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A DETAILED FOCUS ON ETHOSOMAL DRUG DELIVERY SYSTEM AND ITS POTENTIALITIES

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ABSTRACT

Ethosomes are interesting and innovative vesicular systems that have appeared in the field of pharmaceutical technology and drug delivery in recent years. This carrier presents interesting features correlated with its ability to permeate intact through the human skin due to its high deformability. Ethosomes are soft, malleable vesicles tailored for enhanced delivery of active agents. Ethosomes are novel lipid vesicles embodying high concentration (20-45%) of ethanol and are prepared by dissolving the lipids and drug in ethanol. Ethosomes are used mainly for transdermal delivery of drugs. Ethosomes have higher penetration rate through the skin as compared to liposomes hence these can be used widely in place of liposomes. Ethosomes were designed to enhance the delivery of drugs into the deep layers of the skin and through the skin. Ethosomes have become an area of research interest, because of its enhanced skin permeation, improved drug delivery; increased drug entrapment efficiency etc. Ethosomal formulation may contain many drugs such as acyclovir, salbutamol, insulin, cyclosporine, fluconazole, minoxidil, etc.The ethosomes were found to be suitable for various applications within the pharmaceutical, biotechnology, veterinary, cosmetic and nutraceutical markets. This article highlights the recent status of the development of ethosome and summarizes its advantages, composition, and method of preparation, mechanism of skin penetration, characterization, applications and stability.

INTRODUCTION

One of the major advances in vesicle research was the finding that some modified vesicles possessed properties that allowed them to successfully deliver drugs in deeper layers of skin. Transdermal delivery is important because it is a noninvasive procedure for drug delivery. Further, problem of drug degradation by digestive enzyme after oral administration and discomfort associated with parenteral drug administration can be avoided. It is the most preferred route for systemic delivery of drugs to pediatric, geriatric and patient having dysphasia. Hence transdermal dosage forms enjoy being the most patient complaint mode of drug delivery[1].

Transdermal drug delivery (TDD) is the potential route for delivering systemic drugs. The greatest challenge is the barrier nature of stratum corneum.So major aim of transdermal drug delivery system is to cross the stratum corneum. Many techniques have been aimed to disrupt or weaken the barrier property of skin. One method is the use of vesicle formulations as skin delivery systems. Vesicles would 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. Intensive research over the past two decades led to the development of novel carriers, the ethanolic liposomes that have been termed ethosomes. Ethosomes are lipid vesicles containing phospholipids, alcohol (ethanol and isopropyl alcohol) in relatively high concentration and water. **(Fig.1).**It is noninvasive delivery and soft vesicle carrier and can entrap drug molecules with various physicochemical characteristics i.e. of hydrophilic, lipophilic, or amphiphilic. The size range of ethosomes may vary from tens of nanometers to microns[1,2].

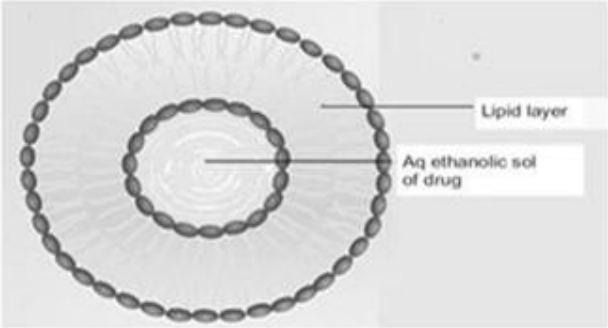

Fig: 1 Structure of Ethosome

ROUTES OF PENETRATION

Human skin comes into contact with sebum, cellular debris, microorganisms and other materials, which somewhat affect the permeation of vesicles. The penetrant permeates by three potential pathways to the viable tissue: (i) Through hair follicles with associated sebaceous glands, (ii) Via

sweat ducts (iii) Across continuous stratum corneum between these appendages. These pathways are important for ions and large polar molecules that struggle to cross intact stratum corneum[3].

RATIONALE FOR TRANSDERMAL DRUG DELIVERY

Transdermal drug delivery offers several distinct advantages including relatively large and readily accessible surface area for absorption, ease of application and termination of therapy. Moreover evolution of better technologies for delivering drug molecules, safe penetration enhancers and the use of vesicular carriers have rejuvenated the interest for designing transdermal drug delivery system for drugs that were thought to be unfit for transdermal delivery[3]**.**

ETHOSOMES COMPOSITION

Ethosomes may contain phospholipids (Vesicles forming component) with various chemical structures like phosphatidylcholine (PC), hydrogenated PC, phosphatidic acid (PA), phosphatidylserine (PS), phosphatidylethanolamine (PE), phosphatidylglycerol (PPG), phosphatidylinositol (PI), hydrogenated PC, alcohol (penetration enhancer and for providing softness to vesicles) like ethanol or isopropyl alcohol, water and propylene glycol (penetration enhancer). Such a composition enables delivery of high concentration of active ingredients through skin. Drug delivery can be modulated by altering alcohol: water or alcohol-polyol: water ratio. Some preferred phospholipids are soya phospholipids such as Phospholipon 90 (PL-90). It is usually employed in a range of 0.5-10% w/w. Cholesterol are used for providing stability to vesicles its concentrations ranging between 0.1- 1% can also be added to the preparation. In addition, non-ionic surfactants (PEG-alkyl ethers) can be combined with the phospholipids in these preparations. Cationic lipids like cocoamide, dodecylamine, cetrimide etc. can be added too. The concentration of alcohol in the final product may range from 20 to 50%. The concentration of the non-aqueous phase (alcohol and glycol combination) may range between 22 to 70%.Carbopol 934 can also be added for converting in to gel form. (Table 1)[2,3,4].

Table.1.Different Additives Employed In Formulation of Ethosomes

Class	Example	Uses
Phospholipid	Soya phosphatidyl choline Egg phosphatidyl choline Dipalmityl phosphatidyl choline Distearyl phosphatidyl choline	Vesicles forming component
Polyglycol	Propylene glycol Transcutol RTM	As a skin penetration enhancer
Alcohol	Ethanol Isopropyl alcohol	For providing the softness for vesicle membrane As a penetration enhancer
Cholesterol	Cholesterol	For providing the stability to vesicle membrane
Dye	Rhodamine-123 Rhodamine red Fluorescene Isothiocynate (FITC) 6- Carboxy fluorescence	For characterization study
Vehicle	Carbopol Đ934	As a gel former

MECHANISM OF DRUG PENETRATION

The drug absorption probably occurs in following two phases **(Fig. 2)** [2,3,5].

1. Ethanol effect 2. Ethosomes effect

1. Ethanol effect:

Ethanol acts as penetration enhancer through skin. It penetrates into intercellular lipids, increases fluidity of cell membrane lipids and decrease density of lipid multilayer of cell membrane.

Ethosome 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.

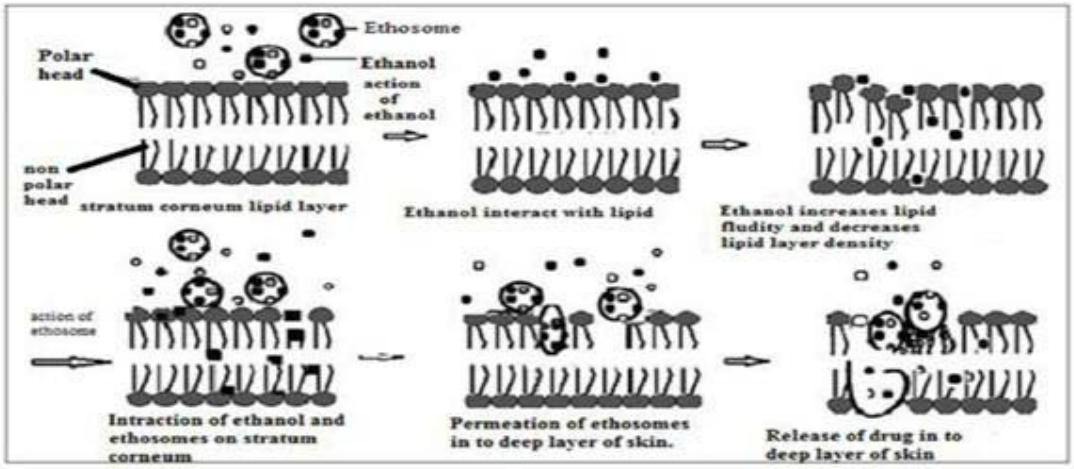

Fig. 2: Mechanism of Action of Ethosomes[29]

METHODS OF PREPARATION ETHOSOMES [6,7,8]

Ethosomes can be prepared by two very simple and convenient methods that are hot method,cold method.

Phospholipids Drugs

Ethanol

Slowly add double distilled water with constant mixing at 700 rpm

Ethanolic solution of Phospholipids

Sonication or extrusion

Figure 3: General Method for the Preparation of Ethosomes

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˚C in water bath. The heated water 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 or extrusion method. Finally, the formulation is stored under refrigeration **(Fig. 4).**

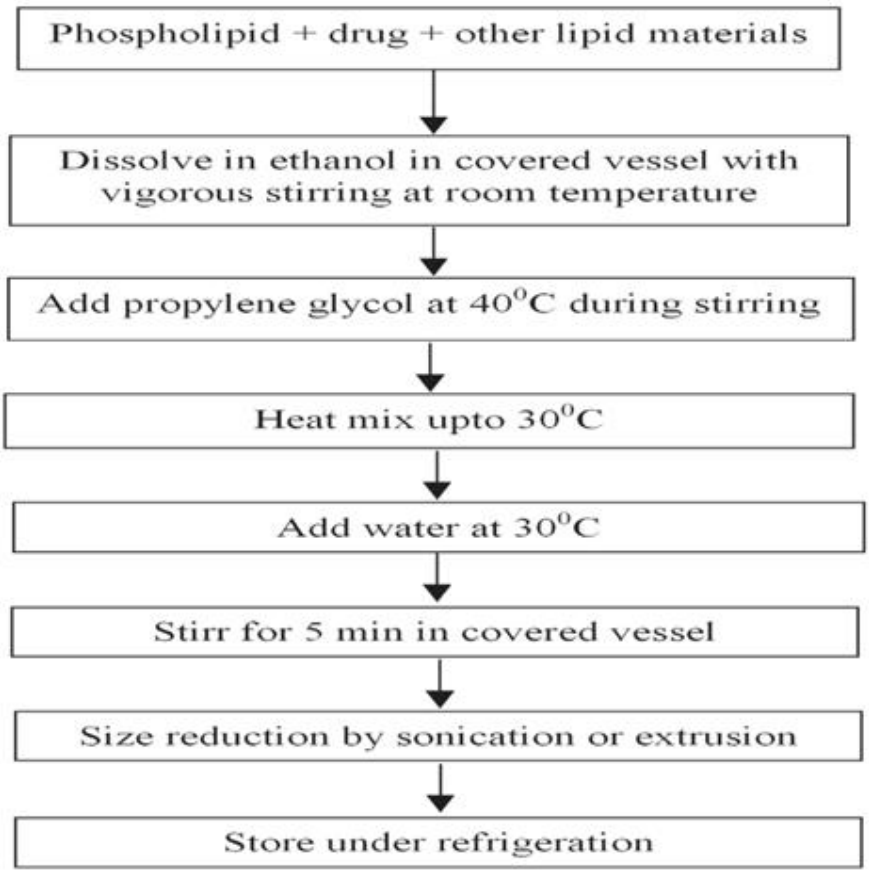

Fig. 4. Cold Method for the Preparation of Ethosomes

2. Hot method:

In this method phospholipid is dispersed in water by heating in a water bath at 40˚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 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 **(Fig. 5).**

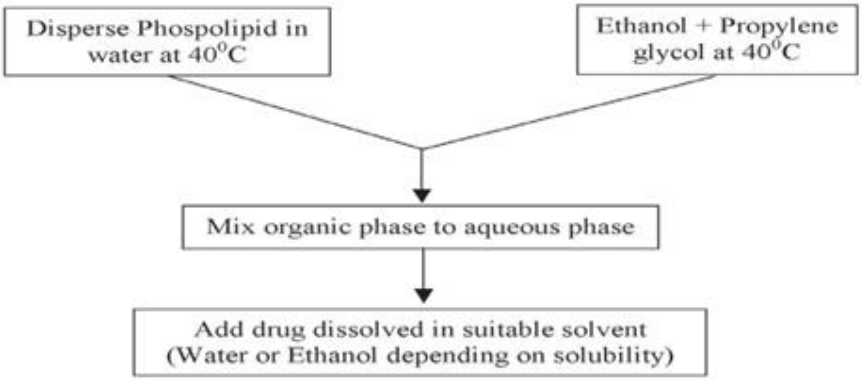

Fig.5 Hot Method for the Preparation of Ethosomes

PROPOSED MECHANISM OF SKIN PERMEATION OF ETHOSOMES

Fig.6 shows the schematic representation of mechanism of skin permeation of ethosomes. The stratum corneum lipid multilayers at physiological temperature are densely packed and highly conformationally ordered**.** Ethosomes formulations contain ethanol in their composition that interacts with lipid molecules in the polar headgroup regions, resulting in an increased fluidity of the stratum corneum lipid [2,9].

The high alcohol content is also expected to partial extract the stratum corneum lipids. These processes are responsible for increasing inter and intracellular permeability of ethosomes. In addition, ethanol imparts flexibility to the ethosomal membrane that facilitates their skin permeation and release drug in the deep layers of skin. The transdermal absorption of drugs could then result from fusion of ethosomes with skin lipids. This is expected to result in drug release at various points along the penetration pathway[3,10.]

CHARACTERIZATIONS OF ETHOSOMES [4,5,11]

1. Visualization

Visualization of ethosomes can be done using transmission electron microscopy (TEM) and by scanning electron microscopy (SEM) for surface morphology of prepared ethosomal vesicles.

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. Differential scanning calorimetry (DSC)

Transition temperature (Tm) of the vesicular lipid systems was determined by using the Mettler DSC 60 computerized with Mettler Toledo star software system (Mettler, Switzerland).The transition temperature was measured by using the aluminium crucibles at a heating rate 10 degree/minute, within a temperature range from 20˚C–300˚C.

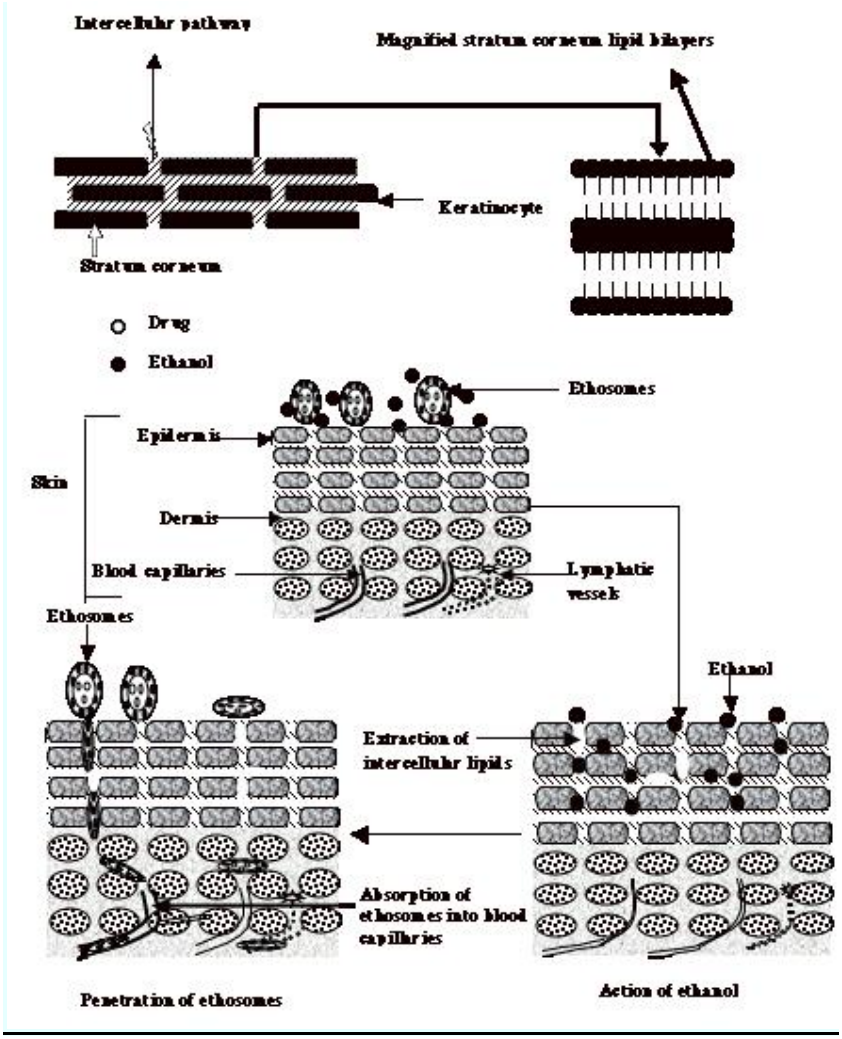

Fig. 6. Proposed mechanism of skin permeation of ethosomes

4. Surface Tension Activity Measurement

The surface tension activity of drug in aqueous solution can be measured by the ring method in a Du Nouy ring tensiometer.

5. Entrapment Efficiency

The entrapment efficiency of drug by ethosomes can be measured by the ultra centrifugation technique. The percentage drug entrapment can be calculated by using the following equation: %Drug entrapment = Amount of entrapped drug recovered/Total amount of drug×100.

6. Penetration and Permeation Studies

Depth of penetration from ethosomes can be visualized by confocal laser scanning.

7. Vesicle Stability

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. Drug Content: Drug can be quantified by a modified high performance liquid chromatographic method **(Table 2).**

TABLE.2. METHODS FOR CHARACTERIZATION OF ETHOSOMAL FORMULATION

Parameters	Methods
Vesicle shape (morphology)	*Transmission electron microscopy* Scanning electron microscopy
Entrapment efficiency	Mini column centrifugation method Fluorescence spectrophotometry
Vesicle size and size distribution	Dynamic light scattering method
Vesicle Skin interaction study Penetration and permeation studies	Confocal laser scanning microscopy Fluorescence microscopy Transmission electron microscopy Eosin-Hematoxylin staining
Phospholipid-ethanol interaction	^{31}P NMR Differential scanning calorimeter
Degree of deformability	Extrusion method
Zeta potential	Zeta meter
Turbidity	Nephelometer
In vitro drug release study	Franz diffusion cell with artificial or biological membrane, Dialysis bag diffusion
Drug deposition study	Franz diffusion cell
Stability study	Dynamic light scattering method Transmission electron microscopy

ADVANTAGES OF ETHOSOMAL DRUG DELIVERY [5,6,12]

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. 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 Ionotophoresis and Phonophoresis and other complicated methods

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

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

9. High market attractiveness for products with proprietary technology. Relatively simple to manufacture with no complicated technical investments required for production of ethosomes.

10. Better stability and solubility of many drugs as compared to conventional vesicles.

11. Relatively smaller size as compared to conventional vesicles.

12. Enhanced permeation of drug molecules to and through the skin to the systemic circulation.

LIMITATIONS OF ETHOSOMES [11,12]

1. Poor yield.

2. In case if shell locking is ineffective then the ethosomes may coalescence and fall apart on transfer into water.

3. Loss of product during transfer form organic to water media.

PATENTED AND MARKETED FORMULATION OF ETHOSOME [13]

● Noicellex TM an anti – cellulite formulation of ethosome is currently marketed in Japan.

● Lipoduction TM another formulation is currently used in treatment of cellulite containing pure grape seed extracts (antioxidant) is marketed in USA.

● Physonics is marketing anti – cellulite gel Skin Genuity in London.

● Nanominox© containing minoxidil is used as hair tonic to promote hair growth is marketed by Sinere.

ETHOSOMES APPLICATIONS [2,3,5,14]

1. In the treatment herpetic infection:

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

2. Transcellular delivery:

Ethosomes as compared to the marketed formulation suggested ethosomes to be an attractive clinical alternative for anti-HIV therapy.

3. Pilosabeceous targeting:

Ethosomes, the high ethanol containing vesicles are able to penetrate the deeper layers of the skin and hence appear to be vesicles of choice for transdermal drug delivery of hydrophilic and impermeable drugs through the skin.

4. Transdermal delivery of hormones:

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

5. Delivery of Anti-arthritis drug:

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

6. Delivery of HIV drugs:

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

7. 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 **(Table 3**).

Table 3 Application of Ethosomes as a drug carrier [15,16]

Drug	Purpose of Ethosomal delivery	Result	Application
Azelaic acid	Improves the sustained release	▪ Prolong drug release	Treatment of acne
DNA	Expression into cells	▪ Better expression of genes ▪ Selective targeting to cells	Treatment of genetic disorders
Diclofenac	Selective targeting the cells	▪ Selective delivery of drug to desired, for prolong period of time	NSAIDS
Erythromycin	Better cellular uptake	Prolonging drug action	Antimicrobial
Zidovudine	Better cellular uptake	▪ Improved transdermal flux ▪ Improved in biological activity two to three times	AntiHIV

		▪ Prolonging drug action ▪ Reduced drug toxicity ▪ Affected histology of skin	
Bacitracin	Better cellular uptake	▪ Improved dermal deposition ▪ Improvedintracellular delivery ▪ Increased bioavailability	Antibacterial
Insulin	GIT degradation	▪ Significant decrease in blood glucose level ▪ Provide control release	Treatment of diabetes
Trihexyphenidyl hydrochloride	4-5 times higher than that from liposome	▪ Improved transdermal flux ▪ Provide controlled release ▪ Improved patient compliance ▪ Biologically active at dose several times lower than the currently used formulation	Treatment of Parkinson's disease
Cannabidol	low bioavailability	▪ Improved skin deposition ▪ Improved biological activity ▪ Prolonging drug action	Rheumatoid Treatment
Acyclovir	Poor skin permeation	▪ Increase skin permeation ▪ Improved in biological activity two to three times ▪ Pharmacodynamic profile	Treatment of Herpes labialis
Enalapril maleate	Low oral bioavailability Major side effects in oral delivery	▪ Increased bioavailability	Treatment of Hypertension
Minoxidil	Pilocebaceous targeting	Accumulation in skin increased	Treatment of baldness
Ammonium glycyrrhizinate	Poor skin permeation Oral bioavailability	▪ Improved dermal deposition exhibiting sustained release ▪ Improved biological anti-inflammatory activity	Treatment of inflammatory based skin diseases
Fluconazole	Poor skin permeation	Improved skin permeation	Treatment of candidiasis
Methotrexate	Poor skin permeation	Improved skin permeation	Treatment of psoriasis
Salbutamol	Enhancing drug delivery through skin with ethosomes	Enhanced drug delivery through skin with ethosomes	Anti-asthmatic Bronchodilator

Marketed Product of Ethosomes [2,3,17]

In 2000, the ethosomes technology began to Commercialize. There are only two companies which developed ethosome products (**Table 4**).

Table. 4. Marketed Products Based On Ethosomal Drug Delivery System

Name of product	Uses	Manufacturer
Cellutight EF	Topical cellulite cream, contains a powerful combination of ingredients to increase metabolism and break down fat	Hampden Health, USA
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
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
Noicellex	Topical anti-cellulite cream	Novel Therapeutic Technologies, Israel
Skin genuity	Powerful cellulite buster, reduces orange peel	Physonics, Nottingham, UK
Supravir cream	For the treatment of herpes virus, formulation of acyclovir drug has a long shelf life with no stability problems, stable for at least three years, at 25°C. Skin permeation experiments showed that the cream retained its initial penetration enhancing properties even after three years	Trima, Israel

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. Ethosomes are soft, malleable vesicles and possible carrier for transportation of drugs. Ethosomes are characterized by simplicity in their preparation, safety and efficacy and can be tailored for enhanced skin permeation of active drugs. Ethosomes have been found to be much more efficient at delivering drug to the skin, than either liposomes or 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.

Introduction of ethosomes has initiated a new area in vesicular research for transdermal drug delivery. Different reports show a promising future of ethosomes in making transdermal delivery of various agents more effective. Further, research in this area will allow better control over drug release in vivo, allowing physician to make the therapy more effective. Ethosomes offers a good opportunity for the non- invasive delivery of small, medium and large sized drug molecules. So it can be smartly concluded that ethosomal formulation has promising future in effective dermal and transdermal delivery of bioactive agents.

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