REVIEW ARTICLE

www.japtr.org

Therapeutic and cosmeceutical potential of ethosomes: An overview

Poonam Verma, K. Pathak

Department of Pharmaceutics, Rajiv Academy for Pharmacy, Mathura, Uttar Pradesh - 281 001, India

DOI: 10.4103/0110-5558.72415

J. Adv. Pharm. Tech. Res.

ABSTRACT

The main disadvantage of transdermal drug delivery is the poor penetration of most compounds into the human skin. The main barrier of the skin is located within its uppermost layer, the stratum corneum (SC). Several approaches have been developed to weaken this skin barrier. One of the approaches for increasing the skin penetration of drugs and many cosmetic chemicals is the use of vesicular systems, such as, liposomes and ethosomes. Ethosomes are phospholipid-based elastic nanovesicles containing a high content of ethanol (20–45%). Ethanol is known as an efficient permeation enhancer and has been added in the vesicular systems to prepare elastic nanovesicles. It can interact with the polar head group region of the lipid molecules, resulting in the reduction of the melting point of the stratum corneum lipid, thereby increasing lipid fluidity and cell membrane permeability. The high flexibility of vesicular membranes from the added ethanol permits the elastic vesicles to squeeze themselves through the pores, which are much smaller than their diameters. Ethosomal systems are much more efficient in delivering substances to the skin in the terms of quantity and depth, than either conventional liposomes or hydroalcoholic solutions. The scope of this small review is to introduce the novel concept of ethosomes and to describe some approaches and mechanisms of stimulating topical and transdermal products with ethosomes.

Key words: Ethosomes, liposomes, novel drug delivery, penetration enhancer, Percutaneous absorption

INTRODUCTION

Human skin is an effective, selective barrier to chemical permeation, although the skin as a route for delivery can offer many advantages, including avoidance of first-pass metabolism, lower fluctuations in plasma drug levels, targeting of the active ingredient for a local effect, and good patient compliance.[1] Water soluble molecules and drugs are normally not able to cross the skin as the skin is a natural barrier to water. The stratum corneum is composed of insoluble bundled keratins surrounded by a cell envelope, stabilized by cross-linked proteins and covalently bound lipids as shown in Figure 1.

In general, the epidermis (specifically the stratum corneum) provides the major control element; most small, water-soluble, and non-electrolytes diffuse into the systemic circulation a thousand times more rapidly when the horny layer is present.[2] Thus, to maximise the flux of the drug, the barrier hinderance is reduced by various approaches. Several technological advances have been made in the recent decades to overcome skin barrier properties. Examples include physical means such as iontophoresis, sonophoresis, microneedles, and chemical means, using penetration enhancers and biochemical means, such as, liposomal vesicles and enzyme inhibition. The physical means like iontophoresis, microneedles, and sonophoresis are relatively complicated to use, and will affect patient compliance.[3] The use of chemical enhancers such as surfactants and organic solvents induce irritation, cause damage, and reduce skin barrier function, therefore, it is desirable to deliver the therapeutic agents that maintain the normal skin barrier function without the aid of a chemical enhancer.[4] One such approach is the use of vesicular systems. In the past decade, topical delivery of drugs by liposomal formulation has evoked considerable interest. Deformable liposomes[5] and transferosomes were the first generation of elastic vesicles introduced by Ceve and Blume, in 1992, and were reported to penetrate intact skin while carrying a therapeutic concentration of drugs, when applied under nonoccluded conditions.[6] The drug, encapsulated in lipid vesicles, prepared from phospholipids and nonionic surfactants is known to be transported into and across the skin. The lipids

Address for correspondence

Ms. Poonam Verma, Department of Pharmaceutics, Rajiv Academy for Pharmacy, Mathura, Uttar Pradesh - 281 001, India. E-mail: poonamrajpatel@gmail.com

present in the skin contribute to the barrier properties of the skin and prevent the systemic absorption of drugs. Due to the amphiphilic nature, lipid vesicles may serve as non-toxic penetration enhancers for drugs.[7] In addition, the vesicles can be used for encapsulating hydrophilic and lipophilic as well as low and high molecular weight drugs. Therefore, these lipid rich vesicles are hypothesized to carry a significant quantity of drugs across the skin, thus enhancing the systemic absorption of drugs.[8] The use of lipid vesicles in the delivery system for skin treatment has attracted increasing attention in recent years, however, it is generally agreed that classic liposomes are of little or no value as carriers for drug delivery, because they do not penetrate the skin deeply, but rather remain confined to the upper layer of the stratum corneum; only specifically designed vesicles are shown to enhance permeation into the stratum corneum barrier. It has been investigated and reported that lipid vesicular systems embodying ethanol in relatively high concentrations, called ethosomes, are very efficient at enhancing the skin permeation of a number of drugs.[9]

ETHOSOMES

Ethosomes were developed by Touitou *et al.*,1997, as additional novel lipid carriers composed of ethanol, phospholipids, and water. They are reported to improve the skin delivery of various drugs.[10] Ethanol is an efficient permeation enhancer that is believed to act by affecting the intercellular region of the stratum corneum. Ethosomes are soft malleable vesicles composed mainly of phospholipids, ethanol (relatively high concentration), and water. These soft vesicles represent novel vesicles carriers for enhanced delivery through the skin. The size of the ethosomes vesicles can be modulated from tens of nanometers to microns as shown in Figure 2.

Ethosomes are non-invasive delivery carriers that enable drugs to reach the deep skin layers and / or the systemic circulation. 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 vesicles membrane; it gives the vesicle the ability to penetrate the stratum corneum. Also, because of their high ethanol concentration, the lipid membrane is packed less tightly than the conventional vesicles, although it has equivalent stability, allowing a more malleable structure and improves the drug distribution ability in the stratum corneum lipids.

Ethosomes as Carriers for Dermal and Transdermal Drug Delivery

Ethosomes were reported to be effective at delivering molecules to and through the skin to the systemic circulation. The ethosomal carrier was previously tested for dermal delivery of the antiviral drug acylovir. The authors in the study reported a two-armed, double-blinded, randomized clinical trial, and demonstrated the efficiency of the ethosomal 5% acylovir system, compared to a 5% acylovir cream (Zovirax, ZC) for the topical treatment of herpetic infection.[5]

Enhanced delivery of chemicals from the ethosomal carrier was observed in permeation experiments with fluorescent probes. The amphiphillic flourescent probe D-289 was used to study skin penetration from trihexphenyl HCl ethosomes into nude mouse skin, after the non-occulusive application (Dayan and Touitou 2000) results showed that classic liposomes did not facilitate probe penetration into this skin, rather, resulted in only a small reservoir in the upper layers of skin. Using hydroethanolic solutions, a relatively deep penetration, but of relatively very low fluorescent activity was observed. The use of the ethosomal system resulted in increase in both depth and fluorescent activity. Ethosmes have also been reported to improve *in vivo* and *in vitro* skin delivery of many drugs both under occlusive and non-occlusive conditions.[11]

Mechanism of Action of the Ethosomal Drug Delivery System

A synergistic mechanism was suggested between ethanol, vesicles, and skin lipids.[12] The enhanced delivery of actives

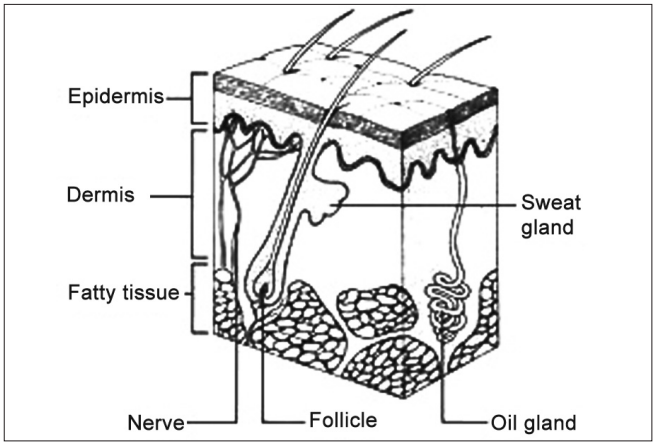

Figure 1: Simplified diagram of skin

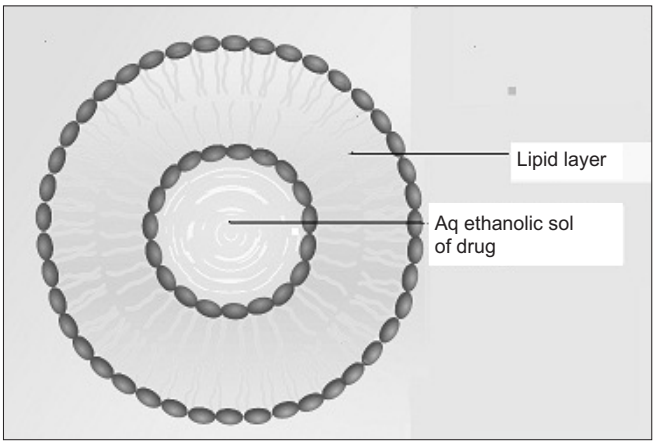

Figure 2: Proposed diagram of Ethosomes

using ethosomes over liposomes can be ascribed to an interaction between ethosomes and skin lipids. A possible mechanism for this interaction has been proposed.

From Figure 3, it is thought that the first part of the mechanism is due to the ethanol effect, where ethanol interacts with the lipid molecules in the polar head group region resulting in a reduction in the transition temperature of the lipids in the stratum corneum, increasing their fluidity and decreasing the density of the lipid multilayer. This is followed by the 'ethosome effect,' which includes lipid penetration and permeation by the opening of new pathways, due to the malleability and fusion of ethosomes with skin lipids, resulting in the release of the drug into the deep layers of the skin. Ethanol may also provide vesicles with soft flexible characteristics, which allow them to penetrate more easily into the deeper layers of the skin. The release of the drug in the deep layers of the skin and its transdermal absorption could then be the result of a fusion of ethosomes, with skin lipids and drug release at various points along the penetration pathway.[13]

Commercial Products Powered by Ethosomes bringing Novelty in the World Market

The commercialization of ethosome technology began in 2000, and it is a rapidly evolving field. There are now two companies exclusively devoted to product development, using ethosomes. Many large pharmaceutical houses and cosmetic firms are also engaged in active research and development. The list of some commercial products based on ethosomal technology are listed in Table 1.

Advantages of Ethosomal Drug Delivery

In comparison to other transdermal and dermal delivery systems,

- Ethosomes enhance permeation of the drug through skin transdermal and dermal delivery.
- Ethosomes are platforms for the delivery of large and diverse groups of drugs (peptides, protein molecules).
- Ethosomal systems are much more efficient at delivering a fluorescent probe (quantum dots) to the skin in terms of quantity and depth.
- Low risk profile — The technology has no large-scale drug development risk, as the toxicological profiles of the ethosome components are well-documented in the scientific literature.
- High patient compliance—The ethosome drugs are administrated in a semisolid form (gel or cream), producing high patient compliance. In contrast, iontophoresis and phonophoresis are relatively complicated to use, which will affect patient compliance.
- High market attractiveness for products with proprietary technology. Relatively simple to manufacture with no complicated technical investments required for the production of ethosomes.
- The ethosomes system is passive, non-passive, and available for immediate commercialization.
- Various applications in the pharmaceutical, veterinary, and cosmetic fields.

Methods of Preparation of Ethosomes

The literature reports various methods for the preparation of ethosomes and some commonly used methods have been compiled in the preceeding text.

Hot method

The drug is dissolved in a mixture of ethanol and propylene glycol and the mixture is added to the phospholipid

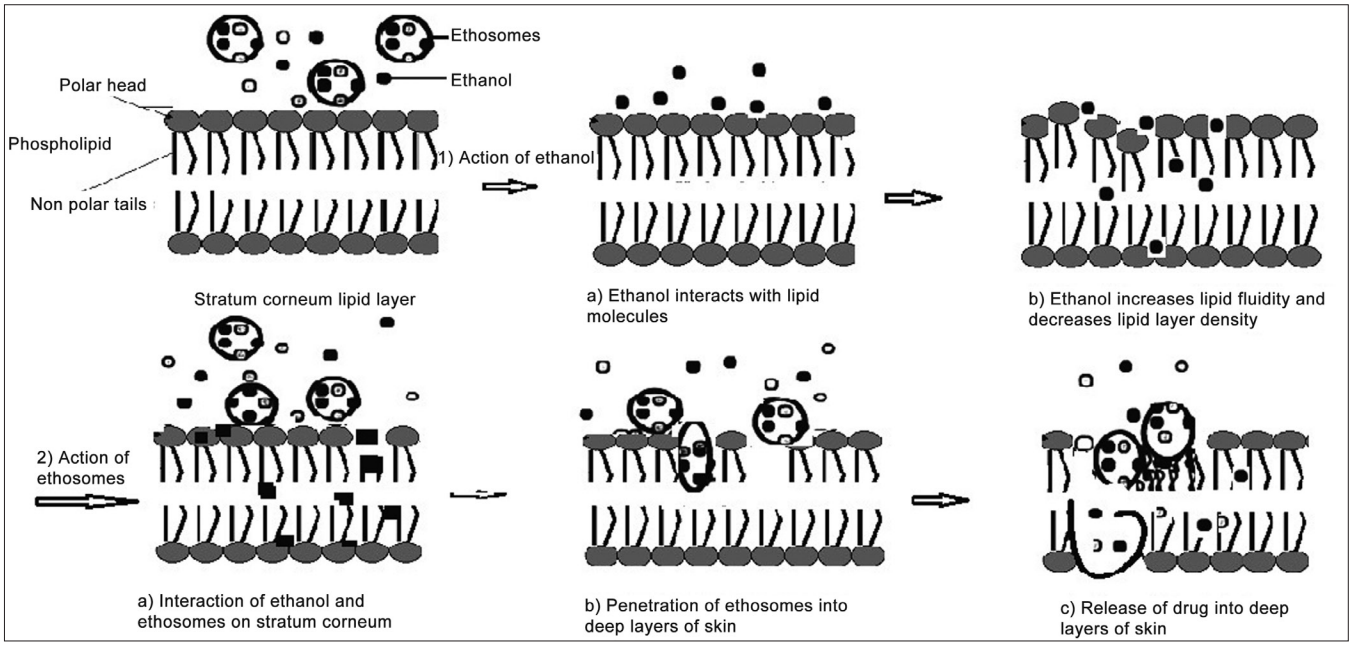

Figure 3: Proposed mechanism of penetration of ethosomal drug delivery system

Table 1: Commercial products based on ethosomal technique

Name of the product	Uses	Manufacturer
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
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
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 hyperpigmentation	Genome Cosmetics, Pennsylvania, U.S.
Noicellex	Topical anti-cellulite cream	Novel Therapeutic Technologies, Israel
Skin genuity	Powerful cellulite buster, reduces orange peel	Physonics, Nottingham, UK

dispersion in water at 40°C. After mixing for five minutes the preparation is sonicated at 4°C for three cycles of five minutes, with a rest of five minutes between each cycle, using the Probe Sonicator. The formulation is then homogenized at 15,000 psi pressure, in three cycles, using a high pressure homogenizer to get nano-sized ethosomes.[14]

Cold method

This is the most common and widely used method for ethosomal preparation. The phospholipids, drug, and other lipid materials are dissolved in ethanol, in a covered vessel, at room temperature, with vigorous stirring. The mixture is heated up to 30°C in a water bath. The water is heated to 30°C in separate vessel, and added to the above mixture and then stirred for five minutes in a covered vessel. The vesicle size of the ethosomal formulation can be decreased if desired, to extend using the sonication or extrusion. Finally the formulation must be properly stored under refrigeration.

Classic Mechanical Dispersion Method

Soya phosphotidylcholine is dissolved in a mixture of chloroform: methanol (3:1) in round bottom flask. The organic solvents are removed using rotary vacuum evaporator above lipid transition temperature to form of a thin lipid film on wall of the flask. Finally, traces of solvent mixture are removed from the deposited lipid film by leaving the contents under vaccum overnight. Hydration is done with different concentration of hydroethanolic mixture containing drug by rotating the flask at suitable temperature.[15,16]

Classic method

The phospholipid and drug are dissolved in ethanol and heated to 30°C±1°C in a water bath. Double distilled water is added in a fine stream to the lipid mixture, with constant stirring at 700 rpm, in a closed vessel. The resulting vesicle suspension is homogenized by passing through a polycarbonate membrane using a hand extruder for three cycles.[17]

Various Methods for Characterization of Ethosomes

The vesicle shape can be easily visualized by using a photomicgrograph, or transmission electron microscopy (TEM) and scanning electron microscopy (SEM) micrographs.[18,19] The vesicle size and zeta potential of the formulation can be measured with the Zeta meter.[20] The size of the ethosomes range between tens of nanometers to microns and it is influenced by the composition of the formulation. Various factors affect the size and zeta potential of the ethosomes. Reduction in mean vesicle diameter is due to the presence of ethanol, as it causes a modification of the net charge of the system and confers it some degree of stearic stabilization that may finally lead to a decrease in the mean vesicle size,[21] while the size of the vesicles increase with increasing the phospholipid concentration. This can be explained in terms of the tendency of lipid coalesces at high lipid concentration.[22,23]

The transition temperature of the vesicular lipid systems can be determined by using differential scanning calorimetry, which also detects ethanol-skin phospholipid interaction, a characteristic attributed to the fluidizing effect of ethanol on the phospholipid bilayers.[24]

The entrapment efficiency of ethosomes can be measured by the ultracentrifugation technique. The ability of ethosomes to efficiently entrap lipophilic and hydrophilic drugs can be explained by the high degree of lamellarity and the presence of ethanol in the vesicles. In addition, ethosomal formulations possess greater entrapment capability than liposomes.[25]

The ability of the ethosomal preparation to penetrate into the skin layers can be determined by using confocal laser

scanning microscopy. *In vitro* and in *vivo* skin permeation studies have demonstrated the ability of the ethosomal formulation to enhance permeation of both hydrophobic and hydrophilic molecules as compared to conventional liposomes. Different workers have reported a 5 – 10 fold better skin permeation of drugs formulated in ethosomes, as compared to the conventional liposomal formulation.[26,27]

Therapeutic Applications of Ethosomes

Mishra *et al*., 2007, reported ethosomes for transcutaneous immunization, and antigen-loaded ethosomes for transcutaneous immunization against Hepatitis B were prepared and characterized, which showed greater entrapment efficiency, optimal size range, and a unilamellar, spherical shape in comparision to conventional liposomes. Spectral bio imaging 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 antigen system, using human cadaver skin, demonstrated a much higher skin permeation of the antigen in comparision to the conventional liposomes and soluble antigen preparation. The topically applied HBsAg-loaded ethosomes in mice showed a robust 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 transverse and target the immunological milieu of the skin finds a potential application in the development of a transcutaneous vaccine against Hepatitis B virus.[28]

Oral administration of hormones is associated with complications like high first pass metabolism, low oral bioavailability, and several dose-dependent side effects such as virilization, acne, and gynecomastia. In addition along with these side effects, oral hormonal preparations rely highly on patient compliance. The risk of failure of treatment is known to increase with each pill missed. Touitou *et al*., 2000,[29] compared the skin permeation potential of testosterone ethosomes (Testosome) across rabbit pinna skin, with the marketed transdermal patch of testosterone (Testoderm® patch, Alza corporation, California). The authors observed nearly 30 times higher skin permeation of testosterone from the ethosomal formulation as compared to the marketed formulation. The AUC and Cmax of testosterone significantly improved after the application of Testosome as compared to Testoderm®. Hence, both *in vitro* and *in vivo* studies demonstrated improved skin permeation and bioavailability of testosterone from the ethosomal formulation. This group, in their further study, designed a testosterone non-patch formulation to reduce the area of application. They found that with ethosomal testosterone formulation, the area of application required to produce the effective plasma concentration was 10 times less than that required by the commercial gel (AndroG, US) formulation.

Lodzki *et al*., 2003,[30] prepared the CBD-ethosomal formulation for transdermal delivery of cannabiol for the treatment of rheumatoid arthritis. Results of the skin deposition study showed significant accumulation of Cannabidiol (CBD) in the skin, and underlying muscles after application of CBD-ethosomal formulation to the abdomen of Mice. A plasma concentration study showed that a steady state level was reached in 24 hours, which was maintained through 72 hours. A significant increase in biological anti-inflammatory activity of CBD-ethosomal formulation was observed when tested by using the carrageenan-induced rat paw edema model. Finally, it was concluded that encapsulation of CBD in ethosomes significantly increased its skin permeation, accumulation, and hence, its biological activity.

In another study, Dayan and Touitou, 2001, prepared ethosomal formulation of the psychoactive drug trihexyphenidyl hydrochloride (THP) and compared its delivery with that with the classical liposomal formulation for the treatment of parkinsons disease. THP is an M1 muscarinic receptors antagonist and used in the treatment of Parkinson disease. THP has a short biological half-life (3 hours) and its oral administration is difficult due to motor disorders and neurogical manifestations associated with parkinsonian syndrome. THP ethosomal formulation, when visualized under transmission and scanning electron microscopes, were viewed as small phospholipid vesicles. The value of the transdermal flux of THP through nude mouse skin from ethosomes was 87-, 51-, and 4.5-times higher than that from liposome, phosphate buffer, and hydroethanolic solution, respectively. The quantity of THP remaining in the skin at the end of 18 hours was significantly higher after the application of ethosomes than after the application of liposome or hydroethanolic solution (control). These results indicated the better skin permeation potential of ethosomal-THP formulation and its use for the better management of Parkinson disease.

Yet another report on methotrexate an anti-psoriatic, anti-neoplastic, highly hydrosoluble agent with limited transdermal permeation was researched by Dubey *et al*. 2007. The authors developed optimized ethosomes-loaded methotrexate and the skin permeation profile of the developed formulation revealed an enhanced permeation of rhodamine red loaded formulation to the deeper layers of the skin. The formulation retained its penetration power after storage and the vesicle skin interaction study also highlighted the penetration enhancing effect of ethosomes, with some visual penetration pathways and corneocyte swelling.

In addition to improved transdermal delivery by ethosomes,

investigations on dermal delivery have also been cited in literature. Paolino *et al*., 2005,[31] investigated the potential application of ethosomes for dermal delivery of ammonium glycyrrhizinate. Ammonium is useful for the treatment of various inflammatory based skin diseases. *In vitro* skin permeation experiments have shown that a significantly higher cumulative amount of drug has permeated from ethosomes (63.2%) than from the hydroalcoholic solution (22.3%) and aqueous solution (8.9%) of ammonium glycyrrhizinate. Ethosomal formulation showed a very good skin tolerability in human volunteers for 48-hour application. Biological anti-edema activity was also significantly enhanced in case of ethosomal formulation as compared to ethanolic or aqueous solution of the drug.

Maiden *et al*., 2004, prepared and evaluated the minoxidil ethosomal formulation. Minoxidil is a lipid-soluble drug used topically on the scalp for the treatment of baldness. The conventional topical formulation has very poor skin permeation and retention properties. 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-fold higher when compared to ethanolic phospholipid dispersion, hydroethanolic solution, and ethanolic solution of the drug, each containing 0.5% of the drug. These results showed the possibility of using ethosomes for pilosebaceous targeting of minoxidil to achieve better clinical efficacy.

Many environmental pathogens attempt to enter the body through the skin. Skin has, therefore, evolved into an excellent protective barrier, which is also immunologically active and able to express the gene. On the basis of the above-mentioned facts another important application of ethosomes, is to use them for topical delivery of DNA molecules, to express genes in the skin cells. Touitou *et al*., 2003, in their study, encapsulated the GFP-CMV-driven transfecting construct into the ethosomal formulation. They applied this formulation to the dorsal skin of five-week-old, male CD-1 nude mice for 48 hours. After 48 hours, the treated skin was removed and penetration of green fluorescent protein (GFP) formulation was observed by Confocal laser scanning microscopy (CLSM). It was observed that the topically applied ethosome-GFP-CMV-driven transfecting construct enabled efficient delivery and expression of genes in the skin cells. It was suggested that ethosomes could be used as carriers for gene therapy applications that required transient expression of genes. These results also showed the possibility of using ethosomes for effective transdermal immunization. Gupta *et al*., 2004,[32] recently reported the immunization potential of using transfersomal formulation. Hence, better skin permeation ability of ethosomes opens the possibility of using these dosage forms for the delivery of immunizing agents. Table 2 is a short compilation of research reports on ethosomes as a carrier for a variety of drugs researched of late.

Cosmeceutical Applications of Ethosomes

The advantage of applying ethosomes in cosmeceuticals 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.[37] However, the compositions and sizes of the vesicles are the main factors to be considered to obtain these advantages of the elastic vesicles for cosmeceutical applications. Topical administration of many antioxidants is one of the several approaches to diminish oxidative injury in the skin for

Table 2: A compilation of research reports on ethosomes as a carrier for topical and transdermal delivery of drugs

Drug	Aim of work	Formulation	Results
Lamivudine (Jain *et al*. 2007)[33]	To improve skin permeation and intracellular uptake of antiviral drug	Suspension	Better intracellular skin delivery, as the ethosomal formulation affected the normal histology of the skin by producing lipid pertubation and increased the intercellular lipid lamellar space in the stratum corneum
Erythromycin (Godin *et al*. 2005)[25]	To treat deep skin and soft tissue bacterial infections by dermal application	Gel	Ethosomal erythromycin applied to the skin of S. aureus infected mice was as effective as systemically administered erythromycin
Gold nanoparticles (Presa *et al*. 2009)[34]	Gold nanoparticles generated in ethosomes bilayers, as revealed by cryo electron tomography	Suspension	Gold nanoparticles encapsulated ethosomes offer a versatile platform for the enhancement of pharmacological efficacy in transdermal and dermal delivery systems
Colchicine (Singh *et al*. 2009)[35]	Elastic liposomal formulation for sustained delivery of colchicine: *In vitro* characterization and *In vivo* evaluation of anti gout activity	Suspension	This reveals that elastic liposomal formulation of colchicine possesses a greater potential to enhance skin accumulation, prolong release, and improve the site specificity of colchicine
Vitamin A palmitate, vitamin e, vitamin c (Koli *et al*. 2008)[36]	Development of anti-oxidant ethosomes of vitamin a palmitate, vitamin e, vitamin c for topical delivery	Gel	The anti oxidation of PC was found to increase due to the synergistic interaction of all three together, as compared to individual use

cosmetic and cosmeceutical applications. However, antioxidants are usually not stable and can be degraded by exposing to light. These antioxidants include vitamin E, vitamin C, and flavonoids. Vitamin E is one of the major exogenous lipophilic antioxidants, which is usually found in tissues. Its topical application can enhance the skin protection from exogenous oxidants. When vitamin E is added to cosmetics and many dermatological products, it is found to decrease the production of lipid peroxides in the epidermis as well as to protect against UV exposure and some destructive chemicals and physical agents. In order to deliver vitamin E into the deeper layer of SC, 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,' and 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 formulations. This has suggested that although elastic and non-elastic liposomes are not beneficial for the delivery of α-tocopherol through the skin, the entrapment of the vitamin either in elastic or non-elastic liposomes can increase its photo-stability under UVB irradiation.[36]

In a study by Esposito *et al*., 2004,[38] ethosomes and liposomes of azelaic acid (Anti-keratinizing agent used in the treatment of acne) were prepared as a topical vehicle (gel) and the result demonstrated that ETHOS 40 could be responsible for a higher azelaic acid, with respect to ETHOS 20 and liposomes.

A USA company, Osmotics Inc., reported new cellulite cream called lipoduction, which used ethosome technology that penetrated the skin lipid barrier and delivered ingredients directly into the fat cells. Ingredients in lipoduction improved the appearance of cellulite by up to 80% in less than 60 days.

Stability of ethosomes

Stability of the formulations was evaluated in terms of the entrapment capacity and the particle size for a specified period. Basically, the proper choice of the lipid composition appeared to be an important factor in obtaining stable ethosomes dispersions with optimum pharmaceutical and therapeutic characteristics. In case of liposomes, upon storage, many different changes could occur. Liposomes tend to fuse and grow into bigger vesicles and this fusion and breakage of liposomes on storage pose an important problem of drug leakage from the vesicles. The absence of electrostatic repulsion is likely to account for the tendency of the neutral liposome to aggregate, but in case of ethosomes, ethanol causes a modification of the net charge of the system and confers it some degree of steric stabilization leading to increased stability of the dispersion against agglomeration that may also lead to a decrease in the mean vesicle size. Increasing the concentration of ethanol from 15 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%) probably makes the vesicle membrane more leaky, thus leading to a decrease in entrapment efficiency. Therefore, it causes destabilization of the ethosomes.

The lipid portion of the ethosomes is derived from natural and / or synthetic phospholipid sources. Phospholipids containing unsaturated fatty acids are known to undergo oxidative reactions. The reaction products can cause permeability changes in the ethosomes bilayers. Oxidative degradation of the lipids in general can be minimized by protecting the lipid preparation from light, by adding antioxidants such as α-tocopherol. Furthermore, hydrolysis of lipids leads to the formation of lyso-PC. The presence of lyso-PC enhances the permeability of ethosomes, and thus, it is essential to keep its level to a minimum in a given preparation.[39,40]

Future perspectives

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 the 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. The results of the first clinical study of the acyclovir-ethosomal formulation support this conclusion. Studies will continue to further improve the skin delivery of drugs using lipid vesicles. Special emphasis seems to be given to the skin delivery of proteins and other macromolecules and for transcutaneous immunization. The near future also holds the emergence of new commercial ethosome-based topical products. NTT, Novel Therapeutic Technology Inc., is a biopharmaceutical company with a portfolio of pharmaceutical formulations based on ethosome technology including formulations for the treatment of alopecia, deep skin infection, herpes, hormone deficiencies, inflammation, postoperative nausea, atopic dermatitis, and erectile dysfunction.

CONCLUSION

The last decade has shown a huge growth in the application of ethosomal technology to monitor skin permeability. The previously proposed mechanism of penetration enhancement with the ethosomal system suggested that the intercalation of ethanol into the polar head group environment results in increased membrane permeability. The understanding of the mechanisms of absorption and enhancement has improved and different determinants at a molecular level are beginning to be understood. With this it should be possible to achieve bioavailabilities comparable

to those expected in oral drug delivery. 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; topically applied ethosomes can increase the residence time of drugs or cosmetic chemicals in the stratum corneun and epidermis and reduce the systemic absorption of drugs or cosmetic chemicals, these properties allow them to penetrate easily into the deeper layers of the skin and circulation. However, the exact mode and nature of deformable vesicle transport into and possibly across the skin is not yet fully understood. Especially the degree of intact vesicle permeation across the stratum corneum and the partitioning of intact vesicles into the viable epidermis remains to be elucidated in more detail. In this respect, there are still inconsistencies in the results. This might be due to differences in vesicle composition, vesicle preparation, or in the skin structure of different species. The optimized developed elastic vesicular formulations, by adjusting their compositions and sizes, can be a promising means for not only cosmeceutical applications, but also for the topical non-invasive treatment of local and systemic disorders of many pharmaceuticals as well. With this possibility, developing safe and effective dermal and transdermal delivery systems should be far more successful.

REFERENCES

1. Barry BW. Novel mechanisms and devices to enable successful transdermal drug delivery. Eur J Pharm Sci 2001;14:101-14.
2. Hadgraft J. Passive enhancement strategies in topical and transdermal drug delivery. Int J Pharm 1999;184:1-6.
3. Thong HY, Zhai H, Maibach HI. Percutaneous enhancers: An overview. J Skin Pharmacol Physiol 2007;2:1-12.
4. Shim J, Kang HS, Park WS, Han SH. Transdermal delivery of minoxidil with ethanolic liposomes. J Cont Rel 2003;97:477-84.
5. Essa AE, Bonner MC, Barry BW. Electroporation and ultradeformable liposomes; Human skin barrier repair by phospholipids. J Cont Rel 2003;92:163-72.
6. Nguyen PL, Bowstra JA. Vesicles as a tool for transdermal and dermal delivery. Drug Disc Tec 2005;2:67-74.
7. Biju S, Talegoankar S, Mishra P, Khar RK. Vesicular systems An overview. Ind J Pharm Sci. 2006;5:141-50.
8. Ceve G. Lipids vesicles and other colloids as drug carrier on the skin. Adv Drug Del Rev 2004;56:675-711.
9. Touitou E, Dayan N, Bergelson L, Godin B, Eliaz M. Ethosomes-novel vesicular carriers for enhanced delivery: Characterization and skin penetration properties. J Control Release 1999;65:403-18.
10. Bendas ER, Tadros MI. Enhanced transdermal delivery of sulbutamol sulfate via ethosomes. AAPS Pharm Sci Tech 2007;8:1-7.
11. Godin B, Touitou E. Mechanism of bacitracin permeation enhancement through the skin and cellular membranes from an ethosomal carrier. J Control Release 2003;94:365-79.
12. Touitou E, Godin B, Dayan N. Intracellular delivery mediated by ethosomal carrier. Biomaterials 2001;22:3055-9.
13. Elsayed MA, Abdallah YO, Naggar FV, Khalafallah NM. Lipids vesicles for skin delivery of drugs: Reviewing three decades of research. Int J Pharm 2006;332:1-16.
14. Bhalaria MK, Naik S, Mishra AN. Ethosomes: A novel system for antifungal drugs in the treatment of topical fungal disease. Ind J Exp Biol 2009;47:368-75.
15. Dubey V, Mishra D, Jain NK. Melatonin loaded ethanolic liposomes: Physicochemical characterization and enhanced transdermal delivery. Eur J Pharm Biopharm 2007;67:398-405.
16. Dubey V, Mishra D, Dutta T, Nahar M, Saraf DK, Jain NK. Dermal and transdermal delivery of an anti-psoriatic agent via ethanolic liposomes. J Control Release 2007;123:148-54 .
17. Jain S, Tiwary AK, Sapra B, Jain NK. Formulation and evaluation of ethosomes for transdermal delivery of lamivudine. AAPS Pharm Sci Tech 2007;8:1-9.
18. Fang YP, Tsai YH, Wu PC, Huang YB. Comparision of 5-aminolevulinic acid-encapsulated liposomes versus ethosomes for skin delivery for photodynamic therapy. Int J Pharm 2008;356:144-52.
19. Godin B, Touitou E. Erythromycin ethosomal systems: Physicochemical characterization and enhanced antibacterial activity. Curr Drug Del 2005;2:269-75.
20. Rao Y, Zheng F, Gao J. *In vitro* percutaneous permeation and skin accumulation finasteride using vesicular ethosomal carrier. AAPS Pharm Sci Tech 2009;9:860-5.
21. Fang JY, Hwang TL, Leu HY. Effect of enhancers and retarders on percutaneous absorption of flurbiprofen from hydrogel. Int J Pharm 2003;250:313-25.
22. Lopez-Pinto JM, Gonzalez-Rodriguez ML, Rabasco AM. Effect of cholesterol and ethanol on dermal delivery from DPPC liposomes. Int J Pharm 2005;298:1-12.
23. Maghraby M, Campbell M, Finnin BC. Mechanism of action of novel skin penetration enhancers phospholipids versus skin lipids liposomes. Int J Pharm 2005;305:90-104.
24. Hu G, Liu J. Advances in studies of phospholipids as carriers in skin topical application. J Nanjug Med Univ 2007;21:349-53.
25. Godin B, Touitou E, Rubinstein E, Athamna A, Athamna M. A new approach for treatment of deep skin infection by an ethosomal antibiotic preparation: An *In vivo* study. J Antimicro Chemother 2005;55:989-94.
26. Verma DD, Verma S, Blume G, Fahr A. Ethosomes increases skin penetration of entrapped and non-entrapped hydrophilic substances into human skin: A skin penetration and confocal laser scanning microscopy studies. Eur J Pharm Biopharm 2003;55:271-7.
27. Vivek D, Dhirendra K. Ethosomes for enhanced transdermal drug delivery of aceclofenac. Int J Drug Del 2010;2:81-92.
28. Mishra D, Mishra PK, Dubey V, Nahar M, Jain NK. Systemic and mucosal immune response induced by transcutaneous immunization using Hepatitis B surface antigen-loaded modified liposomes. J Control Release 2007;33:424-33.
29. Ainbinder D, Touitou E. Testosterone ethosomes for enhanced transdermal delivery. Drug Del 2000;12:297-303.
30. Lodzki M, Godin B, Rakou L, Mechoulam R, Gallily R, Touitou E. Cannabiol-transdermal delivery and anti-inflammatory effect in a murine model. J Control Release 2003;93:377-87.
31. Paolino D, Lucania G, Mardente D, Alhaique F, Fresta M. Ethosomes for skin delivery of ammonium glycyrrhizinate: In vitro percutaneous permeation through human skin and in vivo anti-inflammatory activity on human volunteers. J Control Release 2005;106:99-110.
32. Gupta P, Singh P, Mishra V, Jain S. Topical immunization: Mechanistic insight and novel delivery system. Ind J Bio 2004;3:9-21.
33. Jain S, Umamaheshwari RB, Bhadra D, Jain NK. Ethosomes: A

novel vesicular carriers for enhanced transdermal delivery of an anti HIV agent. Ind J Pharm Sci 2004;66:72-81.

34. Presa P, Rued T, Hernando A. Gold nanoparticles generated in ethosomes bilayers, revealed by cryo electron tomography. J Phys Chem 2009;113:3051-7.
35. Singh HP, Utreja P, Tiwary AK, Jain S. Elastic liposomal formulations for sustained delivery of Colchicine: In vitro characterization and In vivo evaluation of anti gout activity. AAPS Pharm Sci Tec 2008;11:54-64.
36. Koli JR, Lin S. Development of anti oxidant ethosomes for topical delivery utilizing the synergistic properties of Vit A palmitate, Vit E and Vit C. AAPS Pharm Sci Tec 2009;11:1-8.
37. Manosrai A, Jantrawut P, Khositsuntiwong N, Manosroi W, Manosroi J. Novel Elastic Nanovesicles for Cosmeceutical and Pharmaceutical Applications. Chiang Mai J Sci 2009;36:168-78.
38. Esposito E, Menegatti E, Cortesi R. Ethosomes and liposomes as topical vehicles for azeliac acid: A preformulation study. J Cosmet Sci 2004;55:253-64.
39. Rao LS. Preparation of liposomes on the industrial scale: Problems and perspectives. In: Gregoriadis G, editor. Liposome technology, Vol. 1. Florida: CRC Press; 1984.
40. New RRC. Preparation of liposomes. In: New RRC, editor. Liposomes-a practical approach. Oxford: Oxford University Press; 1990.

Source of Support: Nil, **Conflict of Interest:** Nil.