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ETHOSOMES: A NON-INVASIVE APPROCH TO DELIVER DRUG TOPICALLY

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ABSTRACT

Skin acts as a major target as well as a principal barrier for topical/transdermal drug delivery. Despite the many advantages of this system, the major obstacle is the low diffusion rate of drugs across the stratum corneum. Several methods have been tried to increase the permeation rate of drugs temporarily. One simple and convenient approach is application of drugs in formulation with elastic vesicles i.e., ethanolic liposomal vesicles (Ethosomes). Ethosomes have been found to be much more efficient in delivering drug to the skin; 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 an area of research interest, because of its enhanced skin permeation, improved drug delivery, increased drug entrapment efficiency etc.

Keywords: Ethosomes, Transdermal delivery, Skin, Vesicles, Phospholipid, Ethanol

INTRODUCTION:

The skin is one of the most extensive organs of the human body covering an area of about 2 m^2 in an average human adult **[1]**. Dermal drug delivery is the topical

application of drugs to the skin in the treatment of skin diseases and other inflammatory conditions. This has the advantage that high concentrations of drugs can be localized at the site of action, reducing the systemic side effects. In dermal and transdermal delivery, the skin is used as a portal of entry for drugs, for localized and systemic treatment. Transdermal drug delivery offers many advantages as compared to traditional drug delivery systems, including oral and parenteral drug delivery system. Advantages claimed are increased patient acceptability (non-invasiveness), sustained and controlled release thus reduce dosing frequency, avoid hepatic first pass effect, and reduce the fluctuation in plasma drug concentration thus maximum utilization of drug.

The traditional transdermal drug delivery systems involve a patch, in which the drug permeates through various layers of skin, via a passive diffusion pathway. However, this limits the basic potential of these systems, as stratum corneum is the most formidable barrier to the passage of most of the drugs, except for highly lipophilic, low molecular weight drugs. 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.

The ethosomes are soft, malleable vesicular carrier comprising of hydroalcoholic or hydroglycolic phospholipids in which the concentration of alcohols or their combination is relatively high. These “soft vesicles” represent novel vesicular carrier for enhanced delivery to/through the skin. The size of ethosomes vesicles system can be modulated from tens of nanometers to microns. 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 **[1, 2]**.

Composition of Ethosomes:

Ethosomes vesicles are comprises of Phospholipid, Polyglycol, Alcohol, Cholesterol, and Dye which examples and uses are listed in below **Table 1**.

Table 1: Different Additives Employed In Formulation of Ethosomes [2, 3]

Class	Example	Uses
Phospholipid	Soya phosphatidyl choline Egg phosphatidyl choline Dipalmityl phosphatidyl choline Distearyl phosphatidyl choline	Vesicles forming component
Polyglycol	Propylene glycol Polyethylene glycol	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 934	As a gel former

Advantages of Ethosomal Drug Delivery [4]

1. Ethosomes offer enhanced permeation of drug through skin for transdermal and dermal delivery.
2. Since the structure of the ethosome offers place to accommodate hydrophilic, lipophilic as well as ampiphilic drug moieties, they can be used for a variety of drugs.
3. Ethosomes are platform for the delivery of large and diverse group of drugs (peptides, protein molecules)
4. Ethosome composition is safe and the components are approved for pharmaceutical and cosmetic use.
5. The vesicles can act as a depot to release the drug slowly and offer a controlled release.
6. They offer increased efficacy of drug.
7. They are compatible with biomembrane since it is made up of phospholipids.
8. They can increase the oral bioavailability of drugs.
9. The technology has no large-scale drug development risk since the toxicological profiles of the ethosomal components are well documented in the scientific literature.
10. The ethosomal drug is administrated in semisolid form (gel or cream), producing high patient compliance. In contrast, iontophoresis and phonophoresis are relatively complicated to use which will affect patient compliance.
11. They offer high market attractiveness for products with proprietary technology. They are relatively simple to manufacture with no complicated technical investments required.

12. The ethosomal system is passive, non-invasive and is available for immediate commercialization.

Disadvantage of Ethosomal Drug Delivery:

1. Leakage and fusion of encapsulated drug/ molecules.
2. Sometimes phospholipids undergo oxidation and hydrolysis like reaction.
3. It has short half-life.
4. Scale up is difficult and production cost is high.

Proposed Mechanism of Skin Permeation of Ethosomes [5]

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. In comparison to liposomes, ethosomes are less rigid. Thus, the effects of ethanol, which were considered harmful to classic liposomal formulations, may provide the vesicles with soft flexible characteristics, which allow them to more easily penetrate into deeper layers of the skin.

When ethosomal carriers, which contain ethanol and soft vesicles, are applied to the skin a number of concomitant processes may take place, involving the stratum corneum and pilosebaceous pathways. Evidence of existence of the follicular transport pathway taken by by lipid vesicles was reported. The drug absorption probably occurs in following two phase:

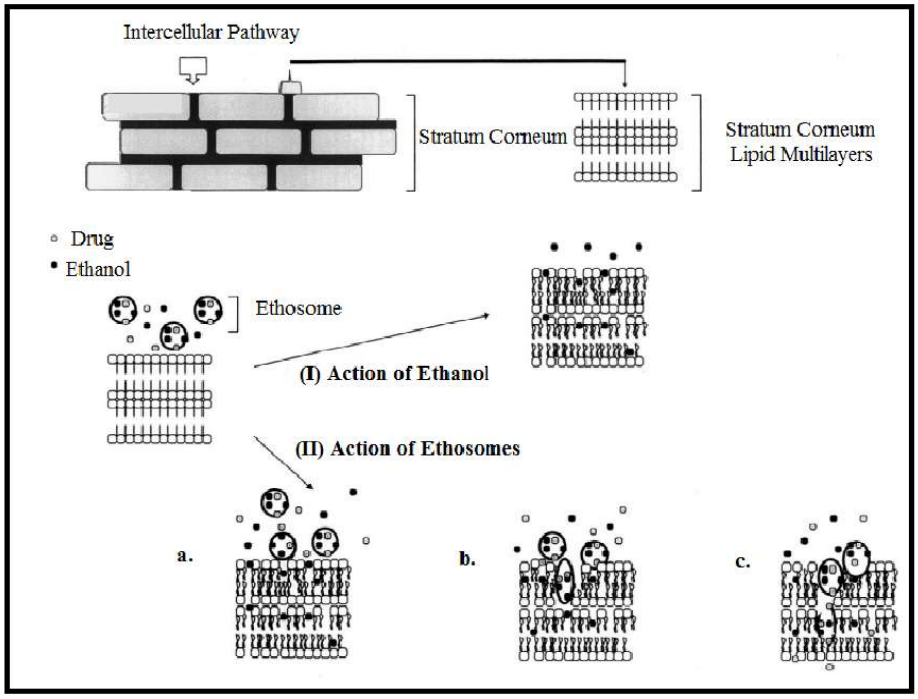

Figure 1: Mechanism of skin permeation of ethosomes

1) Action of Ethanol:

Ethanol penetrates into intercellular lipids and increase the fluidity of cellular membrane lipids and decrease the density of lipid multilayer of cell membrane in other terms ethanol disturbs the organization of the stratum corneum (SC) lipid bilayer and enhances its lipid fluidity.

2) Action of Ethosomes:

The flexible ethosome vesicles can then penetrate the disturbed SC bilayers and even forge a pathway through the skin by virtue of their particular nature. The release of drug in deep layers of skin and its transdermal absorption could then be the result of fusion of ethosomes with the skin lipids and drug release at various points along the penetration pathway.

Another contribution to the high skin penetration from the ethosomal system could be made by the interaction of ethanol and of phospholipid vesicles with the stratum corneum **[6]**. It has been also suggested that mixing of phospholipids with the SC lipids intercellular layers enhances the permeability of skin **[7]**.

Effect of Ethanol concentration:

Several studies investigated the effect of ethanol on physicochemical characteristics of the ethosomal vesicles **[5, 8, 9, 10]**.

One reported characteristic of ethosomes is their small size relative to liposomes, when both are obtained by preparation methods not involving any size reduction steps **[10]**. This reduction in vesicle size could be explained as a result of incorporation of high ethanol concentration. Ethanol confers a surface negative net charge to the liposome which causes the size of vesicles to decrease **[5, 8]**. The size of ethosomal vesicles was reported to increase with decreasing ethanol concentration in the ethanol concentration range of 20–45% **[5]**. Ethosomes have been shown to exhibit high entrapment efficiency for a wide range of molecules including lipophilic drugs. This could be explained by multilamellarity of ethosomal vesicles **[5]** as well as by the presence of ethanol in ethosomes, which allows for better solubility of many drugs.

Effect of Phospholipid Concentration:

The effect of phospholipid concentration on the size of ethosomal vesicles was also investigated. The size of ethosomal vesicles increasing with increasing of phospholipid concentration **[5, 9]**.

Route of administration of ethosome:

Ethosomes mainly administered through transdermal and topical routes. Ethosomes cannot administer orally because of high alcohol content (~20-45%).

For transdermal route, smaller size of ethosomal vesicles and 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 penetration in the skin lipid bilyers.

For topical route, bigger size of ethosomal vesicles compare to transdermal route vesicles, which is responsible for the localized action of drug. Increased ethosomal encapsulation increase the skin residence time leading to faster healing of external lesions and reduction of side effects and duration of therapy.

METHOD OF PREPARATION [2, 3]

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.

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^{o}C in a water bath. The water heated to 30^{o}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.

Hot method

In this method, phospholipid is dispersed in water by heating in a water bath at 40^{0}C until a colloidal solution is obtained. In a separate vessel, ethanol and propylene glycol are mixed and heated to 40°C. Once both mixtures reach 40°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.

EVALUATION AND CHARACTE-RISATION OF ETHOSOMES:

1. Visualization of vesicles

a) By Transmission Electron Microscopy (TEM):

Ethosomes vesicles were visualized using a Philips TEM CM 12 electron microscope (TEM, Eindhoven, Netherlands), with an accelerating voltage of 100 kV. Samples were negatively stained with a 1% aqueous solution of PTA (Phospotugstic acid). Ethosomal solution (10 µl) staining. The excess solution was removed by blotting. After drying, the specimen was viewed under the microscope at a 10-100 k fold enlargement. TEM is for determination of vesicles shape and lamellae **[10, 11]**.

b) By Scanning Electron Microscopy (SEM):

One drop of Ethosomal system was mounted on a stub covered with clean glass. The drop was spread out on the glass homogeneously.

A polaron E5100 sputter-coater (Polaron, UK) was used to sputter-coat the samples with gold, and the samples were examined under a Philips 505 scanning electron microscope (Philips, Eindhoven, Netherlands) at an accelerating voltage of 20 kV. It is for surface morphology, size and shape of ethosomal vesicles **[10, 11]**.

2. Vesicle size and size distribution:

Vesicular size and size distribution was determined by dynamic light scattering (DLS) using a computerized inspection system (Malvern Zetasizer, ZEM 5002, Malvern, UK). The size of ethosomes ranges between tens of nanometers to microns and is influenced by the composition of the formulation **[10, 11]**.

3. Zeta Potential:

Zeta Potential was also determined by dynamic light scattering (DLS) using a computerized inspection system (Malvern Zetasizer, ZEM 5002, Malvern, UK). Zeta potential is an important and useful indicator of particle (vesicle) surface charge, which can be used to predict and control the stability **[10, 11]**.

4. Differential Scanning Calorimetry (DSC) measurements (Calorimetric studies):

The transition temperature (T_m) of the vesicular lipid systems was determined by using the Mettler DSC 30 computerised with Mettler Toledo Star^e software system (Mettler, Switzerland). Samples weighed about 20 mg, and the phospholipid concentration was the same for all samples tested. The heating temperature ranging from -30 to 40 ºC at a rate of 5 ºC/min. The thermogram of the DSC is expected to express the consumption of the energy in phase transfer and fluidity of bilayers, which ascertain the reason of better penetration of ethosomes within the skin efficiently **[11, 12]**.

5. Vesicle Entrapment Efficiency

Entrapment efficiency of ethosomal vesicles was determined by centrifugation method. The vesicles were separated in a high-speed cooling centrifuge at 15,000 rpm for 90 minutes at a temperature maintained at 4ºC. The sediment and supernatant liquids were separated; amount of drug in the sediment was determined by lysing the vesicles using methanol. The vesicles were broken to release the drug, which was then estimated for the drug content. The entrapment efficiency was determined by the following equation (1) **[10, 11]**.

$$\%\textbf{Entrapment efficiency} = \frac{\textbf{Entrapped drug}}{\textbf{Total drug added}} \times 100 \ldots\ldots\ldots\ldots\ldots\ldots (1)$$

Alternatively, the clear supernatant can be used for the determination of free drug spectrophotometrically. The percentage encapsulation efficiency can be calculated from equation (2) below

$$\%\textbf{Entrapment efficiency} = \left[1 - \frac{\textbf{unentrapped drug}}{\textbf{Total drug added}}\right] \times 100 \ldots\ldots\ldots (2)$$

6. Vesicle stability:

Stability of ethosomal vesicles was determined by assessing the size and structure of the vesicles over time. Mean size was measured by dynamic light scattering (DLS) and structural changes were observed by TEM after negative staining with PTA (Phospotugstic acid) **[10, 11]**.

7. Turbidity measurement

Turbidity of ethosomal vesicular suspensions was measured by ELICO-CL 52D Nephalometer. In this method, 500 NTU (Nephalometric Turbidity Units) range is set. Then zero reading is set with Millipore water. After this, formulation is transferred to glass cuvettes of capacity 50 ml and placed in the holder inside the instrument. The method is repeated for each formulation and measurement of turbidity is displayed on the screen and expressed as NTU. Turbidity measurement is an important parameter to study effect of ethanol concentration on lipid bilayer of ethosome vesicles **[10, 11]**.

8. Confocal laser scanning microscopy (CLSM) (Skin penetration study of vesicles):

Depth and mechanism of skin penetration of dye loaded ethosomes was investigated using confocal laser scanning microscopy (CLSM). Skin specimens were analyzed by CSLM at 10 - 20 mm increments through the z-axis and sequential images were collected on the slide film. Fluorescence intensity was analyzed using a Sarastro Phoibos 1000 confocal laser scanning microscope (Molecular Dynamics, Sunnyvale, CA, USA) attached to a universal Zeiss epifluorescence microscope with an oil immersed Plan apo 6331.4 NA objective lens. Optical excitation and fluorescence emission was detected. Samples were observed through the z-axis **[10]**.

9. Skin permeation study of vesicles:

Franz Diffusion Cell was used skin permeation study of vesicles. The receiver compartment with receiver fluid (phosphate buffer, pH 7.4) was used as the receiver medium. A suitable size of skin was cut and mounted in between donor cell and receptor cell of the Franz diffusion cell. The donor compartment was filled with vesicular formulation. The receiver compartment solution was continuously stirred with magnetic stirrer at 100 rpm and equilibrated at 37±1°C with recirculating water bath. Samples were withdrawn through sampling port of the Franz diffusion cell at predetermined time intervals over 24 h and immediately replenished with equal volume of fresh phosphate buffer. Samples were analyzed spectrophotometrically. Sink

condition was maintained throughout the experiment **[13, 14]**.

10. Skin deposition study of vesicles:

After skin permeation study of vesicles amount of drug deposited in the skin. The receptor content was completely removed and replaced by 50% (v/v) ethanol in distilled water and kept for a further 12 hours; then the absorbance was measured spectrophotometrically. This receiver solution diffused through the skin, disrupting any ethosome structure and extracting deposited drug from the skin, thus giving a measure of skin deposition **[13, 14]**.

Different Studies Related to the Application of Ethosomes as a Carrier System: Various studies employing ethosomal formulation have shown better skin permeability of drugs. The uses of ethosomes as carrier system for transdermal/topical drug delivery are summarized below **(Table 2)**.

Table 2: Examples of ethosomes as a drug carrier

Drug	Purpose of Ethosomal delivery	Application
Azelaic acid	Prolong drug release	Treatment of acne
Diclofenac Potassium	Selective delivery of drug to desired side for prolong period of time	NSAIDS
Testosterone	Improved oral bioavailability dose dependent side effects	Steroidal hormone
Trihexyphenidyl hydrochloride	~ Improved transdermal flux ~ Provide controlled release ~ Improved patient compliance ~ Biologically active at dose several times lower than the currently used formulation	Treatment of Parkinson's disease
Zidovudine and lamivudine	Better permeation and intracellular uptake Improved in biological activity two to three times	Anti-HIV
Bacitracin	Improved intracellular delivery Improved dermal deposition Increased bioavailability	Antibacterial
Erythromycin	Improved skin deposition Improved biological activity Prolonging drug action	Antibacterial
DNA	Expression into skin cells	Treatment of genetic disorders
Cannabidol	Efficient systemic delivery of drug Improved bioavailability	Treatment of chronic rheumatoid arthritis
Clotrimazole	More effective for the treatment of local infection compared to marketed preparation	Treatment of Fungal Infection
Acyclovir	Improved skin permeation	Treatment of *Herpes labialis*
Insulin	Improved therapeutic efficacy of drug (GIT degradation)	Treatment of diabetes
Indinavir sulphate	Side effects like Nephrotoxicity and chronic elevations in serum creatinine	Treatment of AIDS
Cyclosporin	Improved therapeutic efficacy of drug (GIT degradation) Improved oral bioavailability	Treatment of Inflammatory skin disease
Ammonium glycyrrhizinate	Improved skin permeation Improved oral bioavailability	Treatment of inflammatory based skin diseases
Fluconazole	Reduce duration of therapy Reduce side effects Increase skin residence time	Treatment of candidiasis

Methotrexate	Improved skin permeation Better compared to conventional liposomes	Treatment of psoriasis
Salbutamol	Enhanced drug delivery through skin. Delivering higher amounts of drug at a controlled release rate through mice skin than classic liposomes	Anti-asthmatic
Minoxidil	Pilocebaceous targeting Increased accumulation in skin	Treatment of baldness
Proteins and Peptides	Large molecules	Overcoming the problems associated with oral delivery
Enalapril Maleate	Improved oral bioavailability Reduce side effects	Treatment of hypertension
ligustrazine	Promote better drug absorption Improved bioavailability.	Treatment of angina pectoris
Ketoconazole	Enhancement of transport across the skin	Treatment of both dermatophytosis and systemic mycosis

FUTURE SCOPE:

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 fluconazole and clotrimazole-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:

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. Ethosomes have been tested to encapsulated hydrophilic drugs, cationic drugs, proteins and peptides. Ethosomal carrier opens a new challenge and opportunities for the development of novel improved therapies.

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