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Ethosomes as Novel Vesicular Carrier: An Overview

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Abstract

Traditional drug delivery (oral route), while having numerous advantages such as ease of administration, has problems such as limited bioavailability and frequent doses, both of which are challenging for patients (low patient compliance), and a high cost. To address all of these drawbacks, a unique drug delivery method with optimum therapeutic efficacy and safety, as well as regulated release to reduce the size and quantity of doses, is required. This can be accomplished by adopting vesicular formulations to bypass these limitations and thereby improve medication delivery through the skin. Ethosomes are non-invasive drug delivery devices that allow medications to go deep into the skin's layers and into the bloodstream. Ethosomes are malleable, flexible vesicles that are used to optimise the administration of active agents. Ethosomes may encapsulate and transmit very lipophilic compounds, as well as cationic medicines, through the skin due to their unique structure. Ethosomal systems are a new concept.

Keywords: Ethosomes; skin; Novel drug delivery; Vsicular lipid carrier; Ethanol

Introduction

The skin is the body's largest and most easily accessible organ, and it may be used to provide drugs with systemic effects. However, the stratum corneum, the outer layer of the skin, is the most resistant barrier to drug penetration through the skin, limiting medication transdermal bioavailability. To overcome the natural skin barrier and transfer medication molecules with various physicochemical characteristics to the systemic circulation, specific carriers are required [1,2]. Transdermal delivery of drugs and vaccines is a feasible alternative to oral and parenteral administration. It is feasible to prevent the liver's "first-pass" inactivation, decrease gastrointestinal pain, ensure uniform drug absorption over long periods of time, and reduce dose frequency, all of which promote adhesion [3].

The transdermal method has gained popularity due to its vast surface area and features that make medication administration easier.

Novel Carriers as Skin Permeability Modification Tools

Effective barrier that protects the insides of our bodies safe while keeping the outside out. The most significant condition and aim for transdermal delivery is to modify this barrier feature, which includes permeability to medicines, chemicals, and bioactive substances. As a result, a variety of methods for increasing the penetration rate of various substances have been tested. One of the options is to use innovative carriers capable of controlled release, drug delivery at a predefined rate, and targeted delivery. This might lead to improved efficacy, security, and patient compliance. For efficient cutaneous and transdermal administration, micro particles, nanoparticles, liposomes, elastic liposomes, noisome, ethosomes, and other new delivery methods or carriers are used. Stable, non-toxic, non-immunogenic, and cost-effective administration techniques are preferred. Pharmaceutically acceptable, stable, biocompatible, and patient-friendly technologies are also required [4-9].

Ethosomes

Ethosomes are soft and pliable ethanolic phospholipid vesicles. They're made to make it easier to distribute agents. Because the skin lipid bilayer organisation is disrupted when ethanol is introduced to a vesicular membrane, it improves the vesicle's capacity to pass through the SC [10]. The lipid membrane in SC lipids is less densely packed than in typical vesicles, yet it is equally stable and improves drug delivery potential [11]. This non-invasive extended delivery system may also be utilised to disseminate cultured cells and microbes. Using an Ethosomal carrier to increase bioactive molecule distribution through the epidermal and cellular membranes poses a number of obstacles as well as opportunities for future study and development of innovative enhanced therapeutics [12-14]. Ethanolic liposomes, also known as ethosomes, are lipid-based delivery vehicles that allow physiologically active substances to reach deeper layers of the skin and/or circulate throughout the body. Phospholipids are a kind of lipid found in the body [15-18].

Benefits of Ethosomal Drug Delivery

- It is possible to transmit large molecules (peptides, protein molecules).
- The raw materials used in the formulation are non-toxic.
- Transdermal medication administration requires improved drug penetration through the skin. Pharmaceuticals, veterinary medicine, and cosmetics are just a few of the applications for the Ethosomal drug delivery method.
- Patient compliance is good due to the fact that the ethosomal medication is supplied in a semisolid form (gel or cream).
- In compared to Iontophoresis, Phonophoresis, and other sophisticated drug delivery systems, this is a simple procedure.
- The ethosomal system is a passive, non-invasive method [19].
- Encapsulation of the medicine in its vesicular structures can be predicted to lengthen the drug's life in systemic circulation and reduce toxicity if selective absorption can be achieved. Compared to standard drug delivery systems, it provides a number of benefits [20,21].

Disadvantages of Ethosomal Drug Delivery

- Ethosomal administration isn't meant to administer a bolus of medication all at once; rather, it's meant to deliver a steady stream of medication over time.
- Adequate drug solubility in both lipophilic and lipophobic conditions allows drugs to penetrate the dermal microcirculation and circulatory system.
- The drug should have a molecular size that allows it to be absorbed via the skin.
- All skin types may or may not adhere well to adhesive.
- It won't be cost-effective.
- The return yield is minimal.
- Skin irritation or dermatitis of the medication distribution structure can be caused by excipients and enhancers.
- When Ethosomes are placed into water and the shell locking fails, the Ethosomes agglomerate and fall apart.
- During the shift from organic to aqueous medium, there is a loss of product.

The main benefit of ethosomes over liposomes is their smaller size, which allows for more drug penetration [22-27].

Composition of Ethosomes

The bulk of Ethosomes are made up of phospholipids (phosphatidylcholine, phosphatidylserine, and phosphatidic acid), ethanol, and water. Water makes up between 22 and 70 percent of the nonaqueous phase. The alcohol might be either ethanol or isopropyl alcohol. Because ethanol is known for damaging the tissue of the skin's lipid bilayer, ethosomes contain a high ethanol content. When integrated into the membrane of a vesicle, it allows the vesicle to burst through the stratum corneum. Because of the high ethanol concentration, the lipid membrane is packed less densely than typical vesicles, yet it has the same strength. It improves structural stability and makes the structure more pliable. The ability of lipids in the stratum corneum to transport medicines many types of additives utilised in the ethosomes preparations [28-38].

Types of Ethosomal Systems

- Classical ethosomes: Classical ethosomes are a kind of liposome that includes phospholipids, water, and high ethanol concentrations of up to 45 percent by weight. Because they were smaller and had a negative charge, traditional ethosomes were considered to be superior to traditional liposomes for transdermal medication delivery. It is possible to boost productivity without cluttering. Classic ethosomes also showed superior skin penetration and stability profiles than regular liposomes. The molecular weights of drugs captured in typical ethosomes ranged from 130.077 Da to 24 kDa.
- Binary ethosomes are a kind of ethosome that has two copies. Binary ethosomes were implemented by Zhou et al. We were created by adding a certain sort of alcohol to the mixture. The antiquity ethosomes: Propylene glycol (PG) and isopropyl alcohol (IPA) are the most often utilised alcohols in binary ethosomes.
- Transethosomes are the newest generation of ethosomal structures, as found by Song et al. in 2012. Traditional ethosomes, as well as an extra substance such as a penetration enhancer or an edge activator, are the basic components of

this ethosomal structure (surfactant). These unique vesicles were produced as a result of transethosomes, which aimed to combine the advantages of regular ethosomes with the deformability of deformable liposomes (transfersomes) in a single formula. Transethosomes have been demonstrated to have better qualities than traditional ethosomes in several research. Various forms of edge activators and penetration enhancers were researched in order to develop better characteristic ethosomal systems. Contains transethosomes. At molecular weights ranging from 130.077 Da to 200–325 kDa, drug entrapment has been discovered [30].

Mechanism of Skin Penetration

- Although the specific mechanism of medication delivery via ethosomes is unknown, the presence of a high quantity of ethanol distinguishes the ethosomes, as ethanol is known to disrupt skin lipid bilayer structure. According to Touitou et al. (2000), ethanol, vesicles, and skin lipids have a synergistic relationship that leads to a more favourable permeability profile. When incorporated into a vesicle membrane, it allows the vesicle to pass through the stratum corneum.
- Ethanol interacts with lipid molecules in the polar hard group area, lowering the transition temperature (Tm) and enhancing the fluidity of stratum corneum lipids. Soft, pliable ethosomes may be able to penetrate deeper into the epidermal layers through this transition [31,32].

After topical application, Ethosomes improve penetration significantly more than pure ethanol, revealing a synergistic process involving ethanol, vesicles, and skin lipids [34]. The main benefit of ethosomes over liposomes is that they allow for greater drug penetration. It's unknown how ethosomes are absorbed, which is one of the drug's mechanisms. The two steps of drug absorption that are most likely to happen are:

1. **Ethanol effect:** Ethanol aids in the penetration of goods into the skin. It has a well-understood mechanism for improving penetration. Intercellular lipids are penetrated by ethanol, which boosts their concentration. Reduce the density of the cell membrane's lipid multilayer and increase the fluidity of cell membrane lipids.
2. **Ethosomes effect:** Increased cell membrane lipid fluidity, which is generated by ethanol in ethanol, leads to increased skin permeability. As a result, ethosomes penetrate deep into the epidermal layers swiftly. It attaches to skin lipids and releases medications when it comes into contact with them [35].

Method of Preparation

Ethosomes may be made in two ways that are both easy and convenient [36,37].

1. **Cold method:** It is the most often used method for ethosomal formulation production. Phospholipids, drugs, and other lipid compounds are dissolved in ethanol in a covered jar at room temperature using a mixer and rapid agitation. During the stirring process, propylene glycol or another polyol is introduced. In a water bath, this combination is heated to 300°C. In a separate pot, heat the water to 300°C and add it to the mixture, which is then agitated for 5 minutes in a covered vessel. The vesicle size of ethosomal preparations can be lowered using sonication or extrusion procedures to obtain the appropriate expansion. Finally, the mixture is placed in the refrigerator [38-40].
2. **Hot method:** The phospholipids are dispersed in water using this process, which involves heating them in a water bath at 400°C until they become a colloidal solution. Ethanol and propylene glycol are combined in a separate vessel and heated to 400 degrees Celsius. The organic phase is introduced to the aqueous phase after both solutions reach 400°C. Depending on the drug's hydrophilic/hydrophobic qualities, it's dissolved in water or ethanol. Using probing sonication or the extrusion approach, the vesicle size of an ethosomal formulation may be reduced to the desired level.

Method of Characterizations of Ethosomal Formulation [41,42]

Vesicle shape: Ethosomes can also be seen using transmission electron microscopy (TEM) and scanning electron microscopy (SEM) (SEM). To see what was going on, electron microscopy was utilised, which revealed that an ethosomal formulation had a vesicular structure with a diameter of 300-400 nm. Because of their asymmetrical spherical shape, the vesicles appear flexible.

Vesicle size and Zeta potential: Particle size and zeta potential may be determined using dynamic light scattering (DLS) using a computerised inspection approach and photon correlation spectroscopy (PCS).

Drug entrapment: The ultracentrifugation technique may be used to determine the entrapment effectiveness of ethosomes.

$$\text{Entrapment Efficiency} = [\langle Q\,t - Q\,s \rangle \div Q\,t] \times 100$$

Where,

Qt is the amount of drug added

Qs Is the amount of drug detected in the supernatant?

Transition Temperature: Differential scanning calorimetry may be used to estimate the transition temperature of vesicular lipid systems.

Drug content: A UV spectrophotometer may be used to assess the drug content in ethosomes. A modified high-performance liquid chromatographic technique can also be used to measure this.

Surface tension measurement: The ring technique in a Du Nouy ring tensiometer can be used to determine the surface tension activity of a medication in aqueous solution.

Stability studies: The size and shape of vesicles over time can be used to determine their stability. DLS is used to determine the average size, while TEM 8 is used to determine the structural change. Experiments on skin permeation: Confocal laser scanning microscopy can be used to assess the ethosomal preparation's capacity to penetrate into the epidermal layers (CLSM).

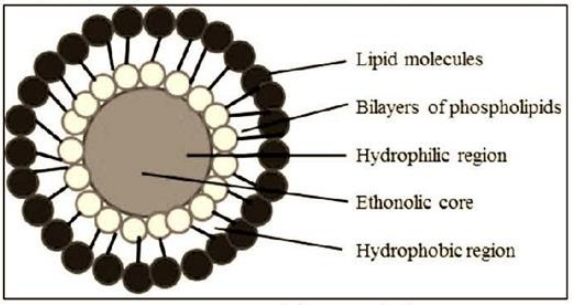

Figure 1: *Structural feature of ethosomes.*

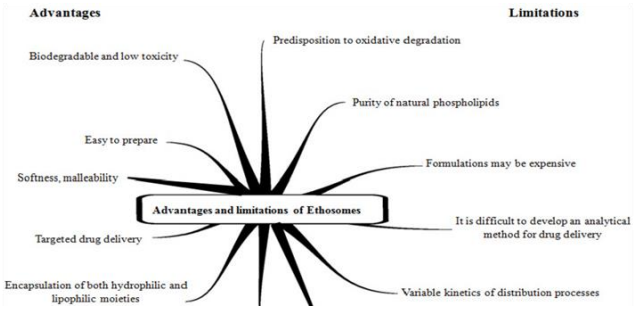

Figure 2: *Advantages and limitations associated with ethosomes.*

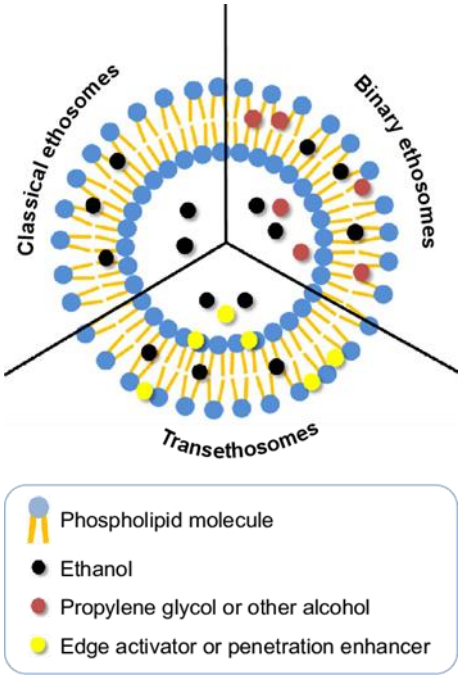

Figure 3: *Types of ethosomes*

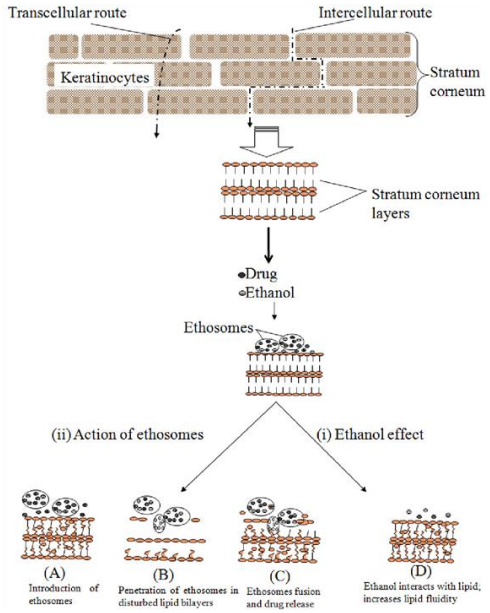

Figure 4: *Proposed mechanisms for skin delivery via ethosomes*

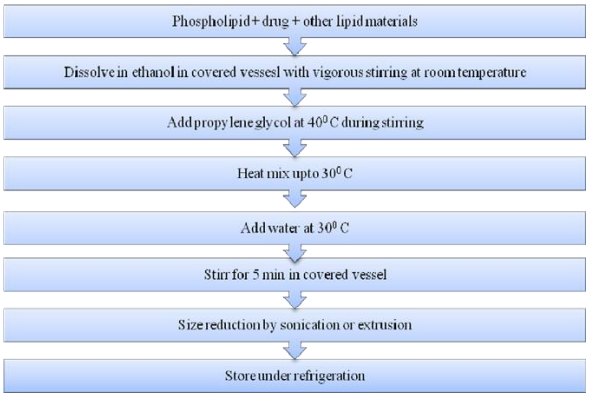

Figure 5: *Cold method for the preparation of ethosomes.*

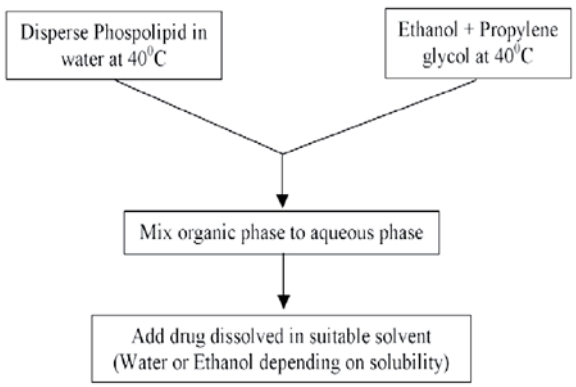

Figure 6: *Hot method for the preparation of ethosomes.*

Table 1: *Various additives are used in the preparation of ethosomes.*

ADDITIVES	USES	EXAMPLES
Phospholipid	Vesicles forming component	Soya phosphatidyl choline, egg phosphatidyl choline
Polyglycol	Skin permeation enhancer	Propylene glycol, polyethylene glycol
Cholesterol	For providing the stability to vesicle membrane	Cholesterol
Alcohol	For providing the softness for vesicle membrane and as a permeation enhancer	Ethanol, isopropyl alcohol
Vehicle	As a gel former	Carbopol 934
Dye	For characterization study	6-Carboxy fluorescence, rhodamine red, fluorescence isothiocyanate

Evaluation of Ethosomes

Filter Membrane-Vesicle Interaction Study by Scanning Electron Microscopy [SEM]

It necessitates vesicle suspension filtration (0.2 mL). In diffusion cells, a membrane with a whole size of 50 nm is placed. The top of the filter is exposed to the sun. The top side should be exposed to sunshine, while the bottom should be immersed in a phosphate saline buffer solution (having pH 6.5). After 1 hour, the filters are removed, and the samples are prepared for SEM examinations by overnight fixation in Karnovsky's fixative at 4°C, followed by dehydration with ethanol solutions of varied concentrations (30%, 50%, 70%, and 80%) in water (90%, 95%). The filters are then mounted, gold-coated, and evaluated under a scanning electron microscope [SEM].

Skin Permeation, Studies

The test animals' hair was cut short (approximately 2 mm) using scissors, and the abdomen skin was removed from the underlying connective tissue with a knife. The skin that had been excised was placed on aluminium foil, and any adhering skin was gently teased off using the dermal side of the skin (fat and/or subcutaneous tissue may be involved). The effective permeation area and receptor cell volume of the diffusion cell were 1.0 cm^2 and 10 mL, respectively. The temperature was held at 32^{0C}. The temperature is -1^{0C}. In the receptor compartment, which contains a saline solution, phosphate buffer was retained (10 mL at pH 6.5). Between the donor's compartment and the receiver's compartment, the skin that had been taken had been mounted. An ethosomal formulation (1.0 mL) was applied to the epidermal surface of the skin. An ethosomal formulation (1.0 mL) was applied to the epidermal surface of the skin. Samples (0.5 mL) were obtained using the sampling system. A high-performance liquid chromatography equipment was used to analyse the data.

Stability Study

The vesicles' stability was tested by storing them at 4°C + 0.5°C. After 180 days, the vesicles' size, zeta potential, and entrapment efficiency were evaluated using the method described before.

Turbidity measurement

The Digital Nephalo-Turbidity Meter was used to determine the turbidity of all of the ethosomal suspensions. The 500 NTU (turbidimetric turbidity unit) range is used in this procedure, with Millipore water as the zero reading. In a 50 mL glass cuvette, the ethosomal formulations were transferred. The holder was then put into the instrument. The turbidity reading was shown on the screen and represented in NTU.

In-vitro release via dialysis membrane

This experiment was carried out using a Franz Diffusion cell. The dialysis membrane was soaked overnight in phosphate buffer 7.4. Between the donor and receiver chambers, the dialysis membrane was clamped down. 5 ml of the ethosomal formulation were uniformly distributed in the donor compartment. 125 mL of phosphate buffer 7.4 was introduced to the receiver compartment. Throughout the experiment, it was stirred continuously at 600 rpm with a Teflon coated magnetic bead, and the temperature was held at 370 ± 0.5°C. To maintain the sink condition, 5 ml of the receiver fluid was withdrawn at each 1-hour interval and refilled with the same amount. A UV spectrophotometer was used to determine the drug content of withdrawn samples.

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