ETHOSOMES: DESIGN, COMPOSITION, AND CHARACTERIZATION IN DIFFERENT DISEASE MANAGEMENT

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Abstract:

Background:
The primary disadvantage of transdermal drug administration is that most medications only slightly penetrate human skin. The stratum corneum, or outer layer, houses the skin's fundamental defense system (SC). Many strategies have been developed to increase the skin barrier's susceptibility. Pharmaceuticals and cosmetics employ ethers to penetrate the skin. Ethosomes are elastic nanovesicles based on phospholipids that contain a high (20–45%) ethanol concentration. It is commonly recognized that adding ethanol to vesicular systems enhances their permeability, drug deposition, substantial flexibility, and deformability, creating elastic nanovesicles. When it comes to the quantity and depth of chemical transport to the skin, the ethosomal systems are noticeably highly successful. Their unique shapes and high ethanol content have made transdermal distribution easier, increasing medication effectiveness and patient compliance. Creams, patches, and gels contain ethosomal dispersions for easy application and stability. Ethosomes have several benefits over typical liposomes in terms of therapeutic drug delivery for illnesses such as psoriasis, acne, alopecia, and hormone imbalances. This is due to their superior composition. The ethosomal system is thoroughly examined in this article, along with an estimation of how well it will work as a nanocarrier to deliver active ingredients to the skin.

Keywords:
Ethosome, Phospholipid, Transdermal delivery, Microemulsion, Parkinsonism.

1. Introduction:

Skin is the largest organ of the human body and can be easily accessed. There are several benefits to delivering drugs with skin over other traditional methods, such as less fluctuation in drug plasma levels, protection against GI issues and first-pass metabolism, and good patient adherence. There are several disadvantages, one of which is that the drug is administrated in a lower amount due to the low permeability of the skin. When the drug is administered topically, because it lowers the quantity of medication bioavailability, the stratum corneum, the skin's outermost layer, acts as the most substantial barrier to drug penetration. Therefore, to get over the skin's natural barrier, it is essential to investigate the different carriers required for systemic pharmaceutical administration [1][2].

To effectively administer a drug through transdermal and topical means, the drug should be able to get through the skin barrier and reach the target site. Over many decades, scientists have methods to damage the skin's protective layer and administer drugs into the body via the intact skin. Chemical skin permeation enhancers, iontophoresis, sonophoresis, electroporation, microneedle, and several methods have been used to increase the efficacy of transdermal transport. Because of their low efficacy, skin irritation, complexity of usage, and high cost, these methods have not been applied broadly nowadays. Lipid-based suspensions such as liposomes, niosomes, and microemulsions have also been used as less-risk drug carriers. However, these drug carriers don't get too deep into the skin, so they aren't beneficial for drug delivery in the transdermal route [3].

ETHOSOMES:

Ethosomes are pliable, soft lipid vesicles mainly composed of water, phospholipids, and ethanol (isopropyl alcohol) in high concentrations (20–45%). Because of its significant deformity, this carrier exhibits intriguing properties associated with its capacity to pass through human skin. These vesicular phospholipids can function as the vesicle-forming element of the ethosomal system due to the physicochemical properties of ethosomes. Phospholipids are employed in concentrations ranging from 0.5 to 10%. Phosphatidylcholine (PC), hydrogenated PC, and phosphatidyl ethanolamine (PE) are examples of phospholipids with different chemical structures [1].

TYPES OF ETHOSOMES:

Ethosomes are classified into three types they are:

Table 1: Types of Ethosomes [2]

Sl no.	Parameter	Classic Ethosomes	Binary Ethosomes	Trans Ethosomes
1	Composition	Ethanol Phospholipid Drug Charge inducer Water	Ethanol Phospholipid Drug Isopropyl Alcohol and propylene glycol Charge inducer	Ethanol Phospholipid Drug Surfactant Penetration enhancer Charge inducer

2	Size	Smaller than classic liposomes	It is the same size or sometimes smaller than classic liposomes	Size is based on the concentration of penetration enhancer or surfactant.
3	Shape	Spherical	Spherical	Spherical in shape but Irregular.
4	Stability	More stable and robust than classic liposomes	Stable than the classic ethosome	Data about stability needs to be revised.
5	Entrapment Efficiency	Higher than conventional liposome	Sometimes higher than conventional ethosomes	Higher than most of the basic ethosomes.

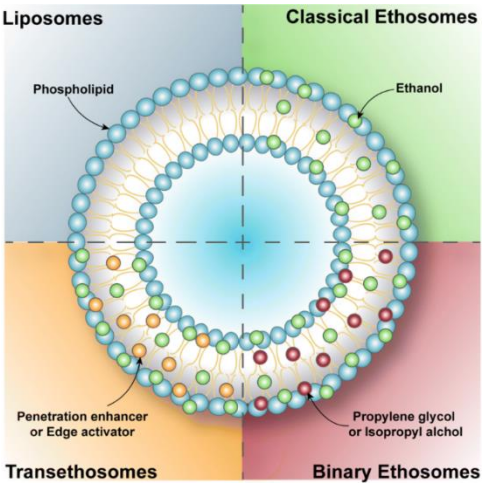

Figure 1. Types of ethosomes- a. Classical Ethosomes, b. Transethosomes, c. Binary Ethosomes [4]

COMPOSITION:

Ethanol, phospholipids, and water are present in the ethosomes. Phosphatidylcholine (PC), phosphatidyl glycerol (PPG), phosphatidyl ethanolamine (PE), hydrogenated PC, phosphatidyl inositol (PI), and other phospholipids are among its chemical structures. Aqueous phase content varies from 22% to 70%. Either isopropyl or ethanol alcohol may be utilized. The dyes employed are 6-carboxy fluorescence, fluorescence isothiocyanate, rhodamine -123, and rhodamine red.

Table 2. Composition of Ethosomes [5]

Class	Example	Uses
Phospholipid	Dipalmitoyl phosphatidylcholine Soya phosphatidylcholine, Egg phosphatidylcholine,	It helps to form vesicles.
Polyglycol	Propylene glycol Transcutol RTM	It can be used as a skin penetration enhancer.

Alcohol	Ethanol Isopropyl alcohol	Keeping the vesicle membrane soft as a penetration enhancer.
Cholesterol	Cholesterol	To stabilize the membrane
Dyes	Rhodamine -123 Rhodamine red Fluorescence isothiocyanate 6-carboxy fluorescence	To perform the characterization study
Vehicle	Carbopol 934	As a gelling agent

PREPARATION METHODS:

There are various methods to prepare ethosome, which are as follows:

1. HOT METHOD:

To disperse the phospholipid in water and to form a colloidal solution, heat the solution in a water bath at 40°C. Mix propylene glycol and ethanol in another container at 40°C (Fig 2). Add the organic phase into the aqueous phase once both mixtures have reached 40°C. Based on whether the drug is hydrophilic or hydrophobic, it dissolves in either water or ethanol. In an ethosomal formulation, vesicle size can be decreased by probe sonication or extrusion [6].

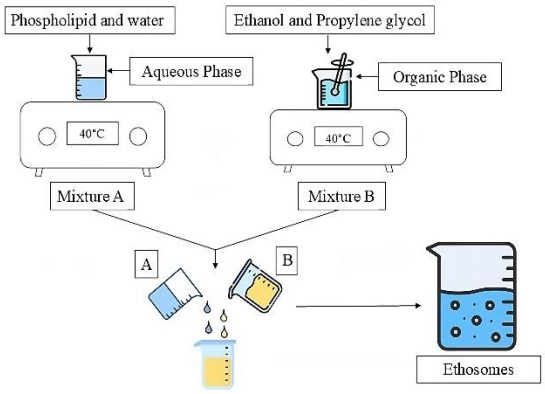

Figure 2. Hot Method

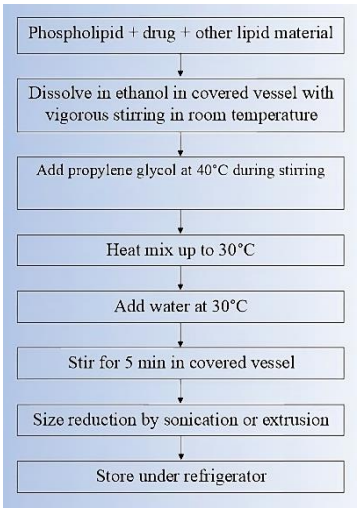

Figure 3: Flow chart of the Hot method process

2. COLD METHOD:

Cold process is the most widely used technique for creating Ethosomal. Phospholipids and other lipophilic substances are dissolved in ethanol at room temperature and rapidly stirred. The organic phase is heated in a water bath to a maximum temperature of 30°C. Before adding the aqueous phase to the organic phase, it is heated to 30°C in a separate vessel and vigorously stirred. The aqueous phase is added, and stirring is continued for an additional time (Fig 4). The aqueous phase may consist of a buffered solution, regular saline, or water [6].

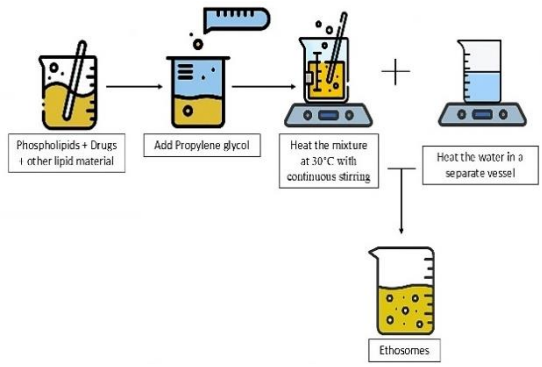

Figure 4. Cold Method

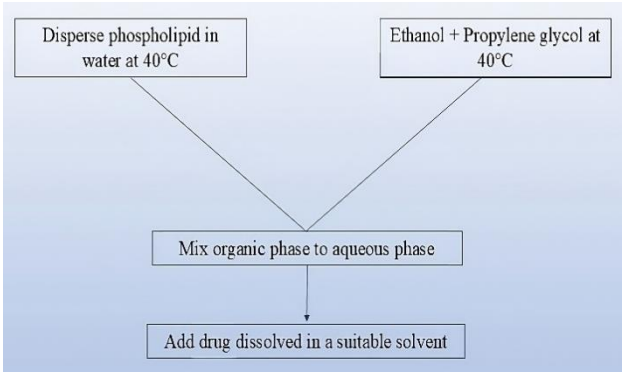

Figure 5. Flow chart for Cold method process

3. THIN FILM HYDRATION METHOD:

The lipids will be dissolved in an organic solvent in a round-bottom flask, and the organic solvent will be removed using a rotary evaporator above the temperature at which the lipid transition occurs. An ethanolic mixture will be used to hydrate the thin film that forms around the inner walls of the flask. The resultant ethosome suspension will then be dispersed using a probe sonicator [7].

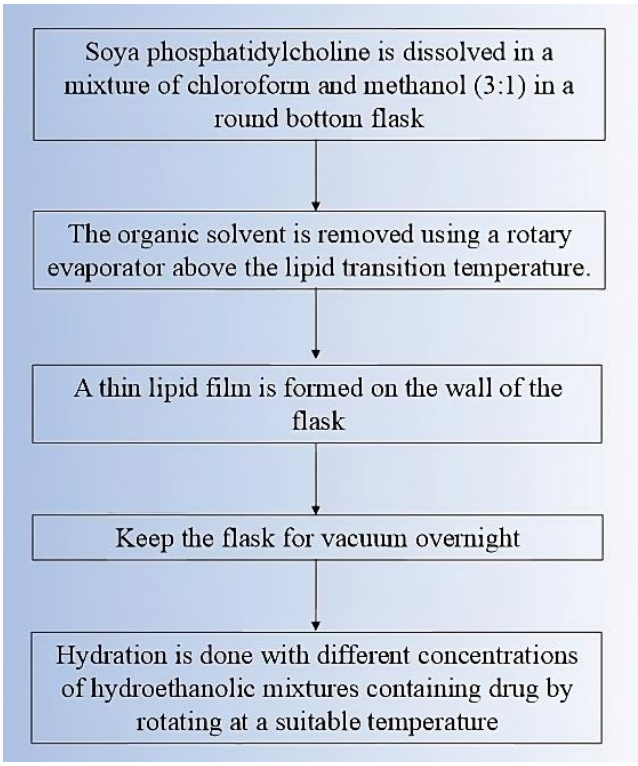

Figure 6: Flow chart for the thin film hydration process

4. CLASSIC METHOD:

After dissolving the medicine and phospholipid in the ethanol, the mixture is heated in a water bath to 30ºC±1ºC. After adding the lipid mixture, double-distilled water is added to a covered jar and stirred at 700 rpm. The resultant vesicle solution is homogenized using a manual extruder and three cycles of polycarbonate membrane [1].

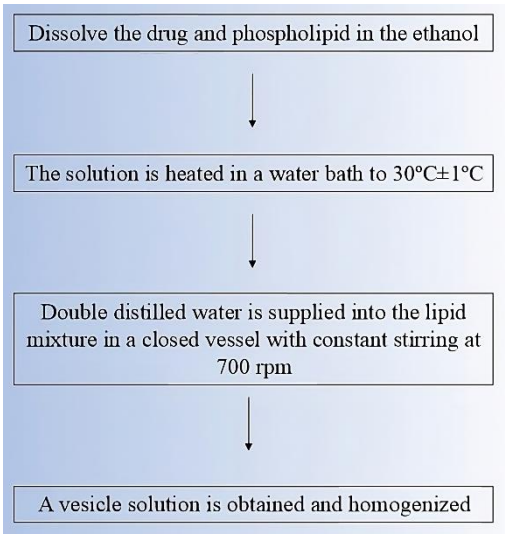

Figure 8: Flow chart for Cold method process

EVALUATION TESTS:

- **Size and morphology:**

In vitro research often employs techniques like Transmission Electron Microscopy (TEM) and Dynamic Light Scattering (DLS) to characterize the size and shape of ethosomes; these techniques help understand the homogeneity and physical characteristics of the vesicles.

- **Encapsulation efficiency:**

Using High-Performance Liquid Chromatography (HPLC), this method measures the quantity of medication encapsulated within the vesicles.

- **Drug release kinetics:**

Fluorescence spectroscopy and dynamic dialysis are two techniques used in in vitro release investigations to determine the controlled release characteristics of the vesicles [8].

- **Stability studies:**

Using the method above, the vesicles' size, zeta potential, and trapping efficiency were measured after 180 days, and their stability was examined at 4°C ± 0.5°C [9].

- **Cellular uptake studies:**

These studies shed light on possible drug delivery pathways and aid in our understanding of how skin cells interact with drugs. Confocal Laser Scanning Microscopy (CLSM) is a method that produces high-resolution pictures by enabling the monitoring of cellular uptake in various cell layers.

- **Skin retention study:**
  Twelve-hour in vitro permeation experiments measure the quantity of medication retained in the skin. Washing with distilled water removes the formulation left behind from the in vitro permeation trial. The drug concentration is calculated after adding 50% v/v ethanol to the receptor content and stirring it for 12 hours. This receiver solution removes the medicine deposited on the skin as it diffuses into the skin, breaking up any liposome and ethosome formations.
- **Percent entrapment efficiency:**
  Using the ultracentrifugation technique, the vesicles' %EE was ascertained. The vesicular dispersion's required volume was spun (by using a Remi cooling centrifuge CPR-30) for three hours at 20,000 rpm and 4 °C. The medication still entrapped in the supernatant solution was removed, and its concentration was determined using a UV spectrophotometer set to 226 nm with a pH 7.4 phosphate buffer. The amount of medication released from liposomes and ethosomes was computed using the following formula: %Entrapment Efficacy(%EE) = (Cd−C) Cd×100 (where Cd = the overall drug concentration and C =the unentrapped drug concentration). Ultra Dialysis was used to calculate the % EE of test gels. Ultra-dialysis was used to extract the free medication from the gels using a dialysis membrane [10].

- **Skin permeation studies:**
  Studies on skin penetration evaluate ethosomes capacity to transfer medications through the epidermal layer. Drug penetration depth and permeation are frequently measured using methods like Franz diffusion cells, which employ animal or human skin that has been excised [11].

- **Drug content**
  The HPLC method can quantify the drug content in the preparation [12].

***In vitro* STUDIES**

- **Skin irritation test:**
  Rabbits were prepared to assess and compare Tβ-4 Ethosome gel and blank Tβ-4 gel skin irritation. Before applying the formulations, the rabbits were put under anesthesia and had their back hair cut 24 hours beforehand. To ensure no injury, saline was used to rinse the exposed back skin. Three groups were created by scratching the skin until blood oozed from it: Tβ-4 Ethosome gel, Tβ-4 gel, and blank gel (control). Every skin region, whether intact or injured, received the same gel treatment. Redness and irritation were measured for several time intervals after application. The Draize scale was used to rate irritation, with 0 representing no reaction and 4 representing a severe reaction.

- **Physical inspection:**
  Each produced formulation's color, homogeneity, consistency, spreadability, and phase separation were evaluated visually.

❖ **Drug release study and drug deposition study:**
Dialysis bag diffusion and Franz diffusion cells with synthetic or biological membranes can be employed for in vitro drug release and ethosomal preparation deposition. [13].

***In vivo* studies:**
In vivo studies check skin's efficacy, safety, and pharmacokinetics properties during drug delivery.

➢ **Animal selection:**
Suitable animal models, such as rats or mice, are chosen based on the desired usage and ethical concerns.

➢ **Application:**
The test animals' skin is treated with the ethosome formulation. The application site is often cleansed and shaved to achieve optimal skin contact.

➢ **Monitoring:**
After applying the ethosome formulation, the animals are observed for immediate negative responses, such as irritation or inflammation.

➢ **Efficacy evaluation:**
Either clinical illness observation or the measurement of pertinent biomarkers is used to evaluate the therapeutic effectiveness of the ethosome formulation [9].

APPLICATION:

1. **Treatment for microbial infection**
When bacitracin and erythromycin ethosomal systems are studied in animals with deep skin infections, ethosomes effectively treat viral and microbial skin infections.
2. **Anti-inflammatory effect:**
It has been discovered that ammonium glycyrrhizinate (AG) ethosomes exhibit anti-inflammatory properties on the skin of human volunteers. Using an erythema index measurement and a reflectance visible spectrophotometer, the study assessed the anti-inflammatory impact and compared it with pharmaceutical solutions that included aqueous or hydroethanolic solutions [14].
3. **Ethosome for menopausal syndrome:**
In vivo tests on rabbits have demonstrated the better efficacy of ethosomal patches in treating menopausal symptoms in women and testosterone deficiency in males [15]. Buspirone hydrochloride (BH) must be used often because of its short half-life of 2.5 hours and poor bioavailability of 3.9% when administered intravenously [16].

4. **Ethosomes for erectile dysfunction:**
   Research suggests that ethosomes, in addition to serving as an analgesic and antipyretic, may be an effective treatment for erectile dysfunction. Primarily affecting older men, erectile dysfunction is a common sexual disorder in which a man is unable to achieve or sustain a penile erection.

5. **Transfer DNA molecule:**
   Studies show ethosomes may be utilized to topically transfer DNA molecules such that specific genes are expressed in skin cells [2].

6. **Ethosomes for Parkinsonism disease:**
   An ethosomal formulation of trihexyphenidyl hydrochloride (THP), an M1 muscarinic receptor antagonist used to treat Parkinson's disease, was compared with a regular liposomal formulation by Dayan and Touitou. Because of its more remarkable skin penetration ability, the ethosomal-THP formulation was discovered to be able to successfully manage Parkinson's disease [17].

7. **Minoxidil ethosomes for hair loss:**
   Millions of people worldwide are impacted by hair disorders such as acne, seborrhoea, and excessive hair loss. Minoxidil is used topically on the scalp to treat pilosebaceous diseases. Minoxidil ethosomes were generated and evaluated in vivo in rats without hair to comprehend how minoxidil targets pilosebaceous units via ethosomes.

8. **Transdermal delivery:**
   Ethosomes improve drug permeability across the stratum corneum barrier, making them suitable for administering medicines with poor skin penetration, limited oral bioavailability, and first-pass metabolism. They can also reduce infection at its root.

9. **Ethosomal delivery for vaginal delivery:**
   Using pH-responsive ethosomes, the antifungal drug metronidazole was developed and tested for vaginal administration. Using a semi-permeable membrane made of regenerated cellulose and a phosphate buffer pH 5.5 solution, the Franz diffusion cell was utilized to investigate permeability in vitro [11].

10. **Anti-hypertensive effect:**
    Topical administration of Valsartan, an antihypertensive medication with limited oral bioavailability, addresses its first-pass impact and poor gastrointestinal absorption. In Wistar rats, ethosomal and nanoethosomal formulations of Valsartan showed more significant antihypertensive effects than oral treatment, resulting in persistent and considerable blood pressure decreases.
11. **Delivery of antibiotics:**
    Applying antibiotics topically has demonstrated superior therapeutic effectiveness. Conventional oral medications may cause adverse effects, including allergic reactions.

The deep epidermal layers and subdermal tissues are not well penetrated by conventional exterior preparations [12]. Ethosomes, which may deliver antibiotics deep into the skin by addressing this issue, are utilized to remedy this problem. Ethosomes may quickly pierce the epidermis, allowing drugs to reach the deeper skin layer and preventing the disease from starting from the ground up [18].

12. Ethosomes for analgesic and antipyretic action:

Transdermal ibuprofen ethosomes showed strong analgesic and antipyretic effects in rats. Fevered rats treated with ibuprofen ethosomal gel showed a progressive drop in body temperature, reaching normal levels within 3 hours and lasting 12 hours. Oral administration resulted in shorter periods of lowered body temperature.

13. Testosterone ethosome for hormonal deficiency:

Because of hepatic first-pass metabolism, oral testosterone therapy is ineffective in treating male hypogonadism. Direct testosterone injections are uncomfortable, but intramuscular injections can produce more significant or lower amounts. Transdermal testosterone delivery eliminates hepatic first-pass metabolism and parenteral administration issues [11][19].

14. Delivery of anti-arthritis drug:

Applying anti-arthritis medication topically resolves problems associated with conventional oral therapy and is preferable to site-specific administration. Cannabidiol, or CBD, is a novel drug option for the treatment of rheumatoid arthritis. Issues like first-pass metabolism, restricted bioavailability, and GIT degradation have been associated with his oral use. Consequently, it was shown that encapsulated CBD significantly improved its skin penetration, accumulation, and biological activities [20].

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Author contributions

JDS conceptualized the original article. JDS contributed to reviewing, editing, and supervision. AG contributed to the drafting, formatting, and referencing of this manuscript.

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Conflicts of interest

The authors declare there are no conflicts of interest.

Ethical approval

Not applicable

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