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### Review on Ethosomes: A novel approach of Liposomes

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#### Abstract

Transdermal drug delivery system is a type of convenient drug delivery system where drug goes to the systemic circulation through the protective barrier i.e. Skin. In transdermal drug delivery system (TDDS) specific dose of medicaments are mainly administered as transdermal patch or skin patch. Over the years it has 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. So now a days, liposomes, niosomes, transferosomes and ethosomes are used to increase the permeability of drug through stratum corneum. Ethosomes have been found to be much more efficient in delivering drug to the skin, than that of liposomes or hydro-alcoholic solution. Present review addresses about ethosomes.

Key-Words: Ethosomes, Liposomes, Ethanol, Phospholipids, Transdermal drug delivery system

#### Introduction

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. The increased permeation of ethosomes is probably due to its ethanolic content. Ethanol increases the cell membrane lipid fluidity which results in increased skin penetrability of the ethosomes. These ethosomes permeates inside the skin and fuse with cell membrane lipids and release the drug. Ethosomes are the non invasive drug delivery carriers that enable drugs to reach the deep skin layers finally delivering to the systemic circulation. For optimal skin delivery, drug should be efficiently entrapped within ethosomal vesicles. Ethosomal drug delivery system is a new state of the art technique and easier to prepare in addition to safety and efficacy. Ethosomes have become a area of research interest, because of its enhanced skin permeation, improved drug delivery, increased drug entrapment efficiency etc. Ethosomes are used to deliver many drug molecules like Acyclovir, Bacitracin, Testosterone, Insulin etc. Ethosomal drug delivery system thus became an active area of research and development for novel therapies and can be important drug delivery tool in the future<sup>1</sup>.

Skin permeation of ethosomal components, ethanol and phospholipid, was demonstrated in diffusion-cell experiments. Ethosomal systems composed of soy phosphatidylcholine 2%, ethanol 30% and water were shown by electron microscopy to contain multilamellar vesicles. P-NMR studies confirmed the bilayer configuration of the lipids. Calorimetry and fluorescence measurements suggested that the vesicular bilayers are flexible, having a relatively low  $T_m$  and fluorescence anisotropy compared with liposomes obtained in the absence of ethanol. Dynamic light scattering measurements indicated that ethanol imparted a negative charge to the vesicles. The average vesicle size, as measured by dynamic light scattering, was modulated by altering the ethosome composition<sup>2</sup>. Experiments using fluorescent probes and ultracentrifugation showed that the ethosomes had a high entrapment capacity for molecules of various lyophilicities.

#### METHODS OF PREPARATION OF ETHOSOMES

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

##### Hot method<sup>2,3</sup>

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

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formulation is then homogenized at 15,000 psi pressure, in three cycles, using a high pressure homogenizer to get nano-sized ethosomes.

#### Cold method<sup>4</sup>

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.

#### LIST OF MATERIALS USED IN THE PREPARATION OF ETHOSOMES

Ethosomes are vesicular carrier comprise of hydroalcoholic or hydro 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

phosphatidylcholine (HPC), phosphatidic acid (PA), phosphatidylserine (PS), phosphatidylethanolamine (PE), phosphatidylglycerol (PG), phosphatidylinositol (PI), 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%.<sup>5</sup>

CLASS	EXAMPLE	USES
Phospholipid	Soyaphosphatidyl choline, Eggphosphatidylcholine, Dipalmitoylphosphatidylcholine, Distearoylphosphatidyl choline	Vesicles forming component
Alcohol	Ethanol, Isopropyl alcohol	For providing the softness for vesicle membrane. As a penetration enhancer.
Polyglycol	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	Carbopol934.	As a gel former

#### LIST OF DRUGS USED IN THE PREPARATION OF ETHOSOMES<sup>6</sup>

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 agent
5	Zidovudine	Better cellular uptake	Anti-HIV
6	Bacitracin	Better cellular uptake	Antibacterial
7	Insulin	GIT degradation	Treatment of diabetes
8	Trihexyphenidyl	4.5-times higher than that from	Treatment of Parkinson's

	hydrochloride	liposome	disease
9	Cannabidiol	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 agent
17	Proteins and Peptides	Large molecules	Overcoming the problems associated with oral delivery

### EVALUATION TESTS

#### Filter Membrane-Vesicle Interaction Study by Scanning Electron Microscopy<sup>7,8</sup>

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% v/v in water). Finally, filters were coated with gold and examined in SEM.

#### Vesicle-Skin Interaction Study by Fluorescence Microscopy<sup>9</sup>

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.<sup>10</sup>

MT-2 cells (T-lymphoid cell lines) were propagated in Dulbecco's modified Eagle medium (HIMEDIA, Mumbai, India) containing 10% foetal calf serum, 100 U/mL penicillin, 100 mg/mL streptomycin, and 2 m.mol/L L-glutamine at 37°C under a 5% CO<sub>2</sub> 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 and 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 (SEM)<sup>11</sup>.

#### 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 v/v) mixture as mobile phase delivered at 1 ml/min by LC 10-AT vp pump (Shimadzu, Kyoto, Japan). A twenty-microlitre 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.

#### Drug Uptake Studies

The uptake of drug into MT-2 cells (1×10<sup>6</sup> cells/mL) was performed in 24-well plates (Corning Inc) in which 100 μL RPMI medium was added. Cells were incubated with 100μL of the drug solution in phosphate buffer solution (pH 7.4), ethosomal formulation, or marketed formulation, and then drug uptake was determined by analyzing the drug content by HPLC assay.<sup>12</sup>

#### 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 cm<sup>2</sup> and 10 mL, respectively. The temperature was maintained at 32°C ± 1°C.<sup>13</sup> The receptor compartment contained phosphate buffer solution (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<sup>14</sup>

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.

### CHARACTERIZATIONS OF ETHOSOMES

#### Visualization

Visualization of ethosomes can be done using transmission electron microscopy (TEM) and by scanning electron microscopy (SEM)<sup>15</sup>.

#### 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).

#### Differential scanning calorimetry (DSC)

Transition temperature (T<sub>m</sub>) 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.<sup>16</sup>

#### 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.<sup>17</sup>

#### Entrapment Efficiency

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

#### Penetration and Permeation Studies

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

#### 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.

### ADVANTAGES OF ETHOSOMAL DRUG DELIVERY<sup>18</sup>

In comparison to other transdermal and dermal delivery systems,

1. Ethosomes enhance permeation of the drug through skin transdermal and dermal delivery.
2. Delivery of large molecules like (peptides, protein, molecules) is possible.
3. Ethosomal systems are much more efficient at delivering a fluorescent probe (quantum dots) to the skin in terms of quantity and depth.
4. 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.
5. High patient compliance–The ethosome drugs are administered 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.
6. High market attractiveness for products with proprietary technology. Relatively simple to manufacture with no complicated technical investments required for the production of ethosomes.
7. The ethosomes system is passive, non-passive, and available for immediate commercialization.

### APPLICATIONS OF ETHOSOMES

#### Transdermal Delivery of Hormones<sup>19</sup>

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. 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.

#### Delivery of anti-parkinson's drugs

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 M<sub>1</sub> 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.<sup>20</sup>

#### 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.

#### **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 show 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 its biological activity.

#### **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. 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 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 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. investigated the potential application of ethosomes for dermal delivery of ammonium glycyrrhizinate. Ammonium glycyrrhizinate is naturally occurring triterpenes obtained from *Glycyrrhiza Glabra* and useful for the treatment of various inflammatory based skin diseases.

#### **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 sub-dermal tissues. 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.<sup>20,21</sup>

#### **PATENTED AND MARKETED FORMULATION OF ETHOSOME<sup>19,20,21</sup>**

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 (NTT) of Hebrew University has been succeeded in bringing a number of products to the market based on ethosome delivery system. Noicellex TM an anti-cellulite formulation of ethosome is currently marketed in Japan. Lipoduction TM another formulation is currently used in treatment of cellulite containing pure grape seed extracts (antioxidant) is marketed in USA. Similarly Physonics marketing anti-cellulite gel Skin Genuity in London. Nanominox containing monoxidil is used as hair tonic to promote hair growth is marketed by Sinere. shows examples of ethosomes as a drug carrier.

#### **Conclusion**

The main limiting factor of transdermal drug delivery system is to overcome the epidermal barrier by ethosomes to significant extent. The ethosomes are more advantageous 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. It is more simple 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 applications in Pharmaceutical, Veterinary and Cosmetic field.

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