Ethosomes - Novel Vesicular Carriers For Enhancing Transdermal Drug Delivery

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ABSTRACT

Skin acts as a major target as well as a principal barrier for topical drug delivery. The major obstacle of this system is the low diffusion rate of drugs across the stratum corneum. Ethosomes are noninvasive delivery enable drugs to reach deep skin layers and to the systemic circulation. Ethosomes are soft, malleable vesicles tailored for enhanced delivery of active agents. Because of their unique structure, ethosomes are able to encapsulate and deliver through the skin highly lipophilic molecules as well as cationic drugs. In this article reviews various aspect of ethosomes including their mechanism of penetration, preparation, advantages, characterization, composition, preparation, application and marketed product. Enhanced delivery of bioactive molecules through the skin and cellular membranes by means of an ethosomal carrier opens numerous challenges and opportunities for the research and future development of novel improved therapies.

Keywords: Ethosomes, transdermal, vesicular carriers, ethanol, phospholipid.

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 drug delivery system to be effective, the drug must obviously be able to penetrate the skin barrier and reach the target site.

Transdermal drug delivery system (TDDS) showed promising result in comparison to oral drug delivery system as it eliminates gastrointestinal interferences and first pass metabolism of the drug but the main drawback of TDDS is it encounters the barrier properties of the Stratum Corneum i.e. only the lipophilic drugs having molecular weight < 500 Dacan pass through it[2-3]. FDA approved the first transdermal patch products in 1981. These delivery systems provided the controlled systemic absorption of scopolamine for the prevention of motion sickness and nitroglycerine for the prevention of angina pectoris associated with coronary artery disease (Transderm-Nitro). Over the last two decades, more than 35 transdermal products have been approved generating sales of $3.2 billion in 2002, which is predicted to rise to $4.5 billion in 2008. More recently, such dosage forms have been developed and/or modified in order to enhance the driving force of drug diffusion (thermodynamic activity) and/or increase the permeability of the skin. These approaches include the use of penetration enhancers, supersaturated systems, prodrugs, liposomes and other vesicles. One of the major advances in vesicle research was the finding that some modified vesicles possessed properties that allowed them to successfully deliver drugs in deeper layers of skin. Transdermal delivery is important because it is a noninvasive procedure for drug delivery. Further, problem of drug degradation by digestive enzymes after oral administration and discomfort associated with parenteral drug administration can be avoided. It is the most preferred route for systemic delivery of drugs to pediatric, geriatric and patients having dysphasia. Despite the promise, there were many

problems that researchers had to face with while attempting successful transdermal drug delivery.

The skin is a multi-layered structure made up of stratum corneum (SC), the outermost layer, under which lies the epidermis and dermis. Within these layers of skin are interspersed fibroblasts, hair follicles and sweat glands that originate in the dermis blood supply. The almost insurmountable nature of SC is a major challenge for systemic delivery of percutaneously applied drugs.[4-6] The Òbrick and mortarÓ arrangement of corneocytes, flattened mononucleated keratinocytes, with interspersed lipids and proteins makes the SC approximately 1000 times less permeable than other biological membranes. Furthermore, it is even more difficult for anything to penetrate to the deeper strata of skin. To overcome the stratum corneum barrier, various mechanisms have been investigated, including use of chemical or physical enhancers such as iontophoresis, sonophoresis, etc. Liposomes, niosomes, transferosomes and ethosomes also have the potential of overcoming the skin barrier and have been reported to enhance permeability of drug through the stratum corneum barrier. 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. 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.[7-10]

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. 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.[11]

The physical means like iontophoresis, microneedles, and sonophoresis are relatively complicated to use, and will affect patient compliance.[12] 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. One such approach is the use of vesicular systems.[13]

In the past decade, topical delivery of drugs by liposomal formulation has evoked considerable interest. Deformable liposomes 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 non occluded conditions. The drug, encapsulated in lipid vesicles, prepared from phospholipids and nonionic surfactants is known to be transported into and across the skin. The lipids 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. 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.[14] 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.[15-18]

Ethosomes

Ethosomes are novel carrier system used for delivery of drugs having low penetration through the biological membrane mainly skin. Ethosomes are the slight modification of well established drug carrier liposome. Ethosomes are soft vesicles made of phospholipids and ethanol (in higher quantity) and water.[19-22] The size range of ethosomes may vary from tens of nanometers to microns (μ). Ethosomes permeate through the skin layers more rapidly

and possess significantly higher transdermal flux in comparison to conventional liposomes. Although, the exact mechanism for better permeation into deeper skin layers from ethosomes is still not clear. The synergistic effects of combination of phospholipids and high concentration of ethanol in vesicular formulations have been suggested to be responsible for deeper distribution and penetrationin the skin lipid bilayers. Ethosomes are mainly used for the delivery of drugs through transdermal route. The transdermal delivery is one of the most important routes of drug administration. The main factor which limits the application of transdermal route for drug delivery is the permeation of drugs through the skin. Human skin has selective permeability for drugs. Lipophilic drugs can pass through the skin but the drugs which are hydrophilic in nature can't pass through. Water soluble drugs either show very less or no permeation.[23-25] Ethosomes can entrap drug molecule with various physicochemical characteristics i.e. of hydrophilic, lipophilic, or amphiphilic.[26, 27]

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Ethosome formulations provide sustained delivery of drugs where ethosomes act as reservoir system for continues delivery of drugs.[30] Visualization by transmission electron microscopy showed that ethosomes could be unilamellar or multilamellar through to the core.[31-33] 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.[34] Contrary to Transfersomes® ethosomes improves skin delivery of drugs both under occlusive and non-occlusive condition.[35-37]

Potential advantages of this system include[38]

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.

Ethosome Composition

The ethosomes are vesicular carrier comprise of hydroalcoholic or hydro/alcoholic/glycolic phospholipid in which the concentration of alcohols or their combination is relatively high. Typically, ethosomes may contain phospholipids with various chemical structures like phosphatidylcholine (PC), hydrogenated PC, phosphatidic acid (PA), phosphatidylserine (PS), phosphatidylethanolamine (PE), phosphatidylglycerol (PPG), phosphatidylinositol (PI), hydrogenated PC, alcohol (ethanol or isopropyl alcohol), water and propylene glycol (or other glycols). Such a composition enables delivery of high concentration of active ingredients through skin. Drug delivery can be modulated by altering alcohol: water or alcohol-polyol: water ratio. Some preferred phospholipids are soya phospholipids such as Phospholipon 90 (PL-90). It is usually employed in a range of 0.5-10% w/w. Cholesterol at concentrations ranging between 0.1-1% can also be added to the preparation. Examples of alcohols, which can be used, include ethanol and isopropyl alcohol. Among glycols, propylene glycol and Transcutol are generally used. In addition, non-ionic surfactants (PEG-alkyl ethers) can be combined with the phospholipids in these preparations. Cationic lipids like cocoamide, POE alkyl amines, dodecylamine, cetrimide etc. can be added to concentration of the non-aqueous phase (alcohol and glycol combination) may range between 22 to 70%.[39-45]

Influence of high alcohol content

Ethanol is an established efficient permeation enhancer [46, 47] and is present in quite high concentration (20-50%) in ethosomes. However, due to the interdigitation effect of ethanol on lipid bilayers, it was commonly

believed that vesicles could not coexist with high concentration of ethanol.[48]

Touitou discovered and investigated lipid vesicular systems embodying ethanol in relatively high concentration and named them ethosomes. The basic difference between liposomes and ethosomes lies in their composition. The synergistic effect of combination of relatively high concentration of ethanol (20-50%) in vesicular form in ethosomes was suggested to be the main reason for their better skin permeation ability. The high concentration of ethanol (20-50%) in ethosomal formulation could disturb the skin lipid bilayer organization. Therefore, when integrated into a vesicle membrane, it could give an ability to the vesicles to penetrate the stratum corneum.[49, 50] Furthermore, due to high ethanol concentration the ethosomal lipid membrane was packed less tightly than conventional vesicles but possessed equivalent stability. This allowed a softer and malleable structure giving more freedom and stability to its membrane, which could squeeze through small openings created in the disturbed stratum corneum lipids. In addition, the vesicular nature of ethosomal formulations could be modified by varying the ratio of components and chemical structure of the phospholipids. The versatility of ethosomes for systemic delivery is evident from the reports of enhanced delivery of quite a few drugs like acyclovir, minoxidil, triphexyphenidyl, testosterone, cannabidol and zidovudine.[51-53]

Method of Preperation of Ethosomes
Ethosomal formulation may be prepared by hot or cold method as described below. Both the methods are convenient, do not require any sophisticated equipment and are easy to scale up at industrial level.[54-58]

A) Hot method
In this method phospholipid is dispersed in water by heating in a water bath at 40^0C until a colloidal solution is obtained. In a separate vessel ethanol and propylene glycol are mixed and heated to 40^0C. Once both mixtures reach 40^0C, the organic phase is added to the aqueous one. The drug is dissolved in water or ethanol depending on its hydrophilic/ hydrophobic properties. The vesicle size of ethosomal formulation can be decreased to the desire extent using probe sonication or extrusion method.

B) Cold Method
This is the most common method utilized for the preparation of ethosomal formulation. In this method phospholipid, drug and other lipid materials are dissolved in ethanol in a covered vessel at room temperature by vigorous stirring with the use of mixer. Propylene glycol or other polyol is added during stirring. This mixture is heated to 30^0C in a water bath. The water heated to 30^0C in a separate vessel is added to the mixture, which is then stirred for 5 min in a covered vessel. The vesicle size of ethosomal formulation can be decreased to desire extend using sonication or extrusion method. Finally, the formulation is stored under refrigeration.

C) 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.[59]

Mechanism of Action of the Ethosomal Drug Delivery System
A synergistic mechanism was suggested between ethanol, vesicles, and skin lipids. The enhanced delivery of actives 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 4, 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.[60-65]

Method of Characterizations of Ethosomal Formulation[66]

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

2. Vesicle size and Zeta potential
Particle size and zeta potential can be determined by dynamic light scattering (DLS) using a computerized inspection system and photon correlation spectroscopy (PCS).

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

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

5. Drug content
Drug content of the ethosomes can be determined using UV spectrophotometer. This can also be quantified by a modified high performance liquid chromatographic method.

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

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

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

Evaluation Test[68]
Filter Membrane-Vesicle Interaction Study by Scanning Electron Microscopy
Vesicle suspension (0.2 ml) was applied to filter membrane having a pore size of 50 nm and placed in diffusion cells. The upper side of the filter was exposed to the air, whereas the lower side was in contact with PBS (phosphate buffer saline solution), (pH 6.5). The filters were removed after 1 hour and prepared for SEM studies by fixation at 4°C in Karnovsky's fixative overnight followed by dehydration with graded ethanol solutions (30%, 50%, 70%, 90%, 95%, and 100% vol/vol in water). Finally, filters were coated with gold and examined in SEM (Leica, Bensheim, Germany).

Vesicle-Skin Interaction Study by Fluorescence Microscopy
Fluorescence microscopy was carried according to the protocol used for TEM and SEM study. Paraffin blocks are used, were made, 5-µm thick sections were cut using microtome (Erma optical works, Tokyo, Japan) and examined under a fluorescence micro Cytotoxicity Assay MT-2 cells (T-lymphoid cell lines) were propagated in Dulbecco's modified Eagle medium (HIMEDIA, Mumbai,India) containing 10% fetal calf serum, 100 U/ml penicillin, 100 mg/ml streptomycin, and 2 mol/l glutamine at 37°C under a 5% CO_2 atmosphere. Cytotoxicity was expressed as the cytotoxic dose 50 (CD50) that induced a 50% reduction of absorbance at 540 nm.

Vesicle-Skin Interaction Study by TEM and SEM
From animals ultra thin sections were cut (Ultracut, Vienna, Austria), collected on formvar-coated grids and examined under transmission electron microscope. For SEM analysis, the sections of skin after dehydration were mounted on stubs using an adhesive tape and were coated with gold palladium alloy using a fine coat ion sputter coater. The sections were examined under scanning electron microscope.

HPLC Assay
The amount of drug permeated in the receptor compartment during in vitro skin permeation experiments and in MT-2 cell was determined by HPLC assay using methanol: distilled-water :acetonitrile (70:20:10 vol/vol) mixture as mobile phase delivered at 1 mL/min by LC 10-AT vp pump (Shimadzu, Kyoto, Japan). A twenty microliter injection was eluted in C-18 column (4.6×150 mm, Luna, 54, Shimadzu) at room temperature. The column eluent was monitored at 271 nm using SPDM10A vp diode array UV detector. The coefficient of variance (CV) for standard curve ranged from 1.0% to 2.3%, and the squared correlation coefficient was 0.9968.

Drug Uptake Studies

The uptake of drug into MT-2 cells (1×106 cells/mL) was performed in 24-well plates (Corning Inc) in which 100 µL RPMI medium was added. Cells were incubated with 100 µL of the drug solution in PBS (pH 7.4), ethosomal formulation, or marketed formulation, and then drug uptake was determined by analyzing the drug content by HPLC assay.

Skin Permeation Studies

The hair of test animals (rats) were carefully trimmed short (<2 mm) with a pair of scissors, and the abdominal skin was separated from the underlying connective tissue with a scalpel. The excised skin was placed on aluminium foil, and the dermal side of the skin was gently teased off for any adhering fat and/or subcutaneous tissue. The effective permeation area of the diffusion cell and receptor cell volume was 1.0 cm2 and 10 mL, respectively. The temperature was maintained at 32°C ± 1°C. The receptor compartment contained PBS (10 ml of pH 6.5). Excised skin was mounted between the donor and the receptor compartment. Ethosomal formulation (1.0 ml) was applied to the epidermal surface of skin. Samples (0.5 ml) were withdrawn through the sampling port of the diffusion cell at 1-, 2-, 4-, 8-, 12-, 16-, 20-, and 24-hour time intervals and analyzed by high performance liquid chromatography (HPLC) assay.

Stability Study

Stability of the vesicles was determined by storing the vesicles at 4°C ± 0.5°C. Vesicle size, zeta potential, and entrapment efficiency of the vesicles was measured after 180 days using the method described earlier [2] 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 leakier, 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.

Applications of ethosomes

Ethosomes can be used for many purposes in drug delivery.Ethosomes are mainly used as replacement of liposomes. Mainly the transdermal route of drug delivery is preferred. Ethosomes can be used for the transdermal delivery of hydrophilic and impermeable drugs through the skin. Various drugs have been used with ethosomal carrier (Table 4).

1. Delivery of Anti-Viral Drugs

Zidovudine is a potent antiviral agent acting on acquired immunodeficiency virus. Oral administration of zidovudine is associated with strong side effects.Therefore, an adequate zero order delivery of zidovudine is desired to maintain expected anti-AIDS effect.[69] Jain *et al.* concluded that ethosomes could increase the transdermal flux, prolong the release and present an attractive route for sustained delivery of zidovudine. Acyclovir is another anti-viral drug that widely used topically for treatment of Herpes labialis.[70] The conventional marketed acyclovir external formulation is associated with poor skin penetration of hydrophilic acyclovir to dermal layer resulting in weak therapeutic efficiency. It is reported that the replication of virus takes place at the basal dermis. To overcome the problem associated with conventional topical preparation of acyclovir .[71] Horwitz *et al.* formulated the acyclovir ethosomal formulation for dermal delivery. The results showed that shorter healing time and higher percentage of

abortive lesions were observed when acyclovir was loaded into ethosomes.

2. Topical Delivery of DNA

Many environmental pathogens attempt to enter the body through the skin. Skin therefore, has evolved into an excellent protective barrier, which is also Immunologically active and able to express the gene.[72] On the basis of above facts another important application of ethosomes is to use them for topical delivery of DNA molecules to express genes in skin cells. Touitou et al. in their study encapsulated the GFP-CMV-driven transfecting construct into ethosomal formulation. They applied this formulation to the dorsal skin of 5-week male CD-1 nude mice for 48 hr. After 48 hr, treated skin was removed and penetration of green fluorescent protein (GFP) formulation was observed by CLSM. It was observed that topically applied ethosomes-GFP-CMV-driven transfecting construct enabled efficient delivery and expression of genes in skin cells. It was suggested that ethosomes could be used as carriers for gene therapy applications that require transient expression of genes.

These results also showed the possibility of using ethosomes for effective transdermal immunization. Recently reported immunization potential using transfersomal formulation. Hence, better skin permeation ability of ethosomes opens the possibility of using these dosage forms for delivery of immunizing agents.

3. Transdermal Delivery of Hormones

Oral administration of hormones is associated with problems like high first pass metabolism, low oral bioavailability and several dose dependent side effects The risk of failure of treatment is known to increase with each pill missed.[73] Touitou *et al.* compared the skin permeation potential of testosterone ethosomes (Testosome) across rabbit pinna skin with marketed transdermal patch of testosterone (Testoderm patch, Alza). They observed nearly 30-times higher skin permeation of testosterone from ethosomal formulation as compared to that marketed formulation.

4. Delivery of anti-parkinsonism agent

Dayan and Touitou prepared ethosomal formulation of psychoactive drug rihexyphenidyl hydrochloride (THP) and compared its delivery with that from classical liposomal formulation. THP is a M1 muscarinic receptors antagonist and used in the treatment of Parkinson disease. The results indicated better skin permeation potential of ethosomal-THP formulation and its use for better management of Parkinson disease.

5. Delivery of Anti-Arthritis Drug

Topical delivery of anti-arthritis drug is a better option for its site-specific delivery and overcomes the problem associated with conventional oral therapy. Cannabidol (CBD) is a recently developed drug candidate for treating rheumatoid arthritis. Lodzki *et al.* prepared CBDethosomal formulation for transdermal delivery. Results shows significantly increased in biological anti-inflammatory activity of CBD-ethosomal formulation was observed when tested by carrageenan induced rat paw edema model. It was concluded encapsulation of CBD in ethosomes significantly increased its skin permeation, accumulation and hence it's biological activity.

6. Delivery of Problematic drug molecules

The oral delivery of large biogenic molecules such as peptides or proteins is difficult because they are completely degraded in the GI tract. Non-invasive delivery of proteins is a better option for overcoming the problems associated with oral delivery .[74] Dkeidek and Touitou 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 applied on the abdominal area of an overnight fated rat. The result showed that insulin delivered from this patch produced a significant decrease (up to 60%) in BGL in both normal and diabetic rats. On the other hand, insulin application from a control formulation was not able to reduce the BGL. Verma and Fahr[75] reported the cyclosporin A ethosomal formulation for the treatment of inflammatory skin disease like psoriasis, atopic dermatitis and disease of hair follicle like alopecia areata etc. Paolino *et al.*[76] investigated the potential application of ethosomes for dermal delivery of ammonium glycyrrhizinate. Ammonium glycyrrhizinate is naturally occurring triterpenes obtained from Glycyrrhizinate Glabra and useful for the treatment of various inflammatory based skin diseases.[77]

7. Delivery of Antibiotics

Topical delivery of antibiotics is a better choice for increasing the therapeutic efficacy of these agents. Conventional oral therapy causes several allergic reactions along with several side effects. Conventional external preparations possess low permeability to deep

skin layers and subdermal tissues.[78] Ethosomes can circumvent this problem by delivering sufficient quantity of antibiotic into deeper layers of skin. Ethosomes penetrate rapidly through the epidermis and bring appreciable amount of drugs into the deeper layer of skin and suppress infection at their root. With this purpose in mind Godin and Touitou prepared bacitracin and erythromycin loaded ethosomal formulation for dermal and intracellular delivery. The results of this study showed that the ethosomal formulation of antibiotic could be highly efficient and would overcome the problems associated with conventional therapy.

8. Pilosebaceous Targeting

Hair follicles and sebaceous glands are increasingly being recognized as potentially significant elements in the percutaneous drug delivery. Interest in pilosebaceous units has been directed towards their use as depots for localized therapy, particularly for the treatment of follicle-related disorders such as acne or alopecia. Furthermore, considerable attention has also been focused on exploiting the follicles as transport shunts for systemic drug delivery.[79] With the purpose of pilosebaceous targeting.

9. 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.[80]

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.[81]

The first commercial product based on ethosome technology was marketed in 2000, and majority of products marketed so far are cosmeceutical products. Nanominox 49©, containing minoxidil (hair growth promoter), marketed by Sinere, is the first minoxidil containing product, which uses ethosome technology. Another product, NoicellexTM, an anti-cellulite ethosome formulation is currently marketing in Japan by an Israel based company Novel Therapeutic Technologies (NTTs). LipoductionTM, another anticellulite 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.

FUTURE PROSPECTS

Introduction of ethosomes has initiated a new area in vesicular research for transdermal drug delivery. Different reports show a promising future of ethosomes in making transdermal delivery of various agents more effective. Further, research in this area will allow better control over drug release in vivo, allowing physician to make the therapy more effective. Ethosomes offers a good opportunity for the non-invasive delivery of small, medium and large sized drug molecules. The results of the first clinical study of acyclovir - ethosomal formulation support this conclusion. Multiliter quantities of ethosomal formulation can be prepared very easily. It, therefore, should be not before long that the corresponding drug formulation would have found their way into clinics to be tested for widespread usage. Thus, it can be a logical conclusion that ethosomal formulations possess promising future in effective dermal/transdermal delivery of bioactive agents.

CONCLUSION

Ethosomal carrier opens new challenges and opportunities for the development of novel improved therapies. Ethosomes are soft, malleable vesicles and potential carrier for transportation of drugs. Ethosomes are characterized by simplicity in their preparation, safety and efficacy and can be tailored for enhanced skin permeation of active drugs. Ethosomes have been found to be much more efficient at delivering drug to the skin, than either liposomes or hydroalcoholic solution.It can be easily concluded that ethosomes can provide better skin permeation than liposomes. The main limiting factor of transdermal drug delivery system i.e. epidermal barrier can be overcome by ethosomes to significant extent. Application of ethosomes provides the advantages such as improved permeation

through skin and targeting to deeper skin   layers for various skin diseases.

Table 1: Composition of Ethosomes for Transdermal delivery

Class	Example	Uses
Phospholipid	Soya phosphatidyl choline Egg phosphatidyl choline Dipalmityl phosphatidyl choline Distearyl phosphatidyl choline	Vesicles forming component
Polyglycol	Propylene glycol Transcutol RTM	As a skin penetration enhancer
Alcohol	Ethanol Isopropyl alcohol	For providing the softness for vesicle membrane As a penetration enhancer
Cholesterol	Cholesterol	For providing the stability to vesicle membrane
Dye	Rhodamine-123 Rhodamine red Fluorescene Isothiocynate (FITC) 6- Carboxy fluorescence	For characterization study
Vehicle	Carbopol Ð934	As a gel former

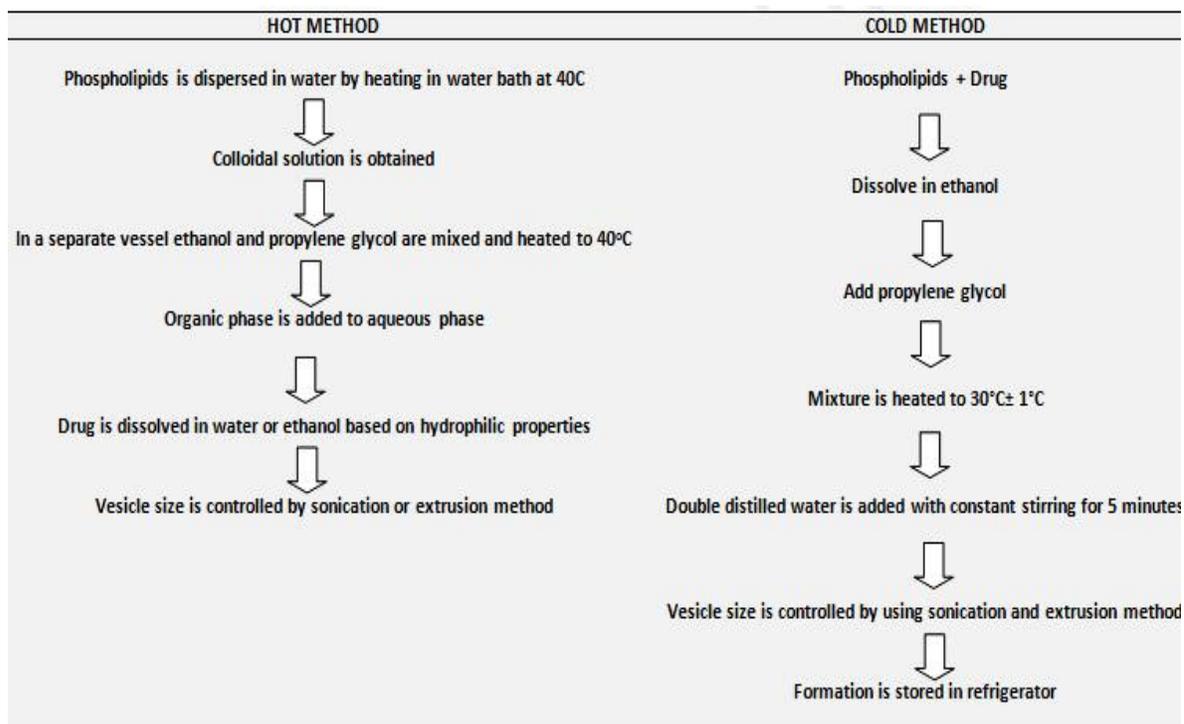**Table 2: Flow chart representation of Hot method and Cold method**

HOT METHOD	COLD METHOD
Phospholipids is dispersed in water by heating in water bath at 40C	Phospholipids + Drug
⇓	⇓
Colloidal solution is obtained	Dissolve in ethanol
⇓	⇓
In a separate vessel ethanol and propylene glycol are mixed and heated to 40°C	Add propylene glycol
⇓	⇓
Organic phase is added to aqueous phase	Mixture is heated to 30°C± 1°C
⇓	⇓
Drug is dissolved in water or ethanol based on hydrophilic properties	Double distilled water is added with constant stirring for 5 minutes
⇓	⇓
Vesicle size is controlled by sonication or extrusion method	Vesicle size is controlled by using sonication and extrusion method
	⇓
	Formation is stored in refrigerator

Table 3: Methods for the Characterization of Ethosomal Formulation

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

Table 4: Application of Ethosomes as a Drug Carrier

| Drug	Results	References
NSAIDS (Diclofenac)	☐ Selective delivery of drug to desired side for prolong period of time	[69, 70]
Acyclovir	☐ Increase skin permeation ☐ Improved in biological activity two to three times ☐ Improved in Pharmacodynamic profile	[75]
Insulin	☐ Significant decrease in blood glucose level ☐ Provide control release	[60]
Trihexyphenidyl hydrochloride	☐ Improved transdermal flux ☐ Provide controlled release ☐ Improved patient compliance ☐ Biologically active at dose several times lower than the currently used formulation	[77]
DNA	☐ Better expression of genes ☐ Selective targeting to dermal cells	[74]
Antibiotic Cannabidol Erythromycin	☐ Improved skin deposition ☐ Improved biological activity ☐ Prolonging drug action	[78]
Bacitracin	☐ Improved dermal deposition ☐ Improved intracellular delivery ☐ Increased bioavailability	[67]
Anti-HIV agents Zidovudine Lamivudine	☐ Improved transdermal flux ☐ Improved in biological activity two to three times ☐ Prolonging drug action ☐ Reduced drug toxicity ☐ Affected the normal histology of skin	[79]
Azelaic acid	☐ Prolong drug release	[80]
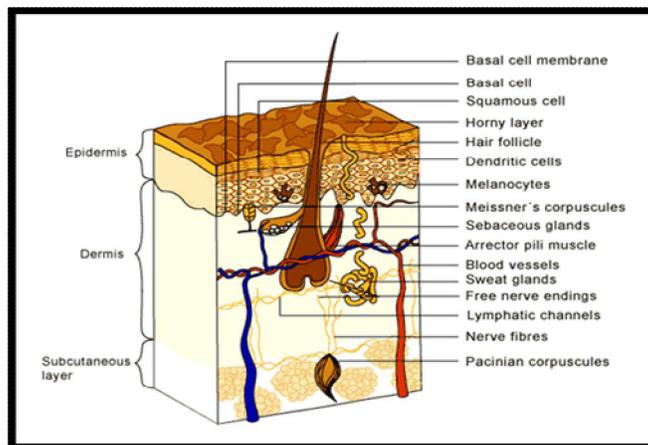
Fig. 1: Structure of skin
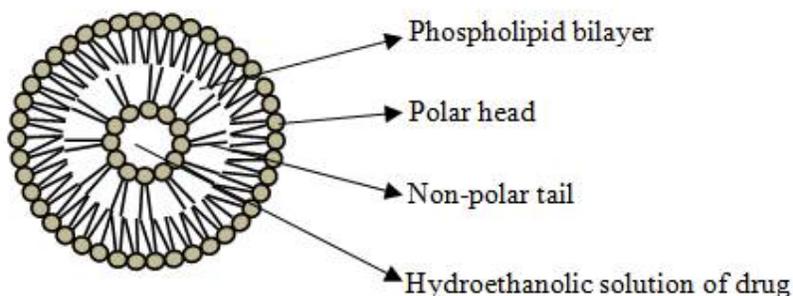
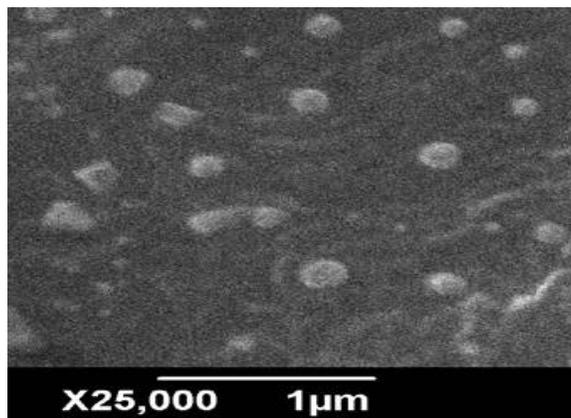**Fig. 2: Proposed diagram of Ethosome vesicle**

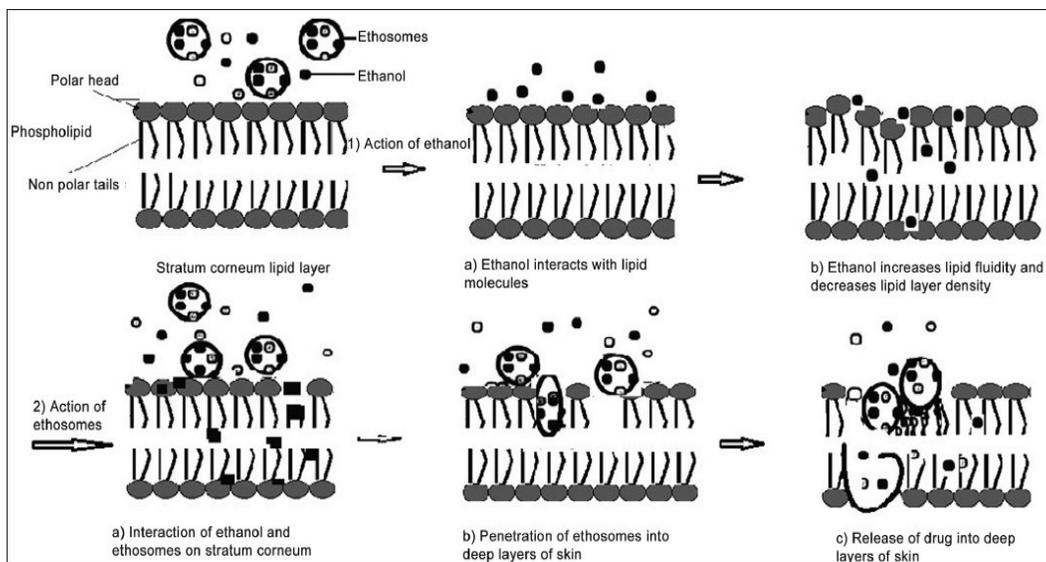**Fig. 3: SEM image of ethosome**

Fig. 4: Mechanism of Penetration of Ethosomal Drug Delivery System

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