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Review Article

ETHOSOMES FOR TRANSDERMAL AND TOPICAL DRUG DELIVERY

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ABSTRACT

Ethosomes are elastic lipid vesicular drug delivery systems embodying relatively high concentration of alcohol. These "soft vesicles" are very efficient in transporting active substances through the stratum corneum into the deeper layers of the skin than conventional liposomes. A proposed mechanism of the percutaneous permeation enhancing effect of ethosomal system is the dual fluidizing effect of alcohol on the ethosomal lipid layers and on the stratum corneum lipids. Ethosomes can act as a carrier for large and diverse group of drugs with different physicochemical properties and found a number of applications in pharmaceutical, biotechnological and cosmetic fields. This article highlights the current status of the development of ethosome and summarizes its advantages, composition, methods of preparation, mechanism of skin penetration, characterization, applications and stability.

Keywords: Ethosomes, Transdermal drug delivery, Lipid vesicular systems, Permeation enhancers, Stratum corneum.

INTRODUCTION

The skin is one of the most extensive and readily accessible organs of the human body and the skin as a route of drug delivery can offer many advantages over traditional drug delivery systems including lower fluctuations in plasma drug levels, avoidance of gastrointestinal disturbances and first-pass metabolism of the drugs, and high patient compliance. One of the greatest disadvantages to transdermal drug delivery is the skin's low permeability that limits the number of drugs that can be delivered in this manner. The skin offers an excellent barrier to molecular transport, as stratum corneum is the most formidable barrier to the passage of most of the drugs, except for lipophilic and low molecular weight drugs. [1] For transdermal and topical drug delivery system to be effective, the drug must obviously be able to penetrate the skin barrier and reach the target site.

During the past several decades, researchers have developed numerous techniques to weaken or disrupt the skin barrier and deliver drugs into the body through the intact skin. Chemical skin permeation enhancers, iontophoresis, sonophoresis, electroporation, microneedles, and many other methods have been investigated to increase the efficacy of transdermal transport. Owing to their limited efficacy, resulting skin irritation, complexity of usage, and/or high cost, none of these methods have been broadly applied to date. [2, 3, 4] Lipid-based suspensions such as liposomes, niosomes, and microemulsions, have also been proposed as low- risk drug carriers, but they do not offer much value in transdermal drug delivery because they do not deeply penetrate the skin, but rather remain on the upper layers of skin strata. [5] Several researchers have developed novel elastic lipid vesicular systems in order to deeply and easily penetrate through the skin. Phospholipids, ethanol, bile salts and many surfactants have been used to prepare these elastic vesicles. The high flexibility of vesicular membranes allows these elastic vesicles to squeeze themselves through the pores in stratum corneum, which are much smaller than their vesicular sizes. [6] In 1992, Cevc et al introduced the first generation elastic lipid vesicular carrier, Transfersomes®, mainly consists of phospholipids and an edge activator (non-ionic surfactant). They were reported to penetrate intact skin and able to deliver the drug into and across the skin, when applied under non-occlusive conditions.[7, 8]

ETHOSOMES

Ethosomes are soft, malleable lipid vesicles composed mainly of phospholipids, alcohol (ethanol or isopropyl alcohol) in relatively high concentration (20-45%) and water. Ethosomes were first developed by Touitou and her colleagues in 1997. [9, 10, 11] This carrier presents interesting features correlated with its ability to permeate intact through the human skin due to its high deformability. The physicochemical characteristics of ethosomes allow this vesicular carrier to transport active substances more efficaciously through the skin in terms of quantity and depth when compared to conventional liposomes that are known mainly to deliver drugs to the outer layers of skin. [10] Furthermore, the ethosome carrier can provide an effective intracellular delivery of hydrophilic, lipophilic or amphiphilic molecules. [11] Basically ethosomes exhibit lipid bilayers like liposomes (Fig 1); however they differ from liposomes in terms of composition (high content of ethanol). In contrast to conventional liposomes, ethosomes shows smaller vesicle size, higher entrapment efficiency as well as improved stability. [12, 13] Ethosome formulations provide sustained delivery of drugs where ethosomes act as reservoir system for continues delivery of drugs. [14] Visualization by transmission electron microscopy showed that ethosomes could be unilamellar or multilamellar through to the core. [15, 16] The size of ethosome vesicles varies from tens of nanometre to a few microns depending on method of preparation, composition and application techniques like sonication. [17, 18] Contrary to Transfersomes® ethosomes improves skin delivery of drugs both under occlusive and non-occlusive conditions. [19]

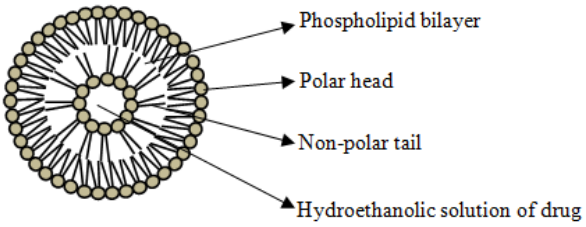

Fig. 1: Proposed diagram of ethosome vesicle

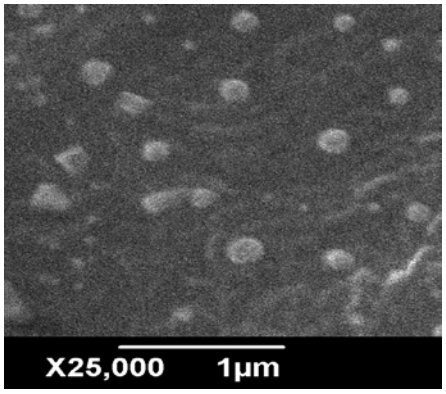

Fig. 2: SEM image of ethosomes

Phospholipids are the vesicle forming component of ethosomal system. Phospholipids with various chemical structures like phosphatidylcholine (PC), hydrogenated PC, phosphatidylethanolamine (PE) are used at concentrations ranging from 0.5-10%. The source of the phospholipids can be egg, soybean, semi-synthetics, and synthetics. Some preferred phospholipids are soya phospholipids such as Lipoid S100, Phospholipon 90 (PL-90). High concentration of alcohol (20-45%) in the formulation provides soft, flexible characteristics and stability to the vesicles and it also disrupts lipid bilayer structure of the skin results in an increase in the membrane permeability. [20] Examples of alcohols, which can be used, include ethanol (commonly used) and isopropyl alcohol. Glycols can also be used in preparations as a penetration enhancer. Among glycols propylene glycol and Transcutol are generally used. [21] For providing further stability to ethosome vesicles cholesterol at concentrations ranging between about 0.1-1% can also be incorporated. [16]

Potential advantages of this system include

1. Ethosome enhance permeation of drugs through skin for dermal, transdermal and intracellular delivery.
2. Deliver various molecules with different physicochemical properties, hydrophilic and lipophilic molecules, peptides and other macromolecules.
3. Ethosome composition is safe and the components are approved for pharmaceutical and cosmetic use.
4. Ethosome formulation has no large scale drug development risk, as the toxicological profiles of the ethosome components are well-documented in the scientific literature.
5. The ethosomal drug is administrated in a semisolid form (gel or cream), providing high patient compliance.
6. The Ethosomal system is passive, non-invasive and is available for immediate commercialization.
7. Various applications in Pharmaceutical, Biotechnology, Veterinary, Cosmetic and Nutraceutical markets.
8. Ease of industrial scale-up: Multiliter quantities of ethosomal formulation can be prepared easily; do not require any sophisticated or specially designed equipments.

Methods of Preparation

Cold method and hot method are the two conventional methods used for the preparation of ethosomes. Classic mechanical dispersion method and transmembrane pH-gradient active loading method [22] are also reported in various literatures. Among this cold method is the most common method used.

1. Cold method

Dissolve phospholipid and other lipid material in ethanol in a covered vessel at room temperature by vigorous stirring. Add propylene glycol or other polyol during stirring. Heat the mixture up to 30^0C in a water bath. Heat the water up to 30^0C in a separate vessel and add to the above mixture slowly in a fine stream. The drug can be dissolved in water or ethanol depending on its hydrophilic/hydrophobic properties. Continue stirring for another 5 min and cool the resultant vesicle suspension at room temperature. The vesicle size of ethosomal formulation can be modulated to desire extend using sonication or extrusion method. Finally, the formulation should be stored under refrigeration. [23]

2. Hot method

In this method, disperse the phospholipid in water by heating in a water bath at 40^0C until a colloidal solution is obtained. In a separate vessel mix ethanol and glycols and heat this mixture up to 40^0C. Once both mixtures reach 40^0C, add the organic phase to the aqueous one. Continue stirring for another 5 min and cool the resultant vesicle suspension at room temperature. The drug can be dissolved in water or ethanol depending on its hydrophilic/ hydrophobic properties. Modulation of ethosomal vesicle size can be done using sonication or extrusion method.[24]

3. Classic mechanical dispersion method

Dissolve phospholipid in an organic solvent or a mixture of organic solvents in a round-bottom flask (RBF). Remove the organic solvent using a rotory vacuum evaporator above lipid transition temperature to form a thin lipid film on the wall of the RBF. Traces of the solvent should be removed from the deposited lipid film by leaving the contents under vacuum overnight. Hydrate the lipid film with hydroethanolic solution of drug by rotating the flask at suitable temperature with or without intermittent sonication and finally, cool the resultant ethosomal suspension at room temperature. The formulation should be stored under refrigeration. [25]

Mechanism of skin penetration

Many mechanisms have been suggested by researchers for enhanced skin delivery potential of ethosomes. The exact process of drug delivery by ethosomes remains a matter of speculation, most likely, a combination of processes contribute to the enhancing effect. [26] When ethosomal carriers, which contain high ethanol concentration are applied to the skin a number of concomitant processes may take place, involving the stratum corneum and pilosebaceous pathways. The permeation enhancement from ethosomes is much greater than would be expected from ethanol alone or from conventional liposomes, suggesting some kind of synergistic mechanism between ethanol, vesicles and skin lipids. [27] It is thought that the first part of the mechanism is due to the 'ethanol effect' (Fig 3). Ethanol has long been known to have permeation enhancing properties and it disturbs the organization of the stratum corneum lipid bilayer, enhances its lipid fluidity and decreases the density of the lipid multilayer, which results in an increase in membrane permeability. Ethanol also supposed to extract the stratum corneum lipids thereby the barrier function of the stratum corneum. [28, 29] Furthermore, ethanol can act as a 'blending' agent for lipid vesicles with increasing their distribution in various skin layers. [17] This is followed by the 'ethosome effect', where the flexible ethosome vesicles interact with the disturbed stratum corneum bilayers and even forge a penetration pathway through the skin by virtue of their particulate nature. The release of drug in the deep layers of the skin and its transdermal absorption could then be the result of fusion of ethosomes with skin lipids. [27]

Methods of characterization

Shape and surface morphology of ethosome vesicles can be studied by scanning electron microscopy (SEM) and transmission electron microscopy (TEM). Results of various studies showed that ethosomes are spherical or nearly spherical in shape (Fig 2) and it could be unilamellar or multilamellar through to the core. Vesicle size, size distribution and zeta potential (ζ) of the ethosomal formulation can be measured by Dynamic light scattering technique (DLS). Size of ethosomal vesicles is important as it can influence the skin permeation. Vesicle size of ethosomal formulation is mainly depend upon the composition of the formulation; usually, as the ethanol concentration increases there is a significant decrease in vesicle size can be observed whereas an increase in vesicle size can be observed with the increasing concentration of phospholipid. [30, 31] Zeta potential is the electric potential of the vesicles including its ionic atmosphere (stern layer), and which influences both vesicular properties such as stability as well as skin-vesicle interaction. High zeta potential (positive or negative) of ethosomes prevents the aggregation of vesicles owing to electrostatic repulsion and increase the inter bilayer distance, hence enhances their physical stability. [21, 24]

The entrapment efficiency of the ethosomes can be measured by various methods, include Ultracentrifugation method, Size-exclusion gel chromatography and Dialysis method. Measuring the entrapment capacity give the quantity of drug in three regions of the vesicular system: the quantity adsorbed onto the vesicle membrane, the quantity incorporated into the membrane bilayer and the quantity incorporated in the internal core phase. The entrapment capacity of vesicles is influenced by both the lamellarity of vesicles and the solubility of the drug in the ethosomal medium. [26]

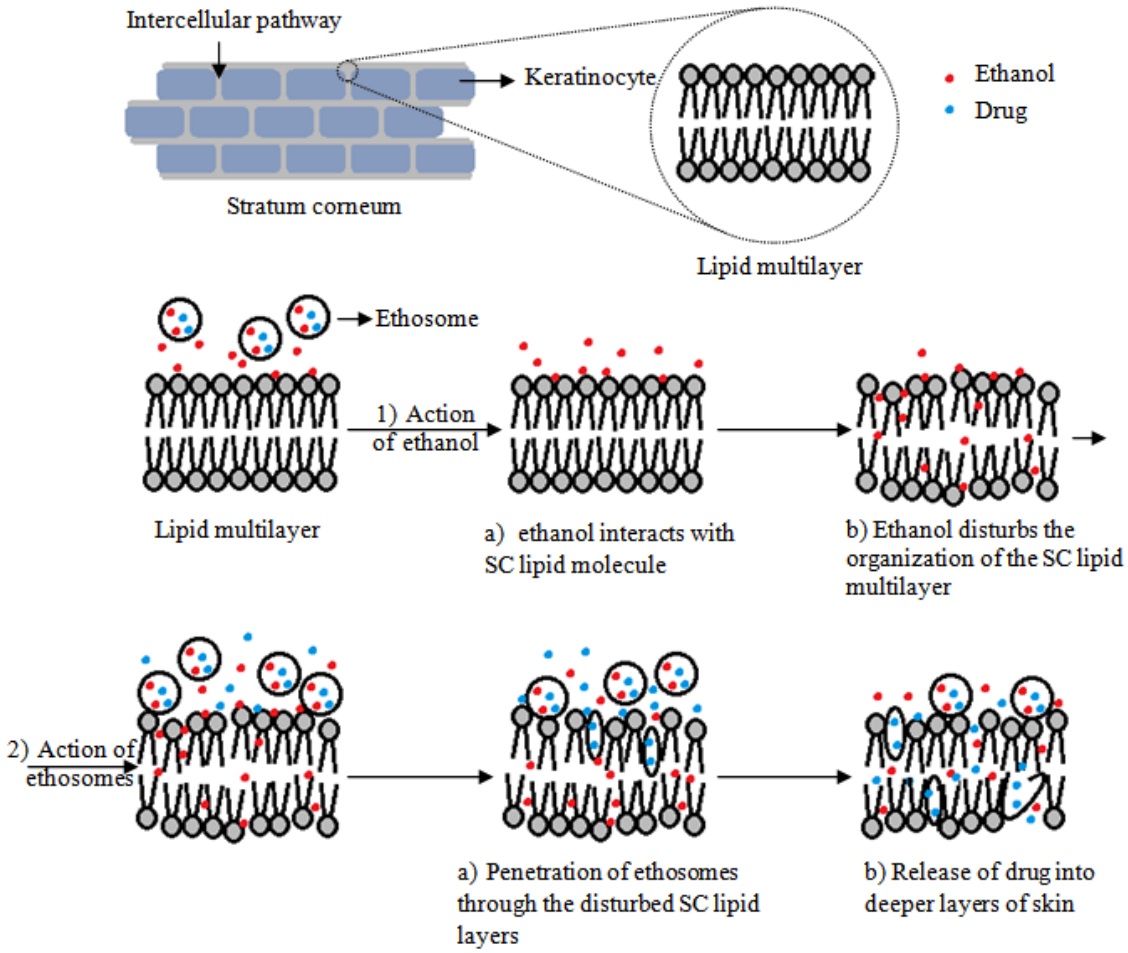

Fig. 3: Proposed mechanism for skin delivery of ethosomal systems

The depth of skin penetration from ethosomal systems can be assessed by confocal laser scanning microscopy (CLSM). For skin penetration studies various fluorescent probes with different physicochemical properties, like rhodamine red, rhodamine B, β-carotene (βC), rhodamine 6G, can be entrapped within the ethosomal vesicles. [12, 23, 32] The transition temperature (*Tm*) of the lipid in the vesicular systems can be determined as a measure of vesicle softness. *Tm* can be determined by differential scanning calorimetry (DSC). Both the drug and concentration of ethanol influence the transition temperature of vesicular lipids. Storage stability of ethosomal systems can be determined by comparing the shape, average size and entrapment capacity of the vesicles over time at different storage conditions. Based on various stability studies performed, researchers suggest refrigerated condition (4-8^{0}C) as the suitable storage condition for ethosomal formulations. Higher temperatures may cause degradation of vesicular lipids, lose of structural integrity of vesicles and an accelerated leakage of the entrapped contents. [26, 33]

Table 1: Methods for the characterization of ethosomal formulation

Parameters	Methods/Apparatus
Vesicle shape and surface morphology	Scanning electron microscopy (SEM) [15]
	Transmission electron microscopy (TEM) [15, 16]
Vesicle size and zeta potential	Dynamic light scattering technique (DLS) [21, 30]
Entrapment efficiency	Ultracentrifugation method [10]
	Size-exclusion gel chromatography [34]
	Dialysis method [18]
In vitro skin permeation and skin deposition	Franz diffusion cells [26]
	Side-by-side diffusion cells [35]
	Keshry-chien diffusion cells [14]
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]
Phospholipid-ethanol interaction	^{31}P NMR [12]
	Differential scanning calorimetry (DSC) [36]
Lipid transition temperature (*Tm*) of vesicular system	Differential scanning calorimetry (DSC) [23, 26]
Degree of deformability	Extrusion method [21]
Turbidity	Nephalometry [24]
Vesicle stability	Dynamic light scattering technique (DLS) [25, 26]
	Transmission electron microscopy (TEM) [26]

Applications

1. Applications of ethosomes in pharmaceuticals

Dubey et al (2010) prepared an ethosomal formulation of Indinavir, an anti-HIV drug, and investigated their enhanced transdermal delivery potential. Indinavir, as a protease inhibitor with variable pH-dependent oral absorption, short biological half life and extensive first-pass metabolism presents a challenge with respect to its oral administration. Transdermal delivery could be a better alternative that could provide sustained levels of drug for a greater time period, precludes first-pass metabolism, and hence increase patient compliance. In their study, the prepared indinavir ethosomes were characterized for vesicle size, shape, polydispersity, and entrapment efficiency. Permeation studies of indinavir conducted across human cadaver skin showed an enhanced transdermal flux from ethosomes that was at least 2.06, 4.32, and 8.5 times that of ethanolic solution, liposomes, and plain drug solution, respectively. Additionally, Ethosomal formulation showed better skin deposition profile and shortest lag time for indinavir. [30]

Dayan et al (2000) formulated ethosomes bearing trihexyphenidyl hydrochloride (THP); a cationic, anti-parkinsonian drug and compared its transdermal delivery with the liposomal formulation. THP is highly ionisable (pKa 8.7) in nature and has only limited permeation through skin, hence challenge its transdermal delivery. The transdermal delivery of THP is beneficial, as neurological manifestations and motor disturbances of Parkinson's syndrome often result in difficulty in swallowing; making oral administration is more difficult. A transdermal delivery system could potentially overcome the problems of motor disturbances and patient compliance. In their study, ethosomes significantly enhanced the permeation of THP through the skin. The flux of THP through nude mouse skin from THP ethosomes was 87, 51 and 4.5 times higher than from liposomes, phosphate buffer and hydroethanolic solutions, respectively. The skin retention of THP was significantly greater from the ethosomal formulation than from liposomes or a control hydroethanolic solution. The ethosomal systems of THP also showed long-term stability as compared to classic liposomes. [26]

Mina et al (2007) formulated ethosomal formulation of salbutamol sulphate (SS); a hydrophilic drug used as bronchodilator, and compared its transdermal delivery potential with classic liposomes containing different cholesterol and dicetylphosphate concentrations. Study showed a significant decrease in vesicle size by decreasing cholesterol concentration and increasing dicetylphosphate and ethanol concentrations. They also prepared an ethosomal gel formulation by incorporating the optimized SS ethosomal dispersion into Pluronic F 127 gel. In vitro permeation studies via synthetic semipermeable membrane or skin from newborn mice showed that both formulations (SS ethosomal dispersion and gel) were much more efficient at delivering SS than were liposomes or aqueous or hydroalcoholic solutions. [37]

Xu et al (2007) investigated the effects of ethosomes, chemical enhancers and their binary combination on the in vitro permeability enhancement of naloxone (opioid antagonist) through human skin. Propylene glycol, N, N-dimethyl formamide, N, N-dimethyl acetamide, dimethyl sulfoxide, azone and polyethylene glycol 400, were used as the chemical enhancers. Naloxone ethosomes showed 11.68 times increase in steady-state flux compared to phosphate buffer solution (PBS) of naloxone. Ethosomes in combination with chemical enhancers synergistically increased in vitro flux of naloxone. Azone 3% + PG7% pre-treated in ethosomal form dramatically enhanced the skin permeation of naloxone in vitro compared with ethosomes. Ethosomes and their binary combination with chemical enhancers also showed better skin retention for naloxone as compared to that of PBS. [38]

Dubey et al (2007) prepared and evaluated the dermal and transdermal potential of ethosomes bearing methotrexate (MTX), an anti-psoriatic, anti-neoplastic, highly hydrosoluble agent having limited skin permeation. MTX loaded ethosomes were optimized and characterized for vesicular shape and surface morphology, vesicular size, entrapment efficiency, stability, in vitro human skin permeation and vesicle-skin interaction. MTX loaded ethosomal carriers provided an enhanced transdermal flux and decreased lag time across human cadaver skin. The skin penetration profile of the developed formulation was further assessed by CLSM and showed an enhanced permeation of Rhodamine Red (RR) loaded formulations to the deeper layers of the skin. The formulation retained its penetration power after storage and the vesicle interaction study also highlighted the penetration enhancing effect of ethosomes, with some visual penetration pathways and corneocyte swelling. [25]

Rong H et al (2009) prepared fluorescence ethosomes (ES-QDs) by incorporating hydrophilic CdTe fluorescent clusters (quantum dots, QDs) within the ethosome vesicle bilayer. QDs are inorganic nanometric size nanoparticles with unique optical properties. QDs are an interesting alternative to organic fluorophores in some biotechnological and biomedical applications. The prepared ES-QDs were characterized by TEM, SEM, High performance particle sizer (HPPS) and photoluminescence spectra. The optical appearance of ES-QDs and skin specimens were analyzed by CLSM. In vitro experiments to penetrate into human skin scar were performed by using the Franz diffusion cell. Ethosomes-QDs enhanced the cellular binding and internalization of QD for in vitro cell labelling and at the same time preserved the exemplary luminescent characteristics of QD. Moreover, the tight packing of QDs within the ethosome vesicles diminished QDs shell shedding that commonly leads to photodegradation, impeding the widespread diagnostic use of QDs due to weak fluorescence signals. ES-QDs not only have the properties of ethosome to penetrate the skin scar tissues but also have the fluorescence labelling properties of the quantum dots, altogether offering a novel system for potential combinatory therapeutic and diagnostic applications in skin scar. [15]

Oral delivery of hormones is associated with number of drawbacks like low oral bioavailability, high first pass metabolism, serious dose-dependent side effects and low patient compliance. In the past few decades, researchers have developed a number of technologies for the transdermal delivery of hormones. [39] Touitou et al (2000) investigated the efficiency of ethosome carriers for transdermal delivery of testosterone hormone. In their study, they compared the skin permeation potential of ethosomal formulation of testosterone (Testosome) across rabbit pinna skin with marketed transdermal patch of testosterone (Testoderm® patch). They observed nearly 30 times higher skin permeation of testosterone from ethosomal formulation as compared to that of marketed formulation. The amount of drug deposited was significantly higher in case of ethosomal formulation. Both in vitro and in vivo studies demonstrated improved skin permeation and bioavailability of testosterone from ethosomal formulation. This group in their further study designed a testosterone non-patch formulation to reduce the area of application. They have found that with ethosomal testosterone formulation area of application required to produce the effective plasma concentration was 10 times less than required by commercial gel (AndroGel®) formulation. [40]

The oral delivery of large biogenic molecules and biotechnologically derived molecules is difficult as they completely degraded in the GI environment. Transdermal delivery is a better alternative for overcoming the problems associated with oral delivery, but skin's low permeability that limits the diffusion of such large molecules and thereby their transdermal delivery. From various research work conducted it is evident that ethosomes could be a better tool for delivery of such macromolecules through skin. Touitou et al (2001) investigated the effect of ethosomal insulin delivery in lowering blood glucose levels (BGL) in vivo in normal and diabetic SDI rats. In this study, a Hill Top patch containing insulin ethosomes was used. The results showed a significant decrease (up to 60%) in blood glucose level in both normal and diabetic rats and kept the level constant for at least 8 hours. On the other hand, insulin application from a control formulation was not able to reduce the blood glucose level. These experiments, as well as others, showed the promise of ethosomal delivery of macromolecule that do not readily permeable to the stratum corneum. [41]

Sebaceous glands and hair follicles are increasingly being recognized as targets in dermal as well as transdermal delivery of drugs and

targeting specific sites of the hair follicle may represent a feasible therapeutic approach, as several dermatological abnormalities are known to originate at the hair follicle. Furthermore, considerable attention has also been focused on exploiting the follicles as transport shunts for systemic delivery of drugs. [42] Maiden et al (2004) prepared and evaluated the minoxidil ethosomal formulation with the purpose of pilosebaceous targeting. Minoxidil is a lipophilic drug used topically on the scalp for the treatment of baldness. The conventional topical formulation has very poor skin permeation and retention properties. From this study it was found that the quantity of minoxidil accumulated into nude mice skin after application of its ethosomal formulation was 2.0, 7.0 and 5.0-folds higher when compared to ethanolic phospholipid dispersion, hydroethanolic solution and ethanolic solution of the drug. These results showed the possibility of using ethosomes for pilosebaceous targeting of minoxidil to achieve better clinical efficacy. [16]

Ethosomes can be tailored to improve the delivery of a number of molecules to the cellular membranes. Delivery of active agents through biological membrane by means of vesicular systems has become one of the most important research areas in recent years. Because of its physiological homeostatic function, the cell membrane is a barely permeable barrier. Most of the molecules of interest are unable to penetrate into cells, and intracellular delivery is difficult to achieve. [43] Godin et al (2005) 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 of mice induced by *Staphylococcus aureus* was compared with intraperitoneal erythromycin administration and with local application of hydroethanolic erythromycin solution. The in vivo experiments demonstrated a very efficient healing of *S. aureus*-induced deep dermal infections when the mice were treated with ethosomal erythromycin. [44]

Transcutaneous immunization (TCI) offers a new method for the delivery of vaccines, that relies on the application of antigen with adjuvant onto the outer layer of the skin and subsequent delivery to underlying Langerhans cells that serve as antigen-presenting cells. This mode of vaccination presents a novel and attractive approach for needle-free immunization that is safe, noninvasive, and overcomes many of the limitations associated with needle-based administrations. [45] Mishra et al (2007) evaluated the efficiency of ethosomes for transcutaneous immunization (TCI) against hepatitis B. Spectral bioimaging and flow cytometric studies showed an efficient uptake of HBsAg-loaded ethosomes by murine dendritic cells in vitro, reaching a peak by 180 minutes. The transcutaneous delivery potential of the antigen-loaded ethosomal system, using human cadaver skin, demonstrated a much higher skin permeation of the antigen in comparison to the conventional liposomes and soluble antigen preparation. The topically applied HBsAg-loaded ethosomes in mice showed an improved systemic and mucosal humoral immune response compared to the intramuscularly administered alum-adsorbed HBsAg suspension, the topically applied plain HBsAg solution, and the hydroethanolic (25%) HBsAg solution. HBsAg-loaded ethosomes are able to generate a protective immune response and their ability to traverse and target the immunological milieu of the skin finds a potential application in the development of a transcutaneous vaccine against Hepatitis B virus (HBV). [46]

Touitou et al (2003) investigated the feasibility of dermal delivery of DNA molecules to express genes in the skin cells using ethosomes. They encapsulated the GFP-CMV-driven transfecting construct into ethosomal formulation and studied in vivo in nude mice. The skin penetration of green fluorescent proteins (GFP) was observed by CLSM studies. It was observed that ethosomes enabled efficient delivery and expression of genes in the skin cells, suggesting that ethosomes could be used as a carrier for gene therapy applications that required transient gene expression. [47]

Paolino et al (2005) studied ethosomes as carriers for the topical application of a natural anti-inflammatory agent such as ammonium glycyrrhizinate. Both in vitro and in vivo skin permeation studies have shown that a significantly higher cumulative amount of the drug has permeated from ethosomes (63.2%) than from hydroalcoholic solution (22.3%) and aqueous solution (8.9%) of ammonium glycyrrhizinate. The in vivo studies showed that ethosomes were able to significantly enhance the anti-inflammatory activity of ammonium glycyrrhizinate compared to the ethanolic or aqueous solutions. [10]

Yan Zhou et al (2010) prepared binary ethosomes containing total alkaloids extracted from *Sophora alopecuroides* (TASA) using a novel transmembrane pH-gradient active loading method at the temperature below the phase transition temperature of the phosphatidyl choline. The TASA binary ethosomes were characterized for shape, vesicle size, and encapsulation efficiency. The percutaneous absorption study of TASA binary ethosomes showed that more than 90% sophoridine, 47% matrine, 35% sophocarpine, and 32% lemannine in TASA were entrapped within 1 h at 40°C, with an efficiency improvement of 8.87, 8.10, 7.63, and 7.78-fold than those observed in passive loading method. Transdermal experiments showed that the penetration depth and fluorescence intensity of Rhodamine B from binary ethosome prepared by pH-gradient active loading method were much greater than that from binary ethosome prepared by passive loading method or hydroalcoholic solution. [22]

2. Applications of ethosomes in cosmeceuticals

Esposito et al (2004) prepared ethosomal gel of azelaic acid (AA); an anti-keratinizing agent used for the treatment of acne and compared the in vitro release with conventional liposomes. The release rate was more rapid from ethosomal systems than from liposomal systems. Ethosomes produced by the highest ethanol concentration released AA more rapidly than other azelaic acid ethosomal formulation and liposomes. [48]

Koli et al (2008) have formulated 'Anti-oxidant ethosomes for topical delivery utilizing the synergistic properties of vitamin A palmitate, Vitamin E, and Vitamin C'. Topical administration of many antioxidants is one of several approaches to diminish oxidative injury in the skin for cosmetic and cosmeceutical applications. But, antioxidants are usually not stable and can be degraded by exposing to light. The findings have revealed that the synergistic interaction of Vitamin C in the aqueous core and vitamin A and E in the lipid bilayer, provide complete protection from the oxidation of the ethosome formulation. [49]

The first commercial product based on ethosome technology was marketed in 2000, and majority of products marketed so far are cosmeceutical products. Nanominox©, containing minoxidil (hair growth promoter), marketed by Sinere, is the first minoxidil containing product, which uses ethosome technology. Another product, Noicellex™, an anti-cellulite ethosome formulation is currently marketing in Japan by an Israel based company Novel Therapeutic Technologies (NTTs). Lipoduction™, another anti-cellulite formulation, containing pure grape seed extract (antioxidant) is marketed in USA. Similarly, Physonics is marketing anti-cellulite gel Skin Genuity in London. Many large pharmaceutical companies and cosmetic firms are now engaged in active research in product development using ethosome technology.

Stability of ethosomes

Ethosomes offer better stability as compared to conventional pharmaceutical liposomes. [65, 66] In case of liposomes, upon storage they tend to fuse and grow into larger vesicles and this fusion and breakage of liposome vesicles on storage pose an important problem of drug leakage from the vesicles. [67] The absence of electrostatic repulsion is likely to account for the tendency of neutral liposomes to aggregate, whereas in ethosomes, ethanol causes a modification of the net charge of the system (impart negative charge to the system) and confers it some degree of steric stabilization leading to increased stability of vesicles against agglomeration and drug leakage from vesicles. Increasing the concentration of ethanol from 20 to 45% increases the entrapment efficiency owing to an increase in the fluidity of the membranes. However, a further increase in the ethanol concentration (>45%) destabilizes the vesicles and probably makes the vesicle membrane more leaky, thus leading to a decrease in entrapment efficiency. [68]

Another problem is purity of phospholipids, phospholipids containing unsaturated fatty acids undergo oxidation and the reaction products can cause permeability changes in the ethosome bilayers. [69, 70]Oxidative degradation of the lipids can be minimized by protecting the lipid preparation from light, by using phospholipids which contain saturated fatty acids, by adding antioxidants such as α-tocopherol. Furthermore, hydrolysis of lipids leads to the formation of lyso-lecithin. The presence of lyso-lecithin in lipid bilayers greatly enhances the permeability of ethosomes, hence it causes leakage of drug from ethosomal vesicles, and thus it is important to start with phospholipids which are free of lyso-lecithin and also of any phospholipases. [71]

Table 2: Compilation of reported works on ethosome

Active Ingredients	Formulations	Applications	Comments
Ketoprofen (2011) [50]	Suspension	Treatment of arthritis related inflammatory pain and musculoskeletal pain	Enhanced transdermal delivery
Linoleic acid (2011) [51]	Suspension	Treatment of melasma	Improved skin permeation and accumulation
Ligustrazine (2011) [34]	Patch	Treatment of angina pectoris	Better drug absorption and Increased bioavailability, Patches showed good storage stability
Buspirone (2010) [23]	Gel	Treatment of Menopausal syndrome (anxiety and hot flushes)	Enhanced transdermal flux, Non-fluctuated and sustained delivery of drug, Reduced side effects
Betamethasone-17-Valerate (2010) [52]	Suspension	Treatment of eczema and psoriasis	Significantly improved the skin penetration
Triptolide (2010) [53]	Suspension	Treatment of skin inflammation	Enhanced skin permeation and biological activity, Better skin accumulation
Ibuprofen (2010) [54]	Gel	Treatment of rheumatoid arthritis	Improved transdermal flux
5-aminolevulinic acid (2009) [55]	Suspension	Treatment of psoriasis	Improved drug penetration in hyperproliferative murine skin in vivo.
Distamycins (2009) [13]	Suspension	Treatment of cancer	Enhanced drug activity, Reduced side effects
Gold nanoparticles (2009) [56]	Suspension	Treatment of skin cancer, Used as a diagnostic agent	High encapsulation efficiency of the gold nanoparticles, Improved pharmacological efficacy
Matrine (2009) [57]	Suspension	Treatment of psoriasis and eczema	Improved the percutaneous permeation and anti-inflammatory activity
Benzocaine (2009) [58]	Gel	Topical anaesthesia	Improved skin penetration and therapeutic efficacy
Flucanazole (2009) [59]	Gel	Treatment of candidiasis	Better antifungal activity compared to marketed formulation
Finasteride (2008) [31]	Suspension	Treatment of androgenetic alopecia	Enhanced skin penetration and accumulation
Melatonin (2007) [60]	Suspension	Treatment of delayed sleep phase syndrome	Enhanced transdermal flux, Reduced lag time, Low skin irritancy potential
Bacitracin (2004) [61]	Suspension	Treatment of dermal infections	Improved dermal and intracellular delivery, Reduced drug toxicity
Zidovudine (2004) [14]	Suspension	Treatment of AIDS	Improved transdermal flux, Reduced drug toxicity
Cyclosporine A (2004) [62]	Suspension	Treatment of psoriasis, atopic dermatitis and alopecia areata	Enhanced skin penetration and deposition
Cannabidiol (2003) [63]	Suspension	Treatment of rheumatoid arthritis	Better skin permeation and accumulation, Improved biological activity, Prolonged drug action
Acyclovir (1999) [64]	Suspension	Treatment of herpes labialis	Improved clinical efficacy in comparison with commercial acyclovir formulation (Zovirax cream)

CONCLUSION

As mentioned above, numerous studies have been published showing that ethosomes can substantially improve the permeation of drugs through the stratum corneum and thereby their efficacy. The versatility of ethosomes for transdermal as well as topical drug delivery is evident from the research reports of enhanced delivery of quite a few drugs like minoxidil, testosterone, trihexyphenidyl hydrochloride, bacitracin, indinavir, salbutamol sulfate, azelaic acid and insulin. Delivery of Hepatitis B surface antigen (HBsAg) and DNA via ethosomes opens new opportunities to transcutaneous immunization (TCI) and gene therapy. Several excellent phytochemicals and herbal extracts have been successfully delivered via ethosomes and showed some distinct advantages over conventional drug delivery systems. As an alternative to conventional transdermal permeation enhancement techniques ethosomes are superior by offering safety, efficiacy, long term stability, simplified industrial manufacture as well as better patient compliance. Thus, it can be a logical conclusion that ethosomes can become a promising drug carrier in future for not only topical treatment of local and systemic disorders, but also for the cosmetic and cosmeceutical fields.

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