



Review Article

ETHOSOMES: A RECENT VESICLE OF TRANSDERMAL DRUG DELIVERY SYSTEM

Tarun Parashar^{1*}, Soniya¹, Roopesh Sachan¹, Vishal Singh¹, Gaurav Singh¹, Satyanand Tyagi², Chirag Patel³, Anil Gupta⁴

1. Himalayan Institute of Pharmacy and Research, Rajawala, Dehradun, Uttarakhand, India-248002.
2. President, Tyagi Pharmacy Association & Scientific Writer (Pharmacy), Chattarpur, New Delhi, India-110074.
3. Department of Pharmaceutics, Maharishi Arvind Institute of Pharmacy, Mansarovar, Jaipur, Rajasthan, India-302020.
4. Research Scholar, Pharmaceutical Sciences and Research Center, Bhagwant University, Ajmer Rajasthan, India-305004.

*Corresponding Author: Email parashar89tarun@gmail.com

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ABSTRACT

Transdermal drug delivery system was first introduced more than 30 years ago. The technology generated tremendous excitement and interest amongst major pharmaceutical companies in the 1980s and 90s. By the mid to late 1990s, the trend of transdermal drug delivery system merged into larger organizations. Ethosomes are the ethanolic phospholipid vesicles which are used mainly for transdermal delivery of drugs. Ethosomes have higher penetration rate through the skin as compared to liposomes hence these can be used widely in place of liposomes. Ethosomes have become an area of research interest, because of its enhanced skin permeation, improved drug delivery, increased drug entrapment efficiency etc. The purpose of writing this review on ethosomes drug delivery was to compile the focus on the various aspects of ethosomes including their mechanism of penetration, preparation, advantages, composition, characterization, application and marketed product of ethosomes. Characterizations of ethosomes include Particle size, Zeta potential, Differential Scanning Calorimetry, Entrapment efficiency, Surface tension activity measurement, Vesicle stability and Penetration Studies etc.

Keywords: Ethosome, Ethanol, Transdermal delivery, Phospholipid, Vesicle

INTRODUCTION

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 Da can pass through it [1, 2]. To improve the permeation of drugs through the skin various mechanisms have been

investigated, including use of chemical or physical enhancers, such as iontophoresis, sonophoresis, etc. Liposomes, niosomes, transfersomes and ethosomes also have been reported to enhance permeability of drug through the stratum corneum barrier. Permeation enhancers increase the permeability of the skin, so that the drugs can cross through the skin easily. Unlike classic liposomes, [3] that are known mainly to deliver drugs to the outer layers of skin, ethosomes can enhance

permeation through the stratum corneum barrier [4, 5]. Ethosomes permeate through the skin layers more rapidly and possess significantly higher transdermal flux in comparison to conventional liposomes [6-8].

Ethosomes (**Fig. 1**) are lipid vesicles containing phospholipids, alcohol (ethanol and isopropyl alcohol) in relatively high concentration and water. Ethosomes are soft vesicles made of phospholipids and ethanol (in higher quantity) and water [1, 6]. Ethosomes can entrap drug molecule with various physicochemical characteristics i.e. of hydrophilic, lipophilic, or amphiphilic. The size range of ethosomes may vary from tens of nanometers to microns () [9, 10].

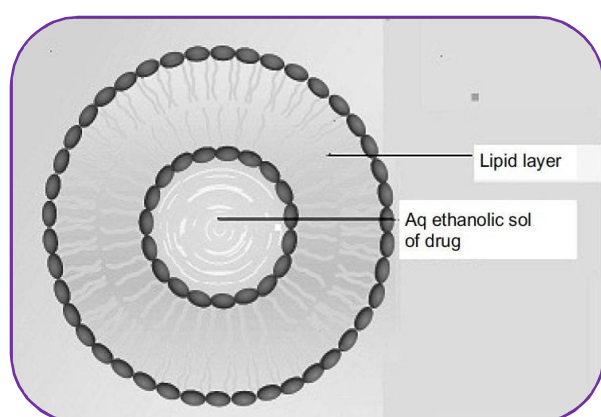


Figure 1: Diagram of Ethosomes

ADVANTAGES OF ETHOSOMAL DRUG DELIVERY

1. Delivery of large molecules (peptides, protein molecules) is possible.
2. It contains non-toxic raw material in formulation.
3. Enhanced permeation of drug through skin for transdermal drug delivery.
4. Ethosomal drug delivery system can be applied widely in Pharmaceutical, Veterinary, Cosmetic fields.
5. High patient compliance: The ethosomal drug is administered in semisolid form (gel or cream) hence producing high patient compliance.
6. Simple method for drug delivery in comparison to iontophoresis and Phonophoresis and other complicated methods
7. The Ethosomal system is passive, non-invasive and is available for immediate commercialization [1].

ETHOSOMES COMPOSITION [2]

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 too. The concentration of alcohol in the final product may range from 20 to 50%. The concentration of the non-aqueous phase (alcohol and glycol combination) may range between 22 to 70% (**Table 1**).

MECHANISM OF DRUG PENETRATION

The main advantage of ethosomes over liposomes is the increased permeation of the drug. The mechanism of the drug absorption from ethosomes is not clear. The drug absorption probably occurs in following two phases:

1. Ethanol effect
2. Ethosomes effect

1. Ethanol effect:

Ethanol acts as a penetration enhancer through the skin. The mechanism of its penetration enhancing effect is well known. Ethanol penetrates into intercellular lipids and increases the fluidity of cell membrane lipids and decrease the density of lipid multilayer of cell membrane.

Table: 1 Different additive employed in formulation of Ethosomes [2]

Class	Example	Uses
Phospholipid	Soya phosphatidyl choline Egg phosphatidyl choline Dipalmityl phosphatidyl choline Distearyl phosphatidyl choline	Vesicles forming component
Alcohol	Ethanol Isopropyl alcohol	For providing the softness for vesicle membrane As a penetration enhancer
Polyglycol	Propylene glycol Transcutol RTM	As a skin penetration enhancer
Cholesterol	Cholesterol	For providing the stability to vesicle membrane
Dye	Rhodamine-123 Rhodamine red Fluorescence Isothiocyanate (FITC) 6- Carboxy fluorescence	For characterization study
Vehicle	Carbopol D934	As a gel former

2. Ethosome effect:

Increased cell membrane lipid fluidity caused by the ethanol of ethosomes results increased skin permeability. So the ethosomes permeates very easily inside the deep skin layers, where it got fused with skin lipids and releases the drugs into deep layer of skin [1].

METHODS OF PREPARATION ETHOSOMES

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

1. Cold Method:

This is the most common method utilized for the preparation of ethosomal formulation. In this method phospholipid, drug and other lipid materials are dissolved in ethanol in a covered vessel at room temperature by vigorous stirring with the use of mixer. Propylene glycol or other polyol is added during stirring. This mixture is heated to 300°C in a water bath. The water heated to 300°C 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 [11, 12].

2. Hot method:

In this method phospholipid is dispersed in water by heating in a water bath at 400C until a colloidal solution is obtained. In a separate vessel ethanol and propylene glycol are mixed and heated to 400°C. Once both mixtures reach 400°C, the organic phase is added to the aqueous one. The drug is dissolved in water or ethanol depending on its hydrophilic/hydrophobic properties. The vesicle size of ethosomal formulation can be decreased to the desire extent using probe sonication or extrusion method [11, 12].

CHARACTERIZATIONS OF ETHOSOMES**1. Visualization**

Visualization of ethosomes can be done using transmission electron microscopy (TEM) and by scanning electron microscopy (SEM) [11].

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) [13].

3. Differential scanning calorimetry (DSC)

Transition temperature (T_m) of the vesicular lipid systems

was determined by using the Mettler DSC 60 computerized with Mettler Toledo star software system (Mettler, Switzerland). The transition temperature was measured by using the aluminium crucibles at a heating rate 10 degree/minute, within a temperature range from 20°C–300°C [14, 15].

4. Surface Tension Activity Measurement

The surface tension activity of drug in aqueous solution can be measured by the ring method in a Du Nouy ring tensiometer [14, 15].

5. Entrapment Efficiency

The entrapment efficiency of drug by ethosomes can be measured by the ultra centrifugation technique [15].

6. Penetration and Permeation Studies

Depth of penetration from ethosomes can be visualized by confocal laser scanning [11].

7. Vesicle Stability

The stability of vesicles can be determined by assessing the size and structure of the vesicles over time. Mean size is measured by DLS and structure changes are observed by TEM [11, 14, 15].

EVALUATION TESTS

1. 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) [2, 11, 16].

2. 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- m 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 mmol/L Lglutamine at 37°C under a 5% CO₂ atmosphere. Cytotoxicity was expressed as the cytotoxic dose 50 (CD50) that induced a 50% reduction of absorbance at 540 nm [1, 2, 11].

3. 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 [11, 16].

4. 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 SPD10A 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 [2, 11, 16].

5. 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 L RPMI medium was added. Cells were incubated with 100 L 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 [1, 2, 11, 16].

6. 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 [2, 11].

7. 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].

PATENTED AND MARKETING FORMULATION OF ETHOSOME

Ethosome was invented and patented by Prof. Elka Touitou along with her students of department of Pharmaceutics at the Hebrew University School of Pharmacy. Novel Therapeutic Technologies Inc (NTT) of Hebrew University has been succeeded in bringing a number of products to the market based on ethosome delivery system. Noicellex™ an anti-cellulite formulation of ethosome is currently marketed in Japan. Lipoduction™ another formulation is currently used in treatment of cellulite containing pure grape seed extracts (antioxidant) is marketed in USA. Similarly Physonics is marketing anti-cellulite gel Skin Genuity in London. Nanominox® containing monoxidil is used as hair tonic to promote hair growth is marketed by Sinere [17, 18]. Table 2 shows examples of ethosomes as a drug carrier.

APPLICATIONS OF ETHOSOMES

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 [20]. Jain et al. [7] 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 [21]. 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 [22]. 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 [23]. 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. Gupta et al. 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 [2].

Table 2: Examples of Ethosomes as a Drug Carrier

S. No.	Drug	Purpose of Ethosomal delivery	Application
1	Azelaic acid	Improves the sustained release	Treatment of acne
2	DNA	Expression into skin cells	Treatment of genetic disorders
3	Diclofenac	Selective targeting the cells	NSAIDS
4	Erythromycin	Better cellular uptake	Antimicrobial
5	Zidovudine	Better cellular uptake	Anti-HIV
6	Bacitracin	Better cellular uptake	Antibacterial
7	Insulin	GIT degradation	Treatment of diabetes
8	Trihexyphenidyl hydrochloride	4.5-times higher than that from liposome	Treatment of Parkinson's disease
9	Cannabidol	low bioavailability	Treatment of rheumatoid
10	Acyclovir	Poor skin permeation	Treatment of Herpes labialis
11	Enalapril maleate	Low oral bioavailability Major side effects in oral delivery	Treatment of Hypertension
12	Minoxidil	Pilocebaseous targeting Accumulation in skin increased	Treatment of baldness
13	Ammonium glycyrrhizinate	Poor skin permeation Poor oral bioavailability	Treatment of inflammatory based skin diseases
14	Fluconazole	Poor skin permeation	Treatment of candidiasis
15	Methotrexate	Poor skin permeation	Treatment of psoriasis
16	Salbutamol	Enhanced drug delivery through skin with ethosomes	Anti-asthmatic
17	Proteins and Peptides	Large molecules	overcoming the problems associated with oral delivery

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 [24]. 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 trihexyphenidyl 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 [2].

5. Transcellular Delivery

Touitou *et al.* in their study demonstrated better intracellular uptake of bacitracin, DNA and erythromycin

using CLSM and FACS techniques in different cell lines. Better cellular uptake of anti-HIV drug zidovudine and lamivudine in MT-2 cell line from ethosomes as compared to the marketed formulation suggested ethosomes to be an attractive clinical alternative for anti-HIV therapy [6, 8].

6. 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. Cannabidiol (CBD) is a recently developed drug candidate for treating rheumatoid arthritis. Lodzki *et al.* prepared CBD-ethosomal 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 [2].

7. 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 [25]. Dkeidek and Toutou 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 fasted 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 [26] 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.* [27] 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 [28].

8. 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 [23]. 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 Toutou 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.

DISCUSSION AND CONCLUSION

The main limiting factor of transdermal drug delivery system i.e. epidermal barrier can be overcome by ethosomes to significant extent. The ethosomes more advantages when compared to transdermal and dermal delivery. Ethosomes are the non invasive drug delivery carriers that enable drugs to reach the deep skin layers finally delivering to the systemic circulation. It delivers large molecules such as peptides, protein molecules. Simple method for drug delivery in comparison to iontophoresis and Phonophoresis and other complicated methods. High patient compliance as it is administered in semisolid form (gel or cream) and various application in Pharmaceutical, Veterinary, Cosmetic field.

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