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Review Article

Ethosomes: A review

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ABSTRACT

Transdermal drug delivery, self-contained, discrete dosage forms which, when applied to the intact skin, deliver the drug, through the skin at controlled rate to the systemic circulation. Ethosomes, noninvasive delivery carriers that enable drugs to reach deep into the skin layers or the systemic circulation made up of phospholipids, high concentration of ethanol and water. This review article summarizes structure, advantages, disadvantages, composition and mechanism of drug penetration method of preparation, evaluation and applications of ethosomes.

1. Introduction

Transdermal drug delivery system (TDDS) showed promising result in comparison to oral drug delivery system as it eliminates gastrointestinal interferences and first pass metabolism of the drug but the main drawback of TDDS is it encounters the barrier properties of the Stratum Corneum i.e. only the lipophilic drugs having molecular weight < 500 Da can pass through it[1,2]. To improve the permeation of drugs through the skin various mechanisms have been investigated, including use of chemical or physical enhancers, such as iontophoresis, sonophoresis, etc. Liposomes, niosomes, transferosomes and ethosomes also have been reported to enhance permeability of drug through the stratum corneum barrier. Permeation enhancers increase the permeability of the skin, so that the drugs can cross through the skin easily. Unlike classic liposomes[3], that are known mainly to deliver drugs to the outer layers of skin, ethosomes can enhance permeation through the stratum corneum barrier[4,5]. Ethosomes permeate through the skin layers more rapidly and possess significantly higher transdermal flux in comparison to conventional liposomes[6-8].

1.1 Ethosomes

"Ethosomes are ethanolic liposomes". Ethosomes can be defined as noninvasive delivery carriers that enable drugs to reach deep into the skin layers and/or the systemic circulation. These are soft, malleable vesicles tailored for enhanced delivery of active agents. The vesicles have been well known for their importance in cellular communication and particle transportation for many years. Vesicles would also allow controlling the release rate of drug over an extended time, keeping the drug shielded from immune response or other removal systems and thus 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 of a vesicle derivative, known as an Ethosomes[9].

Ethosomes are the slight modification of well established drug carrier liposome. Ethosomes are lipid vesicles containing phospholipids, alcohol (ethanol and isopropyl alcohol) in relatively high concentration and water. Ethosomes are soft vesicles made of phospholipids and ethanol (in higher quantity) and water. The size range of ethosomes may vary from tens of nanometers (nm) to microns (µ) ethosomes permeate through the skin layers more rapidly and possess significantly higher transdermal flux.

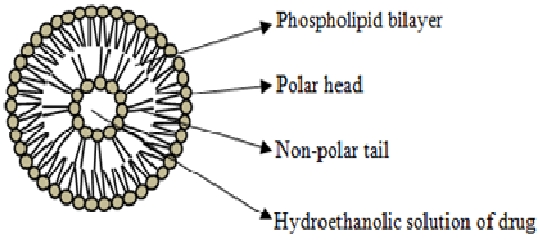

Figure No.1: Structure of ethosome[10]

1.1.1 Advantages of Ethosomal Drug Delivery

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In comparison to other transdermal & dermal delivery systems, Ethosomal drug delivery systems contain several advantages. Few advantages are;

1. Delivery of large molecules (peptides, protein molecules) is possible.
2. It contains non-toxic raw material in formulation.
3. Enhanced permeation of drug through skin for transdermal drug delivery.
4. Ethosomal drug delivery system can be applied widely in Pharmaceutical, Veterinary, Cosmetic fields.
5. High patient compliance: The ethosomal drug is administrated in semisolid form (gel or cream) hence producing high patient compliance.
6. Simple method for drug delivery in comparison to Iontophoresis and Phonophoresis and other complicated methods.
7. The Ethosomal system is passive, non-invasive and is available for immediate commercialization**[11]**.

1.1.2 Disadvantages of Ethosomal Drug Delivery

They required High blood levels cannot be administered – limited only to potent molecules, those requiring a daily dose of 10mg or less.

1. Ethosomal administration is not a means to achieve rapid bolus type drug input, rather it usually designed to offer slow, sustained drug delivery.
2. Adequate solubility of the drug in both lipophilic and aqueous environments to reach dermal microcirculation and gain access to the systemic circulation.
3. The molecular size of the drug should be reasonable that it should be absorbed percutaneously.
4. Adhesive may not adhere well to all types of skin.
5. May not be economical.
6. Poor yield.
7. Skin irritation or dermatitis due to excipients and enhancers of drug delivery systems.
8. In case if shell locking is ineffective then the ethosomes may coalescence and fall apart on transfer into water.
9. Loss of product during transfer from organic to water media.
10. The main advantage of ethosomes over liposomes is the increased permeation of the drug**[12-17]**

1.1.3 Composition of Ethosomes

Ethosomes are composed mainly of phospholipids, (phosphatidylcholine, phosph-atidylserine, phosphatitidic 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**[18-19]**

1.1.4 Advantage of high alcohol content

Ethanol is an established efficient permeation enhancer and is present in quite high concentration (20-50%) in ethosomes. However, due to the interdigitation effect of ethanol on lipid bilayers, it was commonly believed that vesicles could not coexist with high concentration of ethanol. Touitou**[20]** discovered and investigated lipid vesicular systems embodying ethanol in relatively high concentration and named them ethosomes. The basic difference between liposomes and ethosomes lies in their composition. The synergistic effect of combination of relatively high concentration of ethanol (20-50%) in vesicular form in ethosomes was suggested to be the main reason for their better skin permeation ability. The high concentration of ethanol (20-50%) in ethosomal formulation could disturb theskin lipid bilayer organization. Therefore, when integrated into a vesicle membrane, it could give an ability to the vesicles to penetrate the SC. Furthermore, due to high ethanol concentration the ethosomal lipid membrane was packed less tightly than conventional vesicles but possessed equivalent stability. This allowed a softer and malleable structure giving more freedom and stability to its membrane, which could squeeze through small openings created in the disturbed SC lipids. In addition, the vesicular nature of ethosomal formulations could be modified by varying the ratio of components and chemical structure of the phospholipids. The versatility of ethosomes for systemic delivery is evident from the reports of enhanced delivery of quite a few drugs like acyclovir, minoxidil, triphexyphenidyl, testosterone, cannabidol and zidovudine.

1.1.5 Mechanism of Drug Penetration

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:

1. Ethanol effect
2. Ethosomes effect

1. Ethanol effect

Ethanol acts as a penetration enhancer through the skin. The mechanism of its penetration enhancing effect is well known. Ethanol penetrates into intercellular lipids and increases the fluidity of cell membrane lipids and decrease the density of lipid multilayer of cell membrane.

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**[21]**.

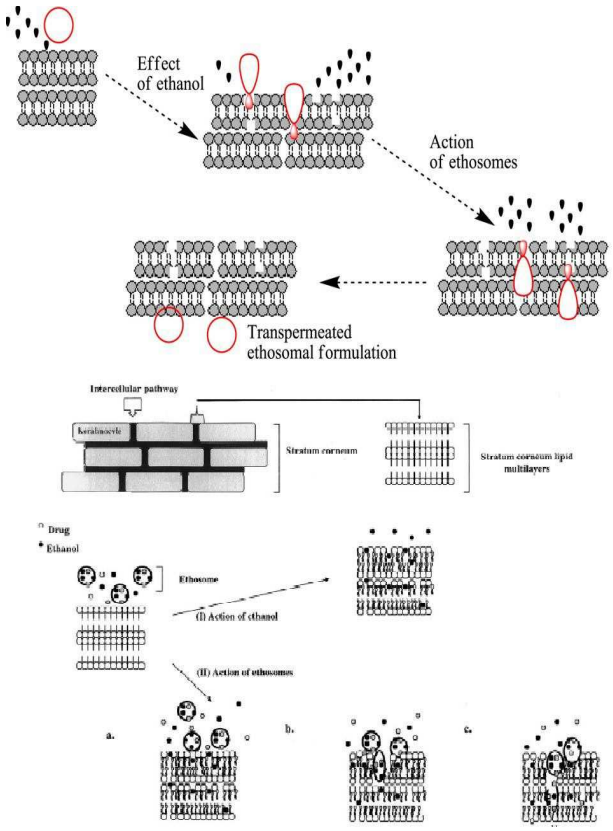

Figure No. 2: Proposed mechanism for skin delivery of ethosomal systems

Table No. 1 Different Additives Employed In Formulation of Ethosomes**[27-30]**

S.No.	Class	Example	Uses
1.	Phospholipid	Soya phosphatidyl choline, Egg phosphatidyl choline, Dipalmityl phosphatidyl choline, Distearyl phosphatidyl choline	Vesicles forming component
2.	Alcohol	Ethanol, Isopropyl alcohol	For providing the softness for vesicle membrane as a penetration enhancer
3.	Polyglycol	Propylene glycol, Transcutol RTM	As a skin penetration enhancer
4.	Cholesterol	Cholesterol	For providing the stability to vesicle membrane
5.	Dye	Rhodamine-123, Rhodamine red, Fluorescene Isothiocynate, (FITC)6-Carboxyfluorescence	For characterization study
6.	Vehicle	Carbopol Đ934	As a gel former

2. Skin

The skin is the largest organ of the body. The skin an average adult body is about 20 square feet and it received about one third of total available blood.

2.1 The skin is multilayered organ composed of three histological tissues

The outermost layer of skin, epidermis is which provides a waterproof barrier and creates our skin tone. Dermis, beneath epidermis, contains tough connective tissue, hair follicles, and sweat glands and deeper subcutaneous tissue (hypodermis) is made of fat and connective tissue**[23].**

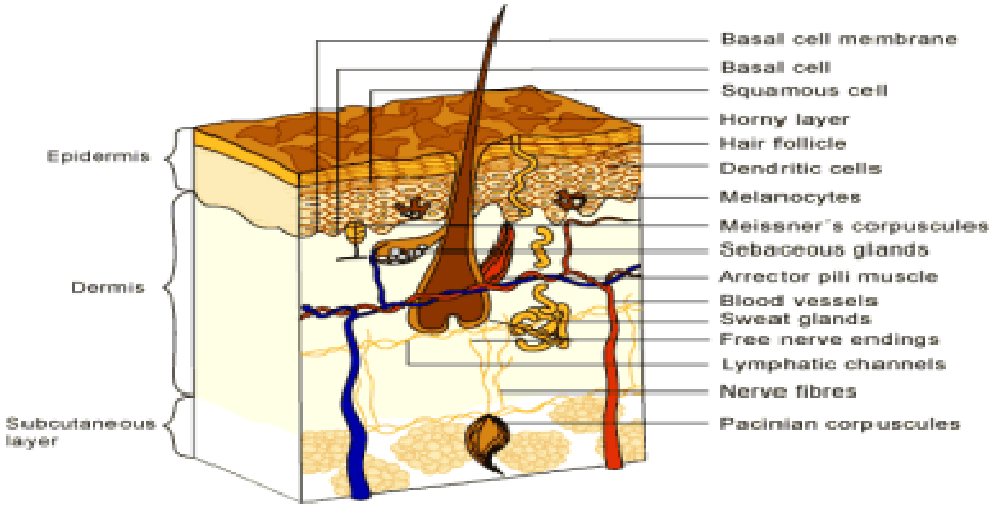

Figure No. 3: Structure of skin**[24]**

1.2.2 Human skin consists of a stratified, cellular epidermis and an underlying dermis of connective tissue

1. Epidermis
2. Dermis

1. Epidermis

- The four layers of the epidermis are:

1. Stratum basale (basal or germinativum cell layer)
2. Stratum spinosum (spinous or prickle cell layer)
3. Stratum granulosum (granular cell layer)
4. Stratum corneum (horny layer).

- The epidermises consist of stratified squamous epithelium. The main cells of the epidermis are known as keratinocytes, which synthesis the protein keratin.

In addition, the stratum lucidum is a thin layer of translucent cells seen in thick epidermis.

2. Dermis

- The dermis varies in thickness, ranging from 0.6 mm on the eyelids to 3 mm on the back, palms and soles. It is found below the epidermis and is composed of a tough, supportive cell matrix.
- Two layers comprise the dermis;

1. A thin papillary layer.
2. A thicker reticular layer.

1.2.3 Function of skin

They Provides a protective barrier against mechanical, thermal and physical injury and nonoxious agents.

- Prevents loss of moisture.
- Reduces the harmful effects of UV radiation.
- Acts as a sensory organ.
- Helps regulate temperature control.
- Plays a role in immunological surveillance.
- Synthesizes vitamin D3 (cholecalciferol)**[25].**

1.3 Methods of preparation of Ethosomes

Ethosomes can be prepared by two very simple and convenient methods that is;

1. Cold method**[26,27]**
2. Hot method

1. Cold Method

This is the most common method utilized for the preparation of ethosomal formulation. 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. This mixture is heated to 30^0C in a water bath. The water heated to 30^0C in a separate vessel is added to the mixture, which is then stirred for 5 min in a covered vessel. The vesicle size of ethosomal formulation can be decreased to desire extend using sonication**[28]** or extrusion**[29]**method. Finally, the formulation is stored under refrigeration **[30].**

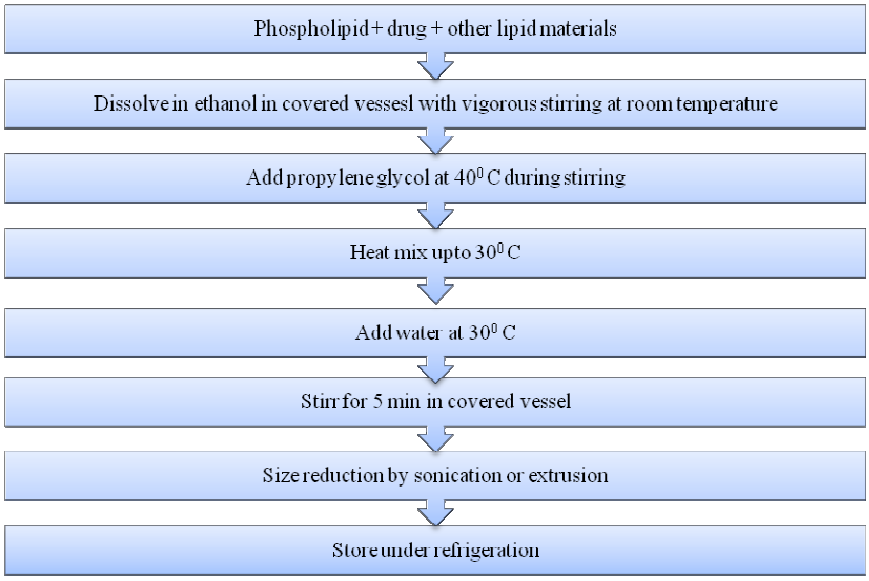

Figure No. 4: Cold method for the preparation of ethosomes

2. Hot method

In this method phospholipid is dispersed in water by heating in a water bath at 40^{0}C until a colloidal solution is obtained. In a separate vessel ethanol and propylene glycol are mixed and heated to 40^{0}C. Once both mixtures reach 40^{0}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**[31].**

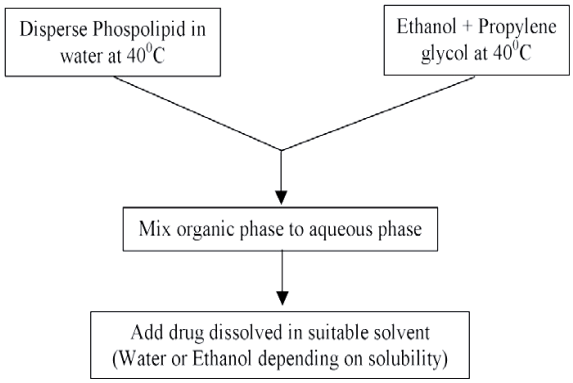

Figure No. 5: Hot method for the preparation of ethosomes

1.4 Method of Characterizations of Ethosomal Formulation[32]

1. Vesicle shape

Visualization of ethosomes can be done using transmission electron microscopy (TEM) and by scanning electron microscopy (SEM). Visualization by electron microscopy reveals an ethosomal formulation exhibited vesicular structure 300-400 nm in diameter. The vesicles seem to be malleable as evident by their imperfect round shape.

2. 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).

3. Drug entrapment

The entrapment efficiency of ethosomes can be measured by the ultracentrifugation technique.

4. Transition Temperature

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

5. 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.

6. 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.

7. Stability studies

The stability of vesicles can be determined by assessing the size and structure of the vesicles over time. Mean size is measured by DLS and structure changes are observed by TEM.

8. Skin permeation studies

The ability of the ethosomal preparation to penetrate into the skin layers can be determined by using confocal laser scanning microscopy (CLSM).

Table No. 2. Characterization of ethosomes[33-39]

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

1.5 Evaluation of Ethosome[40,41]

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 aluminums 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 cm2 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 Lglutamine at 37°C under a 5% CO_2 atmosphere. Cytotoxicity was expressed as the cytotoxic dose 50 (CD50) that induced a 50% reduction of absorbance at 540nm.

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 monitoredat 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).

1.6 Therapeutics application of Ethosomes

Ethosomes can be used for many purposes in drug delivery. Ethosomes are mainly used as replacement of liposomes. Mainly the transdermal route of drug delivery is preferred. Ethosomes can be used for thetransdermal delivery of hydrophilic and impermeable drugs through the skin. Various drugs have been used with ethosomal carrier**[42,43].**

1. Pilosebaceous targeting

Hair follicles and sebaceous glands are increasingly being recognized as potentially significant elements in the percutaneous drug delivery. Furthermore, considerable attention has also been focused on exploiting the follicles as transport shunts for systemic drug delivery. Minoxidil is a lipid-soluble drug used topically on the scalp for the treatment of baldness by pilosebacious delivery. Interest in pilosebaceous units has been directed towardstheir use as depots for localized therapy, particularly for the treatment of follicle-related disorders such as acne or alopecia**[44].**

2. Transcellular Delivery

Touitou et al.**[45]** in their study demonstrated better intracellular uptake of bacitracin, DNA and erythromycinusing CLSM and FACS techniques in different cell lines. Better cellular uptake of anti-HIV drug zidovudine and lamivudine in MT-2 cell line from ethosomes as compared to the marketed formulation suggested ethosomes to be an attractive clinical alternative for anti-HIV therapy.

3. Delivery of problematic drug molecules

Oral delivery of large biogenic molecules such as peptides or proteins and insulin is difficult because they are completely degraded in the GIT tract hence transdermal delivery is a better alternative. But conventional transdermal formulation of biogenic molecules such as peptides or protein and insulin has poor permeation. Formulating these above molecules into ethosomes significantly increase permeation and therapeutic efficacy**[46].**

4. In the treatment herpetic infection

5% acyclovir ethosomal preparation compared herpetic infections**.**

5. Transdermal Delivery of Hormones

Oral administration of hormones is associated with problems like high first pass metabolism, low oral bioavailability and several doses dependent side effects. The risk of failure of treatment is known to increase with each pill missed.

6. Delivery of Anti-Arthritis Drug

Topical delivery of anti-arthritis drug is a better option for its site-specific delivery andovercomes the problem associated with conventional oral therapy.

7. Delivery of Antibiotics

Topical delivery of antibiotics is a better choice for increasing the therapeutic efficacy of this agents.Conventional oral therapy causes several allergic reactions along with several side effects. Conventional external preparations possess low permeability to deep skin layers and subdermal tissues .Ethosomes can circumvent this problem by delivering sufficient quantity of antibiotic into deeper layers of skin. Ethosomes penetrate rapidly through the epidermis and bring appreciable amount of drugs into the deeper layer of skin and suppress infection at their root. With this purpose in mind Godin and Touitou prepared bacitracin and erythromycin loaded ethosomal formulation for dermal and intracellular delivery. The results of this study showed that the ethosomal formulation of antibiotic could be highly efficient and would overcome the problems associated with conventional therapy**[47].**

8. Cosmaceutical Applications of Ethosomes

The advantage of applying ethosomes in cosmaceuticals is not only to increase the stability of the cosmetic chemicals and decrease skin irritation from the irritating cosmetic chemicals, but also for transdermal permeation enhancement, especially in the elastic forms. However, the compositions and sizes of the vesicles are the main factors to be considered to obtain these advantages of the elastic vesicles for cosmaceuticals applications**[48].**

90. 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. Touitou et al. in their study encapsulated the GFP-CMVdriven transfecting construct into ethosomal formulation. They applied this formulation to the dorsal skin of 5-week male CD1 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[49-51]

10. Marketed Product of Ethosomes[52-54]

In 2000, the ethosomes technology began to Commercialize. There are only two companies which developed ethosomes products (Table 3).

Table No. 3. Marketed Products Based On Ethosomal Drug Delivery System

S.No.	Name of product	Uses	Manufacturer
1.	Cellutight EF	Topical cellulite cream, contains a powerful combination of ingredients to increase metabolism and break down fat	Hampden Health, USA
2.	Decorin cream	Anti-aging cream, treating, repairing, and delaying the visible aging signs of the skin including wrinkle lines, sagging, age spots, loss of elasticity, and hyper pigmentation	Genome Cosmetics, Pennsylvania, US
3.	Nanominox	First minoxidil containing product, which uses ethosomes. Contains 4% Minoxidil, well-known hair growth promoter that must be metabolized by sulfation to the active compound.	Sinere, Germany
4.	Noicellex	Topical anti-cellulite cream	Novel Therapeutic Technologies, Israel
5.	Skin genuity	Powerful cellulite buster, reduces orange peel	Physonics, Nottingham, UK
6.	Supravir cream	For the treatment of herpes virus	Trima,Israel

2. Conclusion

It can be easily concluded that ethosomes can provide better skin permeation than liposomes. Ethosomes are more advantages when compared to transdermal and dermal delivery. They are the noninvasive drug delivery carriers that enable drugs to reach the deep skin layers finally delivering to the systemic circulation. It delivers large molecules such as peptides, protein molecules. Ethosomes are characterized by simplicity in their preparation, safety and efficacy and can be tailored for enhanced skin permeation of active drugs. The main limiting factor of transdermal drug delivery system i.e. epidermal barrier can be overcome by ethosomes to significant extent. Ethosomal carrier opens new challenges and opportunities for the development of novel improved therapies. Further, research in this area will allow better control over drug release in vivo and long term safety data, allowing the therapy more effective.

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