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AN OVERVIEW OF ETHOSOMES

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ABSTRACT
Ethosomes are specialized vesicle system containing mainly ethanolic phospholipid vesicles which are commonly used for transdermal drug delivery. Transdermal drug delivery systems are a simple form of drug delivery in which the drug enters the body through a protective barrier (e.g., skin) that is the primary target for topical medications. Vesicular systems are one of the most controversial systems in transdermal drug delivery. Ethosomes are non-invasive transport vehicles that allow drugs to enter the skin and/or body tissues. They have better skin permeability, drug transport and drug encapsulation efficiency than liposomes and therefore can be used more widely than liposomes. Ethosomes are easy to prepare and safe to use and have attracted the attention of researchers due to their superior skin permeability, drug transport and drug encapsulation efficiency. They consist of phospholipids, alcohol, polyethylene glycol and water. Ethanol increases skin penetration by allowing the drug to reach the outer layer of the skin. Ethosomes are easy to make and safe to use. The purpose of this review of ethosome drug delivery is to focus on various aspects of ethosomes, including composition, penetration mechanism, classification, methods of preparation, characterization and evaluation.

KEYWORDS: Ethosomes, Transdermal, Ethanol, Phospholipid.

INTRODUCTION
Ethosomes were first developed by Touitou for the transport of drugs on lipid carriers such as ethanol, phospholipids, and water.[1] They are non-reactive delivery vehicles that enhance drug delivery to skin systems and/or internal organs. The use of ethanol increases the penetration of the drug into the intercellular space of the stratum corneum.[2] There is a slight modification of adult drug liposomes and are therefore also called ethanol liposomes.[3] These are soft, malleable vesicles designed to enhance the delivery of active ingredients. Vesicles can also control the amount of drug released over a long period of time, protecting the drug from the immune system or other elimination, necessitating the release of the drug, and ensuring that the concentration remains constant over a long period of time.[4] The size of ethosomes can vary from 10 nanometers (nm) to microns (μ). Further, they can enter the skin layer faster and have a higher transdermal flux. Therefore, administering ethanol to the body via the transdermal route has an advantage over oral administration as it eliminates the intestinal and first-pass metabolism of drug. In addition, it can deliver large molecules, such as peptides and protein molecules, by increasing the permeability of the drug through the skin, thus improving the skin absorption. It is the best choice for complex methods such as iontophoresis and phonophoresis. However, ethosome delivery is limited to potent molecules, i.e., molecules requiring 10 mg or less per day. In some cases, the product will be damaged by excipients during the transition from organic phase to the aqueous phase, resulting in a low product yield.[5,6] Sometimes antibiotics can cause skin irritation or dermatitis in some cases.[7] To solve this problem, the drug must be encapsulated in "skin-friendly" nanocarriers, which are usually lipid-based vesicles. Ethosomes are lipid vesicles made of natural or synthetic lipids that form emulsions in an aqueous environment. Cholesterol is often added to increase membrane permeability and stability.

Approaches to Drug Loading[7]
Drug loading is done using two strategies; the first hypothesis is that hydrophilic molecules are retained during the formation of the lipid bilayer. The second strategy is to transport drugs into preformed liposomes using pH gradients or ionic differences.

Composition of Ethosomes[4]
Phospholipids with different chemicals, including phosphatidylcholine (PC), hydrogenated phosphatidylcholine (PE), phosphatidylcholine, phosphatidylglycerol (propylene glycol), I-phosphatidyl inositol (PI), hydrogenated phosphatidylcholine, etc., are available. In ethosomes, alcohol falls in two forms that is ethanol and isopropyl alcohol. Amphiphilic fluorophores such as D-289, Rhodamine-123, fluorescein

isothiocyanate (FITC), and 6-carboxyfluorescein are often used to simulate ethosomes.

Mechanism of penetration

Epidermal penetration depth is measured using the ethanol system.[8] The various fluorescent probes with different physical properties, such as rhodamine red, rhodamine-B, β-carotene (C), and rhodamine 6G, can be recorded in alcohol data for skin studies. Lipid change in the vesicular system can be measured by temperature changes in lipid vesicle using Differential Scanning Calorimetry (DSC) which may be due to difference in drug and ethanol concentrations. Stability of ethosome systems can be determined by comparing the composition, culture medium size, and ability to add vesicles over time under different conditions. Based on the results of the stability test, researchers recommend the use of the refrigerator (4–80 °C) as the best place for ethosome production.

Types of ethosomes

On the basis of their chemical composition, ethosomes can be classified into three main, distinct classes.

1. Classical ethosomes

Classical alcoloplasts are modified liposomes containing phospholipids, ethanol (up to 45% w/w), and water.[9,11] These ethosomes are superior to liposomes for transdermal delivery due to their small size, negative delta potential, and higher encapsulation efficiency. Conventional ethosomes are superior to liposome liposomes in terms of skin permeability and stability. The molecular weight of the drug in the classical ethosome is approximately 130.077 Da to 24 kDa.

2. Binary ethosomes

Low drug retention and penetration may be the result of large amounts of ethanol causing drugs to be released outside the vesicles.[12] Therefore, glycol exosomes (BES) containing propylene glycol and isopropyl alcohol (the glycol system) were developed. Zhou et al. introduced binary ethosome.[13] This type of ethosomes are better than the liposomes in terms of simplicity, drug encapsulation effect, drug loading capacity, and intrinsic skin permeability.[14]

3. Transethosomes

They can transport hydrophilic and lipophilic substances in the nose during respiration. Song et al. (2012) introduced some new hyper deformable vesicle (UDV) transplastids with high ethanol content (up to 30%) and ethanol.[15] Transplastosomes can combine the advantages of plastosomes and transferosomes. Penetration through the skin may involve a combination of these two methods. Transethosomes exhibit a heterogeneous spherical shape, with greater values of vesicle elasticity and skin permeability and penetration. A combination of ethanol and edge activators (EA) such as sodium cholate (NaCo), sodium deoxycholate, Span 60, Span 65, Span 80, Tween 20, Tween 60, Tween 80, and dipotassium glycyrrhizinate can cause cysts to form in the lipid skin of these vessels. Fluorescent probes or dyes such as rhodamine red, 6-carboxyfluorescence, isothiocyanate fluorescence, and rhodamine-123 can be included in the UDV for fluorescence microscopy studies.

Methods of Preparation of Ethosomes

Ethosomes are prepared by using various methods, such as the cold method, hot method, ethanol injection method, mechanical dispersion method, thin-film hydration method, and reverse-phase evaporation method.[16] These methods are easy and convenient and do not require sophisticated instruments or processes.

Cold method

This method involves dissolving phospholipids, drugs, and other lipids in ethanol in a covered vessel at room temperature, shaking vigorously with a mixer. Then stirred it in polyols such as propylene glycol. The mixture is heated to 300 °C in a water bath. In a separate vessel, water is boiled to 300 °C before adding it to the mixture and stirred for 5 minutes in a covered vessel. Ethosomal formulation is then sonicated to reduce the size of vesicles for desired extent. Finally, formulation is stored in refrigerator.

Hot method

This method involves heating phospholipid in a water bath at 400 °C until a colloidal solution is produced. Ethanol and propylene glycol are combined and heated to 400 °C in a different tank. The aqueous phase is mixed with the organic phase once both combinations have reached 400 °C. Depending on whether the medication is hydrophilic or hydrophobic, it dissolves in either water or ethanol. Probe sonication or extrusion methods can reduce the ethosomal formulation's vesicle size to the desired degree.

Mechanical Dispersion Method

This method is also termed as thin-film hydration method. In this method, the lipid is dispersed in the bottle by a 3:1 mixture containing chloroform and ethanol, thus hydroethanolic solution is used to hydrate the lipid film.[17,18] In a clean, dry, round-bottom flask, the phospholipid is first dissolved in either chloroform or a chloroform-methanol mixture at a variable ratio. Organic solvents are removed using a rotary vacuum evaporator set to a higher temperature than the lipid-phase transition temperature. The solvent leftovers are then vacuum-extracted from the deposited lipid layer overnight. The lipid film is then hydrated with either a water-ethanol solution or a phosphate-buffered saline-ethanol solution. During the hydration process, the lipid film is spun and heated for as long as possible at the needed temperature, depending on the phospholipid properties.

Ethanol Injection Method
Using an ultrasonic probe, the organic phase containing the phospholipid is dissolved in ethanol and homogenised for five minutes after being injected into the aqueous phase via a 200-flow syringe system at a rate of 38 µl per minute.[19]

Reverse-phase evaporation method
This approach is designed primarily to manufacture massive unilamellar vesicles. The phospholipid is dissolved in diethyl ether to prepare the organic phase, which is then mixed with the aqueous phase in a 3:1 v/v ratio in an ultrasonic bath at 0 °C for five minutes to form an oil-in-water emulsion. The organic solvent is removed under reduced pressure to form a gel, which, when forcefully mechanically agitated, turns into a colloidal dispersion.

The transmembrane pH-gradient technique
This method is based on the pH gradient difference between the basic exterior of the external phase and the acidic interior of the internal phase. This process has three steps: ethosomal blank preparation, active drug loading, and incubation. In the first stage, any of the aforementioned processes are employed to generate the empty ethosomal suspension, but during the aqueous phase hydration process, an acidic buffer is utilised (often citrate buffer, pH 3). During the second stage, the drug is actively added to the ethosomal solution, which is then stirred continuously. To create a pH gradient between the basic exterior phase of the ethosomal system and the acidic internal phase (pH 3), an alkali, commonly a 0.5 M sodium hydroxide solution, is added to the external phase for increasing raise its pH to 7. In the third stage, the ethosomal system is incubated for a set duration and temperature (30 –60 °C) to allow the unionised drug to actively traverse the bilayer of the ethosomal vesicles and get stuck.

Characterization of ethosomes[20,22]
Vesicle size: The size and coating of ethosome nanocarriers are important because these are specifically designed for the delivery of topical and transdermal drugs. The size of the vesicles must be reduced to <300 or 400 nm to administer drug in ethosomal system. Dynamic light scattering (DLS) can measure particle size and zeta potential using a computerized detection system and photon correlation spectroscopy (PCS).

Drug entrapment efficiency (%EE) and drug loading
The ultracentrifugation technique is used to determine the encapsulation efficiency of ethosomes. Vesicles are isolated using a centrifuge at 20,000 rpm for 90 minutes at 4 °C. Separate the pellet and the supernatant. Use methanol to lyse the vesicles to determine the amount of drug in the sediment. The following formula represents the encapsulation function.

$$Entrappment\ efficiency = \frac{De}{Dt}\ X\ 10 \quad \text{.....(1)}$$

Where,
De: Amount of drug in the ethosomal sediment
Dt: - Theoretical amount of drug used to prepare the formulation (equal to amount of drug in supernatant liquid and in the sediment.

Transition Temperature
Differential scanning calorimetry can determine changes in vesicular lipid systems.

Drug content
The drug content of the ethosomes is evaluated with a UV spectrophotometer. This can be quantified using a modified high-performance liquid chromatography (HPLC) approach.

Drug Stability
Modified high-performance liquid chromatography method is used for quantitative analysis. Different temperatures such as25 ± 2 °C, 37 ± 2 °C, and 45 ± 2 °C are used to evaluate drug stability, which is calculated by analyzing vesicle size and morphology using DLS and TEM.

Skin Permeability
A confocal laser scanning microscope (CLSM) is used to measure the permeability of ethanol formulations to the epidermal layer.

Evaluation Tests[23]
Membrane Filter—Vesicle Interaction Study by Scanning Electron Microscopy
The vesicle suspension (0.2 mL) is placed on a membrane filter with a pore size of 50 nm and placed in a diffusion cell. The upper side of the filter is exposed to air, while the lower side is in contact with PBS (phosphate buffered saline, pH 6.5). After 1 h, the membrane filter was fixed in Karnovsky fixative overnight at 4 °C and then dehydrated with a graded ethanol solution (30%, 50%, 70%, 90%, 95%, and 100% vol/vol aqueous solution). Finally, the filters were coated with gold and examined under SEM (Leica, Bensheim, Germany).

Vesicle-Skin Interaction Study by TEM and SEM
Ultracut (Vienna, Austria) is used to cut animals, which are collected on polyformaldehyde-coated grids and viewed under a transmission electron microscope. After dehydration, skin pieces are placed on short sticks using tape for SEM analysis. The skin is then painted with a gold-palladium alloy using high-quality ion-sputtering technology. Check the section under a scanning electron microscope.

Vesicle-Skin Interaction Study by Fluorescence Microscopy
Following fluorescence microscopy, SEM and TEM studies were performed. Paraffin blocks are prepared using a microtome (Erma Optical Works, Tokyo, Japan), and 5-µm-thick sections are analysed under a

fluorescence microscope. Micro T lymphocyte line or cytotoxicity assay MT-2 cells are cultured in Dulbecco's modified Eagle's medium (HIMEDIA, Mumbai, India) at 37°C, 5% CO, 10% foetal bovine serum, 100 U/mL penicillin, and 100 mg. containing streptomycin and 2 mmol/L L-glutamic acid. Cytotoxicity was expressed using cytotoxic dose 50 (CD50); this led to a 50% decrease in absorbance at 540 nm.

Skin Permeation Studies

The rat's hairs are cut short (<2 mm) with scissors and the abdominal skin is removed from the tissue with a knife. The excised skin is placed don aluminum foil and gently combed the dermal side to remove fat or subcutaneous tissue. The effective penetration area of the cell is considered as 1.0 cm^2 at 32 ±1 °C, while the volume of the receptor cell is taken 10 mL. The receptor compartment contains a phosphate-buffered saline solution (10 mL, pH 6.5). The removed skin is placed between the donor and recipient. The ethosome preparation (1.0 mL) is applied to the epidermal skin. 0.5 mLsample (0.5 mL) is collected from the port of the diffusion cell at 1, 2, 4, 8, 12, 16, 20, and 24 hours and analysed using liquid chromatography.

Drug uptake study

Drug absorption of MT-2 cells (1 × 10 cells/mL) is performed in 24-well plates (Corning) with the addition of 100μL of Roswell Park Memorial Institute (RPMI) medium. Drug uptake Is assessed by HPLC analysis of drug content after an incubation time of 100 μL of drug solution in PBS (pH 7.4), alcohol preparation, or commercial preparation.

HPLC Assay

HPLC assay with methanol is used to measure drug penetration into tissue during in vitro skin tests and MT-2 cell cultures. An LC 10AT vp pump (Shimadzu, Kyoto, Japan) delivered a 70:20:10 vol/vol mixture of distilled water and acetonitrile as the mobile phase at 1 mL/min.

Applications of Ethosomes

1. Microbes and Skin diseases[24]

Ethosomes containing antibiotics have been studied in the treatment of skin diseases. The activity of the bacitracin and erythromycin ethosome systems was examined in an animal model of profound dermatitis. The results showed that the erythromycin ethosome system can kill bacteria deep in the skin after injection. Local antibiotic treatment with alcohol may be a better option than injections for treating deep skin infections.

2. Inflammation[25]

Cannabidiol (CBD) is a highly lipophilic drug used to treat rheumatic diseases. Analysis of in vivo skin permeability of the CBD ethosome system and drug accumulation in various organs. CBD alters the alcohol content in the body, resulting in a lack of oral bioavailability, high metabolism in the liver, an unbalanced acidic stomach pH, and low solubility in water (CBD is lipophilic). It can work effectively by overcoming the shortcomings of oral administration, such as Ko/w (8). This also increases patient compliance.

3. Menopausal Syndrome[26]

Testosomes in Menopausal Syndrome are designed to solve the problem of male hormone deficiency in men. An in vivo study compared testosterone blood levels in rabbits after single or multiple (once daily for five days) applications of the Testosome or Testoderm® patch (Alza). A single control did not produce significant differences between the groups studied. After daily application to rabbit ear pinna skin for 5 days, the AUC and Cmax values of Testosome were higher than Testoderm®.

4. Management of Erectile Dysfunction[27]

Natsheh H et al. (2020), administered phospholipid vesicles by dermal, transdermal, and nasal route and studied the effect of surfactants and alcohols on the fluidity of their lipid bilayers and penetration enhancement properties. In a clinical study of 16 men with erectile dysfunction, alcohol prostaglandins were injected into the throat, and they were asked to measure their erectile response and were examined by a physician there. The alcohol administration of prostaglandin E1 (PGE1) suggested that it is a good strategy for treating erectile dysfunction.

5. Analgesic and Antipyretic Ethosome System[28]

Touitou E et al., characterized ibuprofen transdermal ethosomal gel and studied efficiency in animal models. They used special alcohol-bodied ibuprofen gel on the skin of two animal models, namely rats with Brewer's yeast-induced febrile and tail-stroke nociceptive rats. The results showed that the body temperature of the febrile mice gradually decreased.

6. Protein and Peptide Delivery[29]

Peptides and proteins cannot penetrate the subcutaneous layer. Since its oral bioavailability is low, intravenous and subcutaneous administration are recommended. Insulin is an oligomeric protein with a molecular weight of 6,000 daltons per monomer. It may be prescribed for patients with insulin dependent diabetes mellitus (IDDM). Many studies have focused on the transdermal delivery of insulin by physical means, including phonophoresis and iontophoresis. Passive administration using deformable phospholipid vesicles has been shown to increase transdermal absorption of insulin.

7. Hair Loss[30]

Minoxidil is a lipophilic drug that can treat hair loss when applied topically to the scalp. Ethanolic minoxidil was developed to target the pilosebaceous unit and tested in hairless mice. In the study, it was found that minoxidil was more effective on the pilosebaceous unit when the ethosome carrier was used.

8. Miscellaneous[31]

Ethosomes of Finasteride found that more drug accumulated in the deeper layers of the skin (7.4, 3.2, and 2.6 times more than liposomes, aqueous solution, and hydroethanol, respectively). Linoleic acid ethosomes showed good skin permeability for the treatment of hyperpigmentation-related disorders. Many researchers have investigated the use of iodine-containing plastomes as contrast agents for tomography. In one study, ethanol stems were used to capture lipophilic, excited-state intramolecular proton transfer dyes for fluorescence spectroscopy. Alcohol poisoning can cause pneumonia. The nanoethanolosome-based hydrogel composition was successfully treated with methoxsalen to treat vitiligo. The therapeutic effects of ethosomes on HIV and hepatitis have been studied. Synthesis of gold nanoparticles in ethanolide bilayers to improve pharmacological effects. Acetosome, containing phenylethylresorcinol, is a skin treatment that increases tyrosinase inhibition and reduces melatonin without damaging the skin.

Ethosomal dosage forms

Ethosome systems have been used as tools in the development of new drugs, including ethosome gels, transdermal patches, and creams.

Marketed Products

Professor Elka Touitou and her students from the Department of Pharmacy at the Hebrew University developed and patented ethosomes. Lipoduction™ is the current model used in cellulite treatment. This product, sold in the United States, contains pure grape juice, which acts as an antioxidant. Table 1 lists the commercial products of ethosomes. Although this product was produced many years ago, detailed information is not currently available.[32,34]

Table 1: Marketed Products Based on Ethosomal Drug Delivery System.[35]

Sr. No.	Name of product	Uses	Manufacturer
1.	Cellutight EF’	Topical cellulite cream contains a potent combination of ingredients that increase metabolism and break down fat.	Hampden Health, USA
2.	Decorin cream	Anti-aging cream that treats, repairs, and delays the visible aging signs of the skin, such as wrinkle lines, sagging, age spots, loss of elasticity, and hyperpigmentation.	Genome Cosmetics, Pennsylvania, US
3.	Nanominox	The first minoxidil-containing product to use ethosomes. It contains 4% Minoxidil, a well-known hair growth promoter that must be metabolized via sulfation to produce the active compound. Topical Anti-Cellulite Cream	Sinere, Germany
4.	Skin genity	Powerful cellulite buster, reduces orange peel	Physonics, Nottingham, UK
5.	Supravir cream	For the treatment of herpes virus	Trima, Isarael
6.	Noicellex	Topical anti-cellulite cream	Novel Therapeutic Technologies, Israel
7.	Supravir cream[36]	For the treatment of herpes virus, the acyclovir drug formulation has a long shelf life with no stability issues, lasting three years at 25°C. Skin permeation experiments revealed that the cream maintained its initial penetration-enhancing properties even after 3 years.	Trima, Israel
8	Cellutight eF	This topical cellulite cream combines powerful ingredients to boost metabolism and reduce fat.	Hampden Health, USA
9	Decorin cream	This anti-aging cream targets visible skin signs such as wrinkles, sagging, age spots, and hyperpigmentation.	Genome Cosmetics, pennsylvania, US

CONCLUSION

It can be concluded that ethosomes have better skin permeability than liposomes. They provide additional benefits in terms of transfer and dermal delivery. They are non-invasive drug delivery devices that allow the drug to penetrate the skin layer and ultimately enter the body. It carries large molecules, such as peptides and protein molecules. Body lotions are known for their ease of preparation, safety, and effectiveness and can be designed to increase the penetration of active ingredients through the skin. Ethosomes can overcome the main limitation of transdermal drug delivery, which is the epidermal barrier. Ethosome carriers present new challenges and opportunities for innovation and improvement. Additionally, research in this area will lead to better medication management.

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