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ETHOSOME: A RECENT OPTIMIZED TECHNOLOGY FOR TRANSDERMAL DRUG PENETRATION

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ABSTRACT
Ethosome system are novel lipid vesicular carriers containing a relatively high percentage of ethanol. These nanocarriers are especially designed for the efficient delivery of therapeutic agents with different physicochemical properties into deep skin layers and across the skin. Ethosomes have undergone extensive research since they were invented in 1996; new compounds were added to their initial formula, which led to the production of new types of ethosomal systems. Different preparation techniques are used in the preparation of these novel carriers. For ease of application and stability, ethosomal dispersions are incorporated into gels, patches, and creams. Highly diverse in vivo models are used to evaluate their efficacy in dermal/ transdermal delivery, in addition to clinical trials. This article provides a detailed review of the ethosomal systems and categorizes them on the basis of their constituents to classical ethosomes, binary ethosomes, and transethosomes. The differences among these systems are discussed from several perspectives, including the formulation, size, ζ-potential (zeta potential), entrapment efficiency, skin-permeation properties, and stability. This paper gives a detailed review on the effects of ethosomal system constituents, preparation methods, and their significant roles in determining the final properties of these nanocarriers. Furthermore, the novel pharmaceutical dosage forms of ethosomal gels, patches, and creams are highlighted. The article also provides detailed information regarding the in vivo studies and clinical trials conducted for the evaluation of these vesicular system.

KEYWORDS: Ethosome ,cold method,CLSM.

INTRODUCTION
Administration of drugs through the skin has been always an attractive as well as a challenging area for research. The outer layer of the skin, the stratum corneum, represents the most resistible barrier to drug permeation across the skin, Hence reduce the biological availability of drugs. Therefore, special carriers are required to overcome the natural skin barrier to deliver drug molecules Having different physicochemical properties to the systemic circulation. Advances in modern technologies are resulting in a larger number of drugs being delivered transdermally including conventional hydrophobic small molecule drugs, hydrophilic drugs and macromolecules.[1,2]

Transdermal administration of drug moiety eliminates frequent dosing and plasma level fluctuation which are usually associated with oral and parentral dosing .For drugs having short biological half life period can be delivered in transdermal route to maintain a constant drug concentration in therapeutic range.[3,4,5] All this leads to enhanced patient compliance, especially when long-term treatment is required, as in hormonal therapy, pain management, hypertension and smoking cessation therapy. The general acceptability of transdermal products by patients is very high, which is also evident from the increasing market for transdermal products. The transdermal drug delivery market, worth $12.7 billion dollars in 2005, is expected to reach $32 billion in 2017.

A new era of research in this field was opened with the use of liposomes for the topical delivery of triamcinolone, and since then a wide range of novel lipid-based vesicular systems have been developed. Deformable or elastic liposomes, which are currently known as transfersomes, were introduced by Cevc and Blume in 1992 and followed by the innovative work of Touitou et al, which led to the discovery of a novel lipid vesicular system called ethosomes. Ethosomal systems differ from liposomes because they contain relatively high concentrations of ethanol, in addition to phospholipids and water.[5,6] New generations of ethosomal systems have been introduced since then by adding other compounds to the basic ethosomal formula

in an attempt to enhance vesicular characteristics and skin permeation.

In spite of the ongoing research in the field of dermal and transdermal drug delivery, efficient administration of drugs by topical application remains a challenge. One simple and convenient approach is application of drugs in formulation with elastic vesicles .Ethosomes are noninvasive carrier for enhanced skin delivery of drugs which are phospholipid vesicular systems embodying ethanol in relatively high concentrations 20%-45% .it enables the drugs to reach the deep skin layers and/or the systemic circulation. Ethosomes are soft, malleable vesicles tailored for enhanced delivery of active agents.

ETHOSOME

Ethosomes are specially tailored vesicular carriers which were invented by E.Touitou in response to the need for efficient delivery of drugs by topical application on the skin. This system is composed mainly of phospholipids, ethanol (up to 50%) and water.[4] Various phospholipids which are used as vesicle forming component are phosphatidylcholine (for instance: soya phosphatidylcholine, egg phosphatidyl-choline, dipalmitoyl phosphatidylcholine, distearoyl phosphatidylcholine) phosphatidic acid, phosphatidylethanolamine, phosphatidylserine, phosphatidylglycerol, and phosphatidylinositol (PI). In addition, non-ionic surfactants (PEG-alkyl ethers) can be combined with the phospholipids in these preparations. Cholesterol used at a range of 0.1% - 1% provide stability to the vesicle membrane.. The size of ethosomes can vary from thirty nanometers to microns. It was reported that ethosomes had a homogeneous size distribution and were smaller relative to liposomes, although both systems were obtained by preparation methods not involving any size reduction steps.[5] It was further suggested that the vesicle size is affected by the presence of ethanol in the system which imparts a surface negative net charge to the vesicles and induces a decrease in mean vesicular dimensions.[6] With respect to stability, Ethosomes have been reported to be more stable than liposomes because of the presence of ethanol, which provides a net negative charge on the surface.[7]

FIG: 1 Sem Image of Ethosome

POTENTIAL ADVANTAGE OF ETHOSOMAL DRUG DELIVERY SYSTEM[8,9,10]

✓The Ethosome formulation are non invasive in comparison with iontophoresis and phonophoresis. These are administrated in a semisolid form(cream or gel) imparting high patient compliance

✓Drugs entrapped in ethosome having different physic-chemical characterstics and molecular size are showing high degree of permeation compare to other nano-carriers

✓Ethosome formulation in large scale Do not require any sophisticated, designed instruments.

✓Ethosomes show highest transdermal flux enhances the permeation of drug through deeper layers of skin.

✓Due to intense research toxicological profiles of the ethosome components are well-evaluated and documented in the scientific literature thus the ethosome technology has no large-scale drug development risk

✓Ethosomes act as platforms for the delivery of protein and high molecular peptide drugs

✓Ethosomes improve skin delivery under occlusive and non-occlusive conditions.

✓Ethosomal system act as delivery system for a fluorescent probe (quantum dots) to the skin, in terms of quantity and depth.

DISADVANTAGE OF ETHOSOMAL DRUG DELIVERY SYSTEM

- Drugs that require high blood levels cannot be administered –limited to only potent drugs (daily dose -10mg or less)
- Poor practical yield.
- Ethosomes with poor shells may clump together and leads to precipitation.
- Transfer of ethosomes from organic to aqueous layer leads to loss of product.

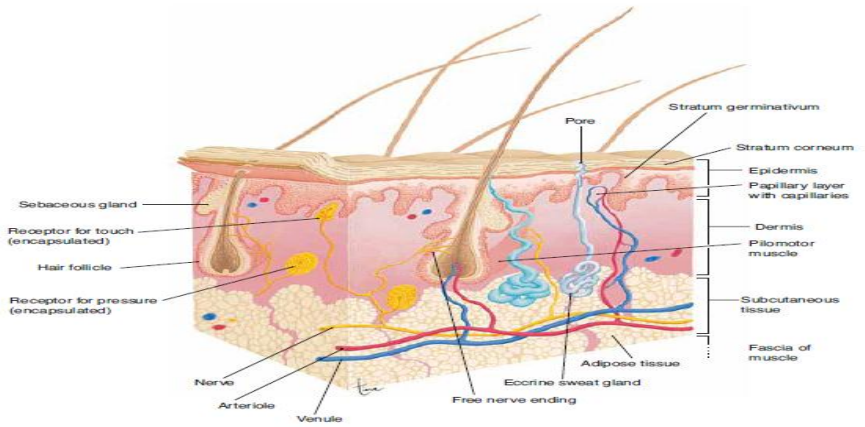

Fig:2 Anatomy of Skin

Table-1 Additives used in the formulation of Ethosome

CLASS	EXAMPLE	USE
Phospholipids	Phosphatidylcholine from soybean (90%), granules, (Phospholipon 90G) Phosphatidylcholine from soybean, agglomerates (Lipoid S100) Phosphatidylcholine content (81.7%), from egg yolk, agglomerates (Lipoid E80) Hydrogenated phosphatidylcholine from soybean (90%), powder (Phospholipon 90H)	It influences on the size,entrapment efficacy, zeta potential and penetration properties of the vesicles.
Alcohol	Ethanol	For providing the softness for vesicle membrane As a penetration enhancer
Other alcohol	Propylene glycol ,Transcutol RTM IPA[24]	Skin permeation enhancer, reduction in particle size[23] Influences entrapment efficiency
Cholesterol	Cholesterol	For providing the stability to vesicle membrane
Edge activators	N-DMSO ,Tween[22], Span	Enhances skin permeability[21]
Dye	Rhodamine-123 Rhodamine red Fluorescene Isothiocynate(FITC) 6 – Carboxy fluorescence	For characterization study
others	Dicetyl phosphate	Prevent aggregation of vesicles[20]

METHODS OF PREPARATION

1. The Cold method

Cold method is a conventional method to formulate ethosome. in this method suitable Phospholipid is mixed with ethanol along with the active moiety and other lipid ingredient the total mixture is heated up to 30°C ± 1°C with constant mixing at 700 rpm with a mechanical stirrer in a closed container and a fine stream of double-distilled water is added slowly into the mixture, while maintaining the system at 30°C ± 1°C.mixing is continued for an additional 5 minutes, The formulation is kept aside to cool at room temperature for 30 min and then it is sonicated using a probe sonicator at 4°C for five cycles of 3 minutes each with a 1-minute rest between cycles to get desired size of ethosome, finally the total formulation must be stored inside refrigerator. The entrapment of the active moiety in ethosome in cold method depends on its partition coeffiecient and other physic-chemical properties.

2. Hot method

Hot method is an unique method to formulate stable ethosome, in this method accurately weighed quantity of active moity is dissolved in required quantity of ethanol and propylene glycol(organic phase) in a seprate vessel maintaining the temperature at 40^0C. In another beaker the phospholipid is dispersed in waterand than it is kept on water bath maintaining the temperature at 40^0C until a colloidal suspension formulation(aqueous phase) is obtained., than the organic phase is added to the aqueous phase slowly with Continue stirring with magnetic stirrer. the final formulation was subjected to

ultrasonication to get evenly dispersed ethosomal vesicles.[21]

3. The thin-film hydration method

This is an alternative method for ethosome formulation.in this method the mixture of chloroform and methanol was prepared at ratio of 3:110 or 2: 186 in a separate dry, round bottom flask ,the phospholipid is dissolved in it. the organic solvents (chloroform:methanol) are removed by subjecting the formulation in RBF at a temperature above the lipid-phase transition temperature. A layer of lipid film is formed inside the RBF, the lipid film is kept under vacuum condition overnight to remove the solvent traces. The lipid film is then hydrated with a water–ethanol solution or phosphate buffered saline–ethanol solution .during hydration process the formulation is maintained at required temperture and rotation speed.

CHARACTERIZATION OF ETHOSOMES

Surface Morphology: The vesicle surface morphology can be easily visualized and analyzed by using a transmission electron microscopy (TEM) or photomicgrograph, and scanning electron microscopy (SEM).[34]

The ethosomal vesicular size and size distribution can be evaluated by using Dynamic light Scattering (DLS) technique using a computerized Nicomp.

Zeta potential measurement of the ehosomal formulation can be done with the Zeta meter. The size of the ethosomes range between tens of nanometers to microns and it is influenced by the composition of the formulation. Physical stability of formulation depend on the charges on the surface of ethosome .due to high zeta potential ethosomes are able to maintain perfect repulsion and inter radial distance between them.[36]

Entrapment efficiency (EE) of the ethosomal system were determinded by various methods like ulracentrifugation method, Dialysis method and gel-chromatography method. the drug entrapped in various segment of the vesicle, it may be incorporated in ethanolic core phase, bilayer membrane or outer vesicle membrane. The entrapment efficiency basically affected by solubility of active moiety in ethosomal medium.

The invitro skin permeation ability of the ethosomal formulation can be evaluated by the use confocal laser scanning microscopy(CLSM). Many fluorescent probe like rhodamine 6G, rhodamine B, beta carotene are incorporated in formulation to know the range of penetration.[38]

The transition temperature of ethosomal formulation can be evaluated by using differential scanning calorimetry(DSC), it is a measure of flexibility of vesicle, the ethanol and active moiety concentration effect the transition temperature of ethosomal formulation. DSC also detects the ethanol-skin phospholipid interaction.[37]

Table:2 Methods of Characterization of Ethosomal formulation

Parameter	Methods	References
Entrapment Efficiency	Ultracentrifugation method Size-exclusion gel chromatography Dialysis method	39,40
Vesicle size and zeta potential	Dynamic light scattering technique (DLS)	41
Vesicle shape and surface morphology	Scanning electron microscopy (SEM) Transmission electron microscopy (TEM)	42
In vitro skin permeation and skin deposition	Franz diffusion cells Side-by-side diffusion cells Keshry-chien diffusion cells	43,44
In vitro skin penetration	Confocal laser scanning microscopy (CLSM)	12,23
Vesicle-skin interaction	Scanning electron microscopy (SEM) Transmission electron microscopy (TEM) Fluorescence microscopy	18
Lamellarity	31P-NMR	12
Phospholipid-ethanol interaction	Differential scanning calorimetry (DSC)	36
Vesicle stability	Dynamic light scattering technique(DLS) Transmission electron microscopy(TEM)	25,26
Degree of deformability	Extrusion method	21
Turbidity	Nephalometry	24

MECHANISM OF SKIN PERMEATION

The basic advantage of ethosomes over liposomes is the increase permeation of drug. The mechanism of penetration of the ethosomes in and through the skin is not yet completely clear. But it is suggested that the drug absorption probably occurs in following two phases.

1. Ethanol effect; according to this mechanism, ethosomal formulations containing ethanol as principal ingredient interacts with intercellular lipid molecules in the polar head group region, thereby enhances its fluidity and decreases the density of the lipid multilayer, which results in an increase in membrane permeability.

2. Ethosomes effect; the high alcohol content is expected to results in increased skin permeability. So the ethosomes permeates very easily inside the deep skin layers, where it got combined with skin lipids and releases the drugs into deep layer of skin.[52]

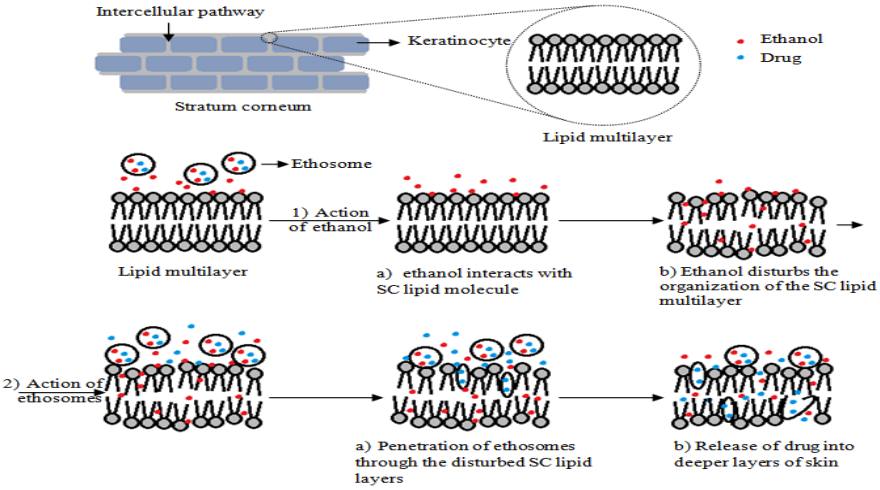

Fig: 3 Mehanism of ethosomal drug penetration through skin

PHARMACEUTICAL APPLICATIONS OF ETHOSOMAL FORMULATIONS

J .Marto et al and his co worker formulated (Griseofulvin) GRF-loaded ethosomes showed to be suitable systems for upper skin delivery of GRF. The ethanol content in the formulation allowed drug solubilization and modified deformable lipid content in startum corneum so that it could easily penetrate between skin corneocytes in SC, resulting in drug skin retention and permeation enhancement. GRF ethosomes were found to be non-cytotoxic on skin. GFR ethosomal formulation showed an antifungal activity on skin in agar diffusion test at 1% concentration From these data, it is possible to conclude about the efficiency and safety of the application of this GRF formulation and, in practical terms, to find out the frequency of application required to maintain a high skin concentration of GRF in order to be within the therapeutic window.

Yu et al and his co worker formulated Ethosomes containing (CPT)Cryptotanshinone for acne treatsment, their research mainly focused on entrapment efficiency, drug(CPT) loading capacity and vesicle size Optimized ethosomes of CPT incorporating Carbomer 974 were formulated as gels and compared with marketed hydroethanolic gels and invitro evaluation for skin permeation and drug deposition were performed. The anti-acne activity and skin irritation of the gel was investigated in rabbits. CPT loaded optimized ethosome formulation were found to exhibit low vesicle size(69.1 ± 1.9 nm), high loading capacity(0.445 ± 0.007 mg/ mL) and encapsulation efficiency (40.31 ± 0.67%) respectively as conventional marketed gel. The optimized ethosome formulation showed better results for in vitro transdermal flux and skin deposition test as compared to conventional gels. The CPT ethosomal gel formulation having better anti-acne effect with only slight skin irritation may be a viable treatment for acne in future.

Chao Fan, et al., worked to explore the feasibility of ethosomes prepared by pH gradient loading method for improving the antiarthritic efficacy of Tetrandrine by topical application. *Ex vivo* permeation and deposition behavior demonstrated that the drugs flux across rat skin and deposition of the drug in rat skin for ethosomes was 2.1 higher and for liposomes 1.7-fold higher. Confocal laser scanning microscopy confirmed that ethosomes could enhance the topical delivery of the drug in terms of depth and quantity compared with liposomes.[55]

Tarek A. Ahmed and his co worker formulated an optimized ethosomal formulation containing glimepiride, the ethosomal formulation for therapeutics merits loaded in trandermal patch In-vivo study was performed following transdermal application on human volunteers. The percent of alcohol was significantly affecting all the studied responses while the other factors and their interaction effects were varied on their effects on each response.The Ex-vivo permeation study of transdermal films loaded with optimized ethosomal formulation was superior to that of the corresponding pure drug transdermal films and this finding was also confirmed after confocal laser microscope study. Permeation of glimepiride from the prepared films was in favor of Higushi-diffusion model and exhibited non-Fickian or anomalous release mechanism. In-vivo study revealed extended drug release behavior and lower maximum drug plasma level from transdermal films loaded with drug ethosomal formulation. So, the ethosomal

formulation could be considered a suitable drug delivery system especially when loaded into transdermal vehicle with possible reduction in side effects and controlling the drug release.[56]

Table:3 PATENTS CLAIMED FOR ETHOSOME FORMULATIONS

Title	Inventor	Patent no	Year	Results
Tretinoin ethosomes gel and preparation method thereof	Hu Chunmei Liu Yan , Wang Jing Li Rong	CN104983675 A	2015	the prepared tretinoin ethosomes gel is an externally-used transdermal delivery preparation
Chinese medicinal ethosome gel patch for treating herpes zoster and preparation method	Bu Ping; Hu Rong; Chen Lin; Wei Rong; Wu Huanhuan; Huang Xiaoli	CN103536700 (A)	2014	Easy in medication and convenient to use, has a good therapeutic effect, quick response,
Ethosome gel film-coating agent with multiple wound repair effects and preparation method of ethosome gel film-coating agent 1	Chen Jie; Huang Changping; Zheng Maoxin; Nie Kaipin	CN103893394 (A)	2014	The Ethosome entrapped film-coating agent helps to promote healing and nutrition supplying of the wound tissue.
Daptomycin ethosome preparation	Li Chong; Liu Xia; Yin Qikun; Wang Xiaoying; Chen Zhangbao	CN103006562 (A)	2013	It is excellent in transdermal performance, drug release and has certain slow-release effect, and the preparation method is simple and convenient, low in cost and good in stability
Ethosome preparation of male hormone	Shu Meng; Jianxin Li; Yanmin Guan;	CN102406605 (A)	2012	To improve transdermal transport of male hormone
Paclitaxel ethosome gel and preparation method there of	Jianping Tan; Lixin Jiang; Tanran Chang; Zhiwen Zhou	CN102579323 (A)	2012	The action of stimulation to the skin can be reduced, and the percutaneous permeation effect is good.
Acyclovir ethosome and preparation method there of 21	Xuewen Wu; Yan Xiong	CN102133183 (A)	2011	Acyclovir ethosome has high stability and narrow particle size distribution
Podophyllotoxin ethosomes and preparation methods there of 22	Nianping Feng; Yanyan Yu; Jihui Zhao; Haiting Weng; Xiaoqin Shi	CN102144972 (A)	2011	The invention discloses two preparation methods for the podophyllotoxin ethosomes
Terbinafine compositions for onychomycosis treatment	E.Touitou	WO2010086723A1	2010	Novel terbinafine topical compositions for the treatment of nail onychomycosis

Table:4 Reported in-vivo results for different ethosomal formulations

Active Ingridient	Dosage form	Subject/Species	Aim	Reported results
Zidovudine	Susp	SD rats	Vesicle–skin interaction study	Ethosomes affected the ultrastructure of the stratum corneum
Vinpocetine	Gel	SD rats	Pharmacokinetics	AUC and elimination half-life of transdermal administration were significantly higher than that by intragastric administration (P<0.01)
Valsartan	Gel	Albino Wistar rats	Antihypertensive activity	Ethosomal gel was found to be effective, with a 34.11% reduction in blood pressure
Tretinoin	Gel	LACA mice	Antipsoriatic activity	Ethosomes were not of much utility for treatment of superficial skin disorders such as psoriasis
Insulin	Patch	SD rats	Blood glucose levels lowering effectiveness	Up to 60% decrease in blood glucose levels

Ketoconazole	Susp	Wistar rats	Antifungal activity	Transethosomes enhanced the antifungal activity in a shorter duration of time than other vesicles
Ligustrazine	Patch	SD rats	Pharmacokinetics	Ethosomal system-enhanced drug absorption and bioavailability
Curcumin	Susp	SD rats	Anti-inflammatory effects	PG liposomes showed the highest and longest inhibition of the development of paw edema, followed by ethosomes and traditional liposomes

SD:Sprague Dawley Susp: suspension AUC :Area Under Curve

CONCLUSION

The enhancement of skin permeability at molecular level as well as bioavailability of active moiety has been accomplished by the use of different nanocarrier. Continuous extensive research has led to the introduction of a new generation of ethosomal systems, As far as stability is concerned, ethosomes are much more stable than liposomes because of the presence of ethanol, which provides a net negative surface charge, which avoids aggregation of vesicles due to electrostatic repulsion. Ethosomes have also been proved to be interesting delivery systems for pharmaceutical and cosmetic products. The incorporation of ethosomal systems in suitable vehicles such as gels, patches and creams represents an important step to get better skin-permeation and therapeutic results. However, more studies are required to enhance the stability of the ethosomal system. The in vivo results and results of different phases of clinical trials are reflecting the potential of ethosomal systems in transdermal delivery of active moiety

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