterone. Testosterone delivery from Testosterone® versus Testoderm® was in-vitro for skin permeation and in-vivo in animals for percutaneous absorption. The results of the in-vitro experiments showed that the amount of Testosterone permeating into the skin from Testosome® patch was significantly higher than from Testoderm®. The in-vivo results presented as AUC of serum Testosterone indicated that Testosome® was able to systemically deliver increased amounts of Testosterone6,13.

Improved Pharmacokinetic release profile

Esposito et al. investigated basic properties and the in vitro release rate kinetics of Azelaic acid, alternatively vehiculated in different phospholipid-based vesicles such as ethosomes or liposomes. Diffusion of Azelaic acid from ethosomal or liposomal dispersions and from ethosomes and liposomes incorporated in a viscous gel was investigated by a Franz cell assembled with synthetic membranes. The release rate was more rapid from ethosomal systems than from liposomal systems26.

Improved Skin retention of Minoxidil

Kim et al. prepared three kinds of topical dosage forms of Minoxidil, namely, vesicle, double emulsions, and an inclusion complex with Hydroxypropyl-g-cyclodextrin (HP-g-CD). The skin retention of Minoxidil in the preparations was evaluated in-vitro using hairless mouse skins. Retention was highest when the drug was encapsulated in cationic vesicles. Nonionic vehicle, the double emulsion and HP-g-CD left no significant amount of drug penetrated through the skin. In-vivo hair growth promotion effect of each dosage form was investigated, in which the sample application on to clipped back of female mice and the subsequent rinsing of the backs was done once a day for 30 days. Only Minoxidil in the cationic vesicles
demonstrated hair growth promotion effect, possibly due to significant skin retention\(^2\).

**Improved permeation activity**

Osmotics Inc., USA, reported a new cellulite cream called *Lipoduction*, which smooth the skin and has a break through permeation technology called ethosomes that penetrate skin lipid barrier and deliver ingredients directly into fat cells. Flexible nanospheres (ethosomes) in *lipoduction* cream have been introduced that allow a cocktail of fat-metabolizing ingredients to reach the fat cells 700\% more effectively than the current cream. Ingredients in *lipoduction* improved the appearance of cellulite by up to 80 percent in less than 60 days\(^6\).

**Newer potentials**

Transdermal absorption of polypeptides is currently under investigation. The high interest of ethosomes in the design of new therapies has been investigated with other drugs such as Propranolol. In this respect ethosomes showed their potential as transdermal dosage forms for prophylaxis of migraine. Moreover the ability of ethosomes to deliver compounds to cells in culture was investigated.

**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. 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.  

Table No 2: Ethosomes as a carrier of various drug molecules

<table>
<thead>
<tr>
<th>Drug</th>
<th>Applications</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acyclovir</td>
<td>Treatment of Herpetic infection</td>
<td>Improved drug delivery</td>
</tr>
<tr>
<td>Zidovudine</td>
<td>Treatment of AIDS</td>
<td>Improved transdermal flux</td>
</tr>
<tr>
<td>Trihexyphenidyl HCl</td>
<td>Treatment of Parkinsonian syndrome</td>
<td>Increased drug entrapment efficiency, reduced side effect &amp; constant systemic levels</td>
</tr>
<tr>
<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>Insulin</td>
<td>Treatment of Diabetes</td>
<td>Improved therapeutic efficacy of drug</td>
</tr>
<tr>
<td>Testosterone</td>
<td>Treatment of male hypogonadism</td>
<td>Enhance skin permeation</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>Minoxidil</td>
<td>Hair growth promotion effect</td>
<td>Higher skin retention</td>
</tr>
<tr>
<td>Bacitracin</td>
<td>Treatment of dermal infections</td>
<td>Reduced drug toxicity</td>
</tr>
<tr>
<td>Diclofenac</td>
<td>Treatment of inflammation</td>
<td>Targeted delivery for prolonged duration</td>
</tr>
<tr>
<td>DNA</td>
<td>Delivery of DNA</td>
<td>Selective and targeted expression of genes</td>
</tr>
<tr>
<td>Azelaic acid</td>
<td>Treatment of acne</td>
<td>Prolonged drug release</td>
</tr>
<tr>
<td>Ammonium glycyrrhizinate</td>
<td>Improved anti-inflammatory activity</td>
<td>Prolonged release</td>
</tr>
</tbody>
</table>
Interest in the formulation and development of ethosomes is increasing tremendously as they are soft, malleable vesicles and potential carriers 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 which can be beneficial for total health care potential of universe.