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Ethosomes: Versatile vesicular carriers for effective medicinal drug transdermal administration

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Abstract
Drugs can be delivered using Ethosomes, which are non-invasive delivery systems, to deep skin layers and/or the systemic circulation. Though conceptually complex, ethosomal systems are distinguished by their ease of preparation, safety, and effectiveness a combination that can greatly increase their utility. Ethosomes are flexible, squishy vesicles designed for better active drug distribution. This page discusses research done using several ethosomal systems and a wide variety of medications using *in vitro*, *in vivo*, animal models, and human subjects. Ethosomes' special structure allows them to enclose and transmit through the skin highly lipophilic substances like propranolol and trihexyphenidil as well as cationic medicines like testosterone and minoxidil. The treatment with the ethosomal acyclovir formulation significantly improved all the examined parameters, according to the findings of a double-blind, two-armed, randomised clinical investigation. It was discovered during preliminary research with insulin and plasmids that the ethosomal carrier may be used to improve the delivery of these medicines. In subsequent research, the ethosomal technique was expanded to allow the introduction of chemicals into microbes and cultivated cells. The research and future development of novel, improved therapeutics are faced with a number of obstacles and opportunities due to the enhanced delivery of bioactive chemicals via the skin and cellular membranes via an ethosomal carrier.

Keywords: Ethosomes, transdermal, vesicular carriers, ethanol, phospholipid

Introduction
The primary function of human skin, which has multiple functions, is to act as a barrier against the entry of xenobiotic material and the outflow of endogenous substances like water (Chemicals and drugs). It is thought of as the body's initial line of defence. The barrier abilities of skin are mostly attributed to the stratum corneum, which is composed of corneocytes.

Transdermal delivery formulations have gained tremendous popularity and rapid advancement over conventional formulations in recent years due to a number of factors, including the ability to avoid fluctuations that occur during gastrointestinal absorption, increase bioavailability of medications by delivering the active ingredients directly into the systemic circulation, avoid hepatic metabolism, and provide a constant, controlled drug input.

Several passive as well as active strategies, such as penetration enhancers, supersaturated systems, vesicles, iontophoresis, electroporation, phonophoresis, usage of microneedles and jet injectors, etc., have been proposed to increase the permeability of the skin for transdermal delivery of pharmaceuticals. Despite all the efforts made to improve penetration, very few bioactive compounds are currently delivered transdermally. Some of these compounds' characteristics include low molecular weight (5500 Da), high lipophilicity, pharmacological activity, and effectiveness at modest doses (5–10 mg/day) for analgesics, steroids, and contraceptives. The use of vesicle formation as skin delivery systems is one of the most lucrative approaches to drug administration over the skin.

Delivery of drugs using vesicular carriers
Phospholipids and non-ionic surfactants, whose wide variety can be used in their formation, make up vesicles. Size, charge, thermodynamic phase, lamellarity, and bilayer elasticity are physicochemical properties of vesicles that play a significant role in their behaviour and ultimately on their efficacy as drug delivery systems, which are ultimately influenced by their composition. Vesicles may have a variety of functions during cutaneous and transdermal distribution. A vesicle performs a variety of tasks, as seen in Figure 1. In addition to showing the effectiveness of vesicles in dermal and transdermal delivery,

research reports from the past few years also describe the long journey that led to their development from conventional to elastic/deformable form to the stratum corneum, which launched an expedition for the passage of therapeutic agents deeply across the skin and eventually led to a breakthrough in the early 1990s. The key application of ethanol as a permeation enhancer, as noted by several studies in the past, was carried out by Touitou (1996) by using it in lipid vesicular systems. Touitou invented the term "Ethosomes" by combining the words "etha- nol" and "somes" following multiple successful exploratory observations (jus- tifying lipid vesicular systems incorporating ethanol in quite high concentrations) (Figure 2). The boost in naproxen's percutaneous absorption, as reported by Valjakka *et al*. in 1998, provided additional support for these findings. In contrast to conventional dosage forms, the study by Caddeo *et al*. (2013) demonstrated the effectiveness of ethanolic vesicles in localising the medicine at the site of inflammation. Ahad *et al*. (2013).'s study demonstrated that valsartan-loaded ethosomes were significantly superior to traditional rigid liposomes in terms of drug release and increment in bioavailability (3.03 times higher), along with oral suspension of valsartan and improved permeation of Rhodamine-Red loaded nanoethosomes to the deeper layers of the skin.

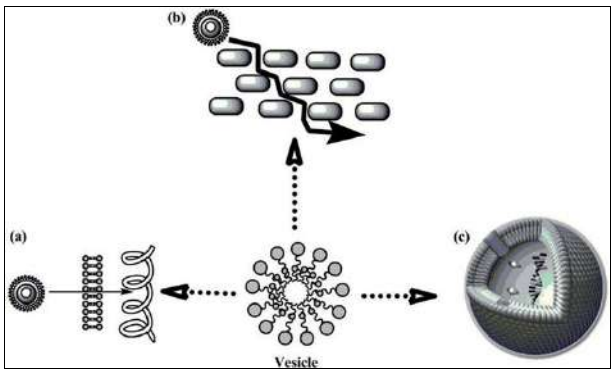

Fig 1: Schematic illustration of a vesicle with multiple functions. delivering medication molecules into or across the skin, penetrating the stratum corneum, and serving as a drug storage chamber.

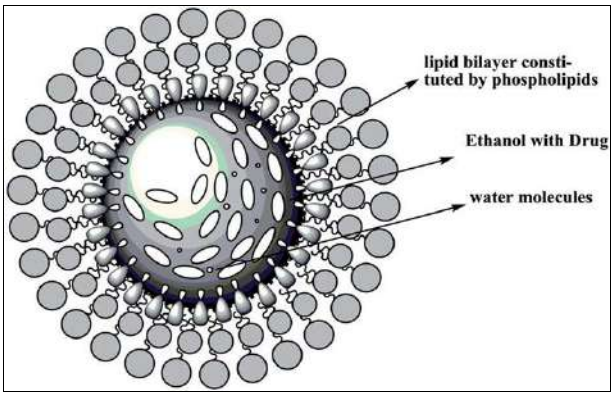

Fig 2: Shows the ethosomes' structure (embodying ethanol and drug molecules).

Structure & Composition of Ethosome

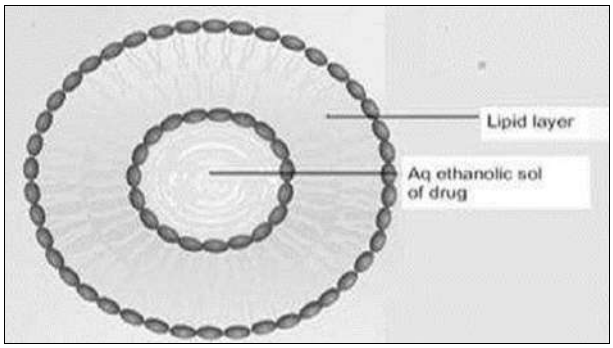

Fig 3: Structure of Ethosomes

Table 1: Composition of Ethosomes

Material	Examples	Uses
Phospholipid	Soya phosphatidyl choline Egg phosphatidyl choline Dipalmityl phosphatidyl choline Distearyl phosphatidyl choline	Vesicles forming component
Polyglycol	Propylene glycol Transcutol RTM	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 Fluorescen Isothiocynate (FITC) 6- Carboxy fluorescence	For characterization study
Vehicle	Carbopol 934	As a gel former

Advantages of ethosomes

- Ethosome formulation uses excipients that are suitable for use in pharmaceutical products, is scale-up-based, and does not require any complex steps. Because ethosomes' vesicles are smaller than those of other vesicular systems and are biodegradable, they have a bigger surface area (when following normal preparation methods excluding any size reduction steps). Since the incorporation of high ethanol concentration results in the formation of liposomes with a negative charge, the size of the vesicles is subsequently reduced, which eventually improves the bioavailability of medicinal substances.
- Ethosomes are highly competent at encapsulating a variety of compounds, including lipophilic medications. By lowering the transition temperature (Tm) of the stratum corneum (SC) fluids, increasing their fluidity, and increasing the membrane permeability by the intercalation of polar head group, ethosomes bear an important advantage of success—fully delivering the therapeutic agents deeply across the highly conformational and densely packed lipid bilayers of the stratum corneum (SC).
- In contrast to the drawbacks of iontophoresis and phonophoresis, ethosomes offer good patient compliance because they can be made in semisolid dosing forms (Gel or Cream).
- Since the toxicological profile of ethosome components is well established in scientific literature, the ethosomal system does not provide an excessive risk for drug development.
- Ethosomes improve drug distribution via transdermal and dermal routes through the skin.
- Ethosome components have received approval for usage in pharmaceutical and cosmetic products. Ethosomes are a platform for the administration of a wide range of medications (peptides, protein molecules).
- Low risk profile: Because the toxicological profiles of the ethosomal components are well-documented in the scientific literature, there is no danger associated with the technology's large-scale medication development.
- High patient compliance – The semisolid (gel or cream) form in which the ethosomal medication is administered results in high patient compliance. Iontophoresis and phonophoresis, in comparison, are relatively difficult to utilise, which will impact patient compliance.
- The demand for items using proprietary technology is high. Ethosome production is rather easy to do and doesn't involve any expensive technical inputs.

Mechanism of Action

Early 1990s research had shown that binding of ethanol to phospholipid bilayers amplifies tangential as well as transverse repulsion between the lipid molecules, which is a problem caused by SC constitution which momentarily restricts the entry of various therapeutics agents and bio-actives across the skin. While the latter causes phosphatidylcholine bilayers in aqueous ethanol solutions to excessively expand, the former pressure increases an ethanol-dependent change in the area per molecule. Ethanol can cause hydrocarbon inter digitation, which increases inter membrane separation in the gel phase. Additionally, ethanol widens the interface, which expands the range and number of lipid head group solvations. In 1996, this property of ethanol saw a breakthrough when it was combined with phospholipid, double-distilled water (DDW), propylene glycols (PG), and other ingredients to establish a platform of formulation from which the face of ethosomes formed. When the ethosomal system is applied to the skin, a number of related processes involving the stratum corneum and pilosebaceous pathways occur. In these processes, ethanol first disturbs the stratum corneum's lipid bilayer's organisation, which is followed by an increase in the layer's lipid fluidity. Due to their particle structure, stretchable ethosomes vesicles then pierce damaged stratum corneum bilayers and even create a passageway through the skin (Figure 4). The fusion of ethosomes with skin lipids and drug release at different locations along the penetration pathway may also contribute to the release of the drug in the deep layers of the skin and its transdermal absorption.

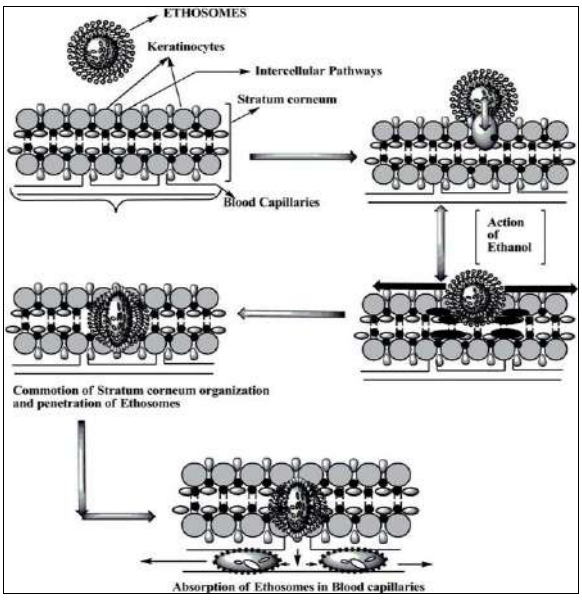

Fig 4: A projected model outlining the skin delivery of ethosomes' mode of action.

Considerations for formulation: Elements and their results

Basically, phospholipids, ethanol, and water make up ethosomes. Phospholipids, which have a hydroalcoholic or hydro/alcoholic/glycolic character with a hydrophilic head and a hydrophobic tail performing the centre segment in making a bilayer constitution, are one of the main vesicle-producing units in ethosomes. The results of several investigations clearly show the diversity of different phospholipids. Ketotifen was delivered to the skin with the use of phosphatidylcholine (PC) from soybean lecithin in a study of ethosomes by Elsayed *et al*. (KT). The new ethosomes have demonstrated better skin delivery of ketotifen over conventional liposomes in terms of vesicle size, entrapment efficiency, stability, *in vitro* penetration, and skin deposition properties. The transdermal permeability of both minoxidil and testosterone significantly increased in the comparative study of the ethosomal system of minoxidil with either ethanolic or hydroethanolic solution or phospholipid ethanolic micellar solution of minoxidil and transdermal delivery of testosterone from an ethosomal patch using soybean phosphatidylcholine (Phospholipon 90).

Alcohol and its high concentrations' effects

Alcohol is a key player in giving the ethosomal system its distinctive personality. The investigation of the phase and packing characteristics of dipalmitoylphosphotidyl choline vesicles or multi-bilayers in the presence of ethanol led to the discovery of the impact of ethanol on lipid systems in the early 1990s, and high alcohol content use followed. According to a brief assessment of several investigative literatures, ethanol essentially fluidizes and engorges the stratum corneum lipids' rigidly structured lipid multilayer system by lowering its Tm, incorporating a high concentration of ethanol into ethosomes causes the lipid vesicular membrane to become less tightly packed, which gives them warmth and malleability. This results in the formation of a deformable and flexible vesicular system that, in contrast to other vesicular systems, could easily pierce through tiny openings formed in the disturbed SC lipids more deeply. Additionally, ethanol is essential in regulating vesicle size. It does this by giving liposomes a surface negative net charge, which reduces the size of the vesicle. However, in terms of vesicle size, entrapment effectiveness, stability, safety, etc., optimization of ethanol concentration in ethosomal systems is essentially required. Numerous research studies have found that ethanol concentrations between 30 and 40% are ideal for the production of successful and durable ethosomes. Studies have shown that ethanol concentrations between 20 and 45% increase the size of vesicles, among other things.

Distilled water and glycols such as propylene glycol and transcutol, among others, can be utilised to promote skin permeability in an ethosomal system. In certain research, cholesterol has been utilised along with other carriers, such as PVP/VA (gels, membranes, and solutions), PVP (gels, membranes, and solutions), carbomer gels, polaxomer, etc. for gel formation, and carbopol, to give the vesicle membrane stability.

Methods for Formulation

At both the pilot and industrial levels, the preparation of ethosomes is based on straightforward scale-up processes without the need for any complex instruments. There are two fundamental ways to prepare ethosomes:

1. Cold Method
2. Hot Method

1. Cold Method

- One of the most popular methods for ethosome preparation, this method just requires two straightforward setups.
- In the first setup, ethanol is heated to 30 °C in a water bath after phospholipid and other lipid material have been thoroughly dissolved in it at room temperature by vigorous stirring using a mixer like a Heidolph mixer and continual addition of polyols like propylene glycol etc.
- In the second setup, water must be heated to 30 degrees Celsius in a separate vessel. After 5 minutes of stirring, the first and second setup mixes must be combined in a covered vessel.
- Using the sonication or extrusion process, the ethosomal formulation's vesicle size can be reduced to the desired extent.
- The formulation is then placed in a refrigerator for storage.

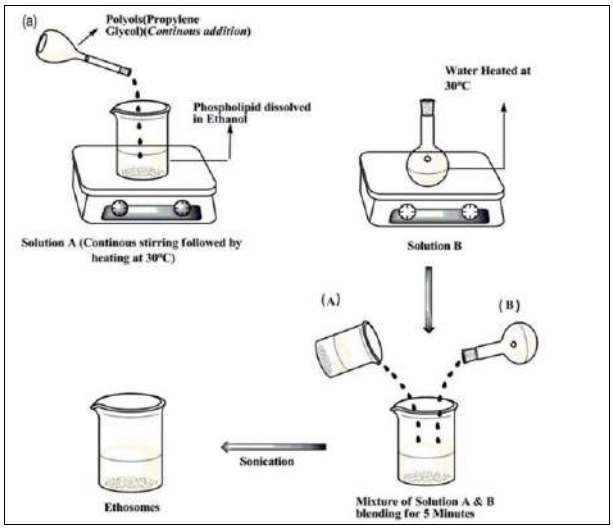

Fig 5: Cold Method

2. Hot Method

- This technique involves heating phospholipid in water to a temperature of 40 °C until a colloidal solution forms.
- Propylene glycol and ethanol are combined and heated to 40 °C in a different tank. The organic phase is added to the aqueous phase once both combinations have reached 40 °C.
- Depending on whether the medication is hydrophilic or hydrophobic, it dissolves in either water or ethanol.
- Using the probe sonication or extrusion approach, the vesicle size of the ethosomal formulation can be reduced to the desired extent.

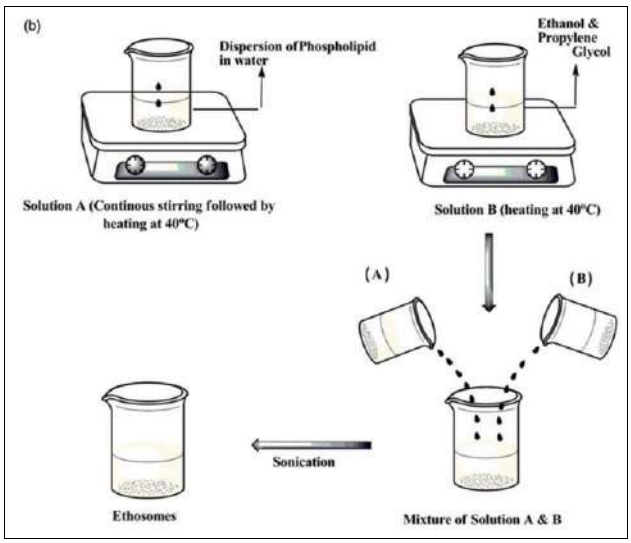

Fig 6: Hot Method

Characterization using Physicochemistry

➢ **Vesicle anatomy**

Transmission electron microscopy (TEM) and scanning electron microscopy (SEM), which involves negatively staining the formulation with aqueous solutions of substances such as phosphotungstic acid etc., can reveal the vesicular morphology of ethosomal systems.

➢ **Size and size distribution of the vesicles**

By using dynamic light scattering (DLS), it is possible to measure the ethosomal system's vesicular size, which ranges between nanometers and microns and is influenced by the formulation's chemical make-up. For example, data from ethosomes prepared with 30% ethanol and 2% phospholipid (PL) revealed a narrow particle size distribution with an average size of 153 4 nm. The size of the vesicles rose as the ethanol concentration decreased in the 20–45% range, with the largest particles in preparations containing 20% ethanol measuring 193 8 nm and the smallest in those containing 45% ethanol measuring 103 9 nm.

For ethosomes containing 30% ethanol and PL concentrations ranging from 0.5% to 4%, the relationship between vesicle size and phospholipid content was discovered. As can be seen, the size of ethosomes very slightly depends on phospholipid concentration. The size of the ethosome doubled from 118 2 to 249 24 nm with an eight-fold increase in phospholipid concentration (from 0.5% to 4%).

➢ **Structure of the vesicular bilayer**

It is imperative to perform an investigative research of the ideal bilayer creation since the ethosomal system's vesicle bilayer determines how well it traps material. By doing Nuclear Magnetic Resonance (NMR) research, this can be accomplished. In contrast to liposomes made without ethanol, Touitou *et al*. (2000) demonstrated that in ethosomes, phosphotidylcholine in the presence of 20–30% of ethanol created bilayers in the form of closed vesicles that are less densely packed and had a higher cation permeability.

➢ **Transition temperature**

Differential scanning calorimetry can be used to determine the transition temperature of the vesicular lipid systems (DSC).

➢ **Drug content**

A UV spectrophotometer can be used to determine the ethosomes' drug content. A modified high performance liquid chromatographic method can also be used to quantify this.

➢ **Surface tension measurement**

A Du Nouy ring tensiometer can be used to assess the surface tension activity of a medication in aqueous solution.

➢ **Vesicle stability studies**

Vesicles' stability can be assessed over time by examining their size and structure. DLS measures mean size, while TEM detects structural changes.

➢ **Skin permeation studies**

Confocal laser scanning microscopy can be used to assess how well the ethosomal preparation permeates the skin layers (CLSM).

➢ **Effectiveness of drug entrapment**

Measuring the entrapment efficiency of ethosomes becomes the next crucial characterization parameter once the investigative studies of the configuration of vesicular bilayer of ethosomal systems are positively verified. This is because it endows the ethosomal system with sustained release characteristics. The two ways that are typically used for this are as follows.

A. Ultracentrifugation

This procedure comprises of two segments. In the first segment, the prepared vesicle is stored overnight before being ultra-centrifuged for a predetermined period of time and RPM. In the second segment, pure drug is evaluated using any highly established technique, such as high-performance liquid chromatography (HPLC), and then the entrapment efficiency is estimated using the formula:

Dt is the theoretical quantity of drug added, Ds is the amount of drug found exclusively in the supernatant,

EE is the entrapment efficiency.

B. Dialysis

A calculated amount of the drug-loaded vesicles or free drug in aqueous solution were placed into the dialysis bag, which was then transferred into 500 mL of phosphate buffer saline (PBS), pH 7.0. The saline solution was kept in the bags for 1 hour before the dialysis to ensure that the entire membrane

was wet. With the use of a magnetic stirrer, the receiver mediums were mixed. To maintain the ideal sink conditions, aliquots of the same quantity were taken out of the receiver medium at predetermined intervals and replaced with equal volumes of PBS solution. Using HPLC techniques, samples were further examined for drug content. The equation described above can subsequently be used to calculate entrapment efficiency.

Low fluorescence anisotropy of the phosphatidyl-choline probe antryl-vinyl phosphatidyl choline (AVPC) in ethosomes reflects entrapment efficacy of ethosomal systems, which is supported by the results of studies on lower *Tm* and fluidizing effect of ethanol on phospholipid bilayers. Following the completion of their investigations using confocal laser scanning microscopy (CLSM) and fluorescence activated cell sorter (FACS), Godin and Touitou demonstrated improved trapping of fluorescent probes by ethosomes.

- **Uniqueness of permeation**

The ability of ethanol to improve permeability has long been acknowledged. However, the permeation enhancement from ethosomes was much greater than would be anticipated from ethanol alone, indicating some sort of synergistic mechanism between ethanol, vesicles, and skin lipids providing flexible characteristics to ethosomes generating enhanced penetration abilities. These two effects are

(a) An increase in thermodynamic activity due to ethanol's evaporation, known as the "push effect," and
(b) A pull effect in which drug molecules are more easily absorbed showed percutaneous flux of 8.2 0.32 (mg/cm^2 h) for paclitaxel-loaded ethosomes, which was 3.2-fold higher than that seen for the paclitaxel present in the physical mixture (paclitaxel and empty ethosomes) and 23.2-fold higher than that seen for the paclitaxel hydroalcoholic suspension (0.037 0.01 mg/cm2 h).

- **Physically Stability**

In attempt to address the stability problems associated with conventional liposomes, a number of innovative techniques, such as proliposomes and niosomes, were created; nevertheless, their usage was restricted to topical distribution and they only partially succeeded as a result of low skin permeability.

Therefore, one of the main issues is stability analysis of ethosomal system because it shows their capacity to maintain their constitution along with active therapeutic drugs. It was previously believed that vesicles might not coexist with high alcohol concentrations due to the alcohol's inter-digitation action on lipid bilayers, which harms vesicular structures. In contrast to this assumption, numerous techniques, including 31P-NMR, TEM, SEM, and DLS, were used to demonstrate the durability of vesicles and the structure of ethosomes. The physical and chemical characteristics of ethosomes, in contrast to other liposomes, make them more effective for drug transport through the stratum corneum into the blood circulation, which is crucial in the development of transdermal drug delivery systems.

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It was also discovered that, when incorporated in the ethosomal formulation, cholesterol plays a crucial function in systemic distribution and that, in the absence of cholesterol, they are more likely to aggregate. The high concentration of ethanol in ethosomes can guarantee the mobility of the vesicles, and a reasonable amount of cholesterol might provide stability. Cholesterol stabilises into the bilayer when ethosomes are maintained in a gel condition. The stability of the ethosome suspension during long-term storage was probably guaranteed by the freeze-drying method. The ethosomes cakes that were freeze-dried were discovered to be glassy, compact, and quickly rehydrated. However, the amount of drug encapsulation within the ethosome was partly influenced by the storage duration, and it was found that following rehydration, there was a 10% drug leakage. The natural and/or synthetic phospholipid sources are used to make the ethosomes' lipid component. Unsaturated fatty acid-containing phospholipids are known to experience oxidative reactions. The permeability of the ethosomes bilayers may change as a result of the reaction products. Protecting the lipid preparation from light and adding antioxidants like a-tocopherol can both reduce the amount of oxidative degradation of the lipids in general. Additionally, lyso-PC is produced as a result of the hydrolysis of lipids. Lyso-PC needs to be kept to a minimum in a given preparation because it increases ethosome permeability when present. It was decided to entrap ()-epigallo-catechin gallate (EGCG) in ethosomes to increase its stability against breakdown. According to the results of a study on the stability of EGCG in solution and in ethosomes subjected to UV or high temperatures, EGCG is stabilised by being trapped in ethosomes. The breakdown of EGCG under UV light was postponed by tocopherol incorporation into ethosome.

Applications for Treatment

a. Transdermal medication delivery using ethosomes

The utilisation of ethosomes can produce a drug delivery efficiency of more than 65% due to their ability to profitably pass through the intact human skin. For the effective treatment of a variety of skin illnesses, ethosomes have been successfully examined for the transport of different medicinal substances across the skin.

b. Provide DNA

Because many environmental infections attempt to enter the body through the skin, which is also immunologically active and capable of expressing the gene, the skin serves as a good protective barrier. These data support another another crucial use of ethosomes: the transfer of DNA molecules to skin cells so they can express genes. Green fluorescence protein (GFP)-driven transfecting construct powered by cytomegalovirus (CMV) was encapsulated into ethosomal formulation by Touitou *et al*. For 48 hours, they used this formulation on the dorsal skin of 5-week-old male CD-1

nude mice. After 48 hours, the treated skin was removed, and CLSM experiments were used to track the penetration of the GFP formulation. It was discovered that ethosomes-GFP-CMV-driven transfecting constructs applied topically allowed for effective gene transport and expression in skin cells.

c. Administration of Hormones

Hormone distribution orally is associated with a number of issues, including high first pass metabolism, low oral bioavailability, and a number of dose-dependent adverse effects. Additionally, oral hormonal preparations also rely heavily on patient compliance in addition to these side effects. Each medication that is missed is known to raise the probability of treatment failure. By comparing the transdermal delivery of testosterone-loaded ethosomes (Testosome) to the transdermal delivery of testosterone patches (Testoderm patch, Alza) across rabbit pinna skin, it was found that ethosomes have a potential for hormonal delivery that is about 30 times greater than that of the patches. At the end of 7 hours, the amount of medication deposited was considerably (p50.05) higher for ethosomal formulation (130.76 18.14 and 18.32 4.05 mg). After applying Testosome instead of Testoderm, the area under the curve (AUC) and Cmax of testosterone considerably increased. The enhanced skin permeability and bioavailability of testosterone from ethosomal formulation were thus demonstrated by both *in vitro* and *in vivo* investigations. In their follow-up research, this group develops a testosterone non-patch formulation to minimise the area of application. It has been discovered that the ethosomal testosterone formulation required a 10 times less area of application to obtain the effective plasma concentration than the commercially available gel (AndroGel) formulation.

d. Distribution Through Cellular Means

In successful clinical research, ethosomes have been shown to be an effective penetration enhancer and carrier system for the transcellular administration of a variety of medicinal medicines. Through their investigation into the transcellular transport of chemicals from ethosomes, phospho-lipid vesicular carriers containing ethanol, to Swiss albino mice 3T3 fibroblasts, Touitou *et al*. demonstrated the effectiveness of ethosomes in transcellular delivery. The chosen probes were fluorescent PC, rhodamine red dihexadecanoylglycerophosphoetha- nolamine (RR), and 4-(4-diethylamino) styryl-N-methylpyridinium iodide (D289). By using CLSM and FACS investigations, the penetration of these fluorescent probes into fibroblasts and the skin of naked mice was investigated. The high-intensity fluorescence visible on CLSM micrographs demonstrated that ethosomes aided the entry of all probes into the cells.

In contrast, almost any fluorescence was seen when the substance was included in hydroethanolic solution or conventional liposomes. After 3 min of incubation, it was clear that each of the three probes under test had intracellular presence. Experiments using the hydrophilic calcein and lypophilic RR to permeate full naked mouse skin revealed that the ethosomal carrier enhanced the transport of molecules to the skin. From ethosomes, hydroethanolic solution, and liposomes, calcein penetrating the skin to depths of 160, 80, and 60 mm, respectively. Using a Franz's cell, Zhang *et al*. examined the percutaneous penetration of 5-Flourouracil loaded ethosomes in human hypertrophic scar (HS) and skin *in vitro*. The amount of 5-fluorouracil that was most abundant in ethosomes via HS was the amount that permeated HS and skin after 24 hours (E-Scar), followed by ethosomes via skin (E-Skin), hydroethanolic solution via HS (H-Scar), and hydroethanolic solution via skin (H-Scar) (H-Skin). Using confocal laser scanning microscopy, ethosomes fluorescently labelled with rhodamine 6GO were used to examine the penetration of ethosomes in HS and skin. Following application for 24 hours, the fluorescence intensity was highest in E-Scar, followed by E-Skin, H-Scar, and H-Skin, indicating that HS had the greatest ethosome penetration. In a study on the behaviour of hydrophilic CdTe fluores- cent clusters (quantum dots, QDs) loaded ethosomes (ES-QDs) over human skin scars, He *et al*. found that the depth of the ES-QDs particles' penetration into the scar showed the highest penetration efficiency.

Ethosomal particles demonstrated outstanding drug delivery and labelling capabilities into the various levels of the skin scar using nanoparticles like quantum dots. The quantum dots of ethosomes have fluorescent labelling characteristics, according to the study's findings.

e. Various Medicinal drugs are delivered

Results from multiple research studies show that ethosomes have been successfully employed to deliver a variety of medicinal substances.

f. Treatment of Viral and Microbiological Skin Infections

Antibiotic-containing ethosomal systems have been studied for the treatment of various skin infections. Animal models of deep skin infections were used to simulate and assess the effectiveness of the bacitracin and erythromycin ethosomal systems. In immunocompetent ICR male mice, S. aureus was intradermally injected to assess the topical therapies' pharmacodynamic effects. Seven and ten days following the start of the experiment, S. aureus colonies were isolated from the skin wounds. Results demonstrated that on days 7 and 10, respectively, mice treated with the etho-somal erythromycin system had no S. aureus bacteria in the inoculation sites compared to mice not treated with the system, which had 0.90 107 and 0.57 107 cfu/g tissue. Additionally, a histological study of the injured skin tissue on days 7 and 10 of the treatment demonstrated the preservation of normal skin structures and the absence of dermatonecroses. In contrast, examination of the injured areas in mice treated with erythromycin hydroethanolic solution and those left untreated showed progression of the infection, leading to significant dermatonecroses of the skin and surrounding tissues and the formation of an initial crust over the necrotic area (Fig. 3). These findings show that the erythromycin ethosomal system effectively eliminates the germs at the injection site in the deep skin layers. Therefore, topical use of an antibiotic ethosomal system could be a good substitute for systemic administration of the drug via injection in the treatment of deep skin infections.

In a pilot clinical research, another antibiotic using an ethosomal system was explored. The effectiveness of a new clindamycin etho-somal gel (CLSA) for the treatment of mild to moderate acne vulgaris was examined in this study, which involved forty patients. Clindamycin phosphate and salicylic acid are combined in CLSA's etho- somes.

Treatment with CLSA gel twice a day for eight weeks considerably improved the acneic condition by reducing the amount of comedones, pustules, and overall lesions in comparison to placebo. Seventyone percent of the participants said that their condition was getting better, with no reports of it getting worse. In addition, because to its enhanced tolerability and diminished adverse effects, the clindamycin ethosomal gel was favoured by 14 out of the 17 individuals who had previously had topical treatment. Acyclovir (ACV), a synthetic acyclic nucleoside analogue, was developed and studied for the treatment of Herpes labialis, another skin infection. The effectiveness of an ethosomal formulation, a commercial acyclovir cream (Zovirax®, GlaxoSmithKline S.p.A.), and a solution of the free medication were compared in this randomised double-blind clinical investigation in 40 participants who had 61 assessable episodes. There were 31 participants in the parallel arm; 12 received ethosomal acyclovir (EA), 10 received Zovirax® cream (ZC), and 9 received a vehicle (V). In the crossover arm, 8 people received EA before receiving ZC treatment, and 7 participants received ZC before receiving EA treatment. In this study, we assessed the proportion of lesions in which reported pain intensity decreased from day 1 to day 2 and from day 1 to day 3 as well as the time (in days) to crust formation, time (in days) to loss of crust, the proportion of abortive lesions of all assessable lesions, the time (in days) to first reduction of reported pain intensity, the time (in days) to absolute resolution of pain, and the time (in days) to first reduction of reported pain intensity. All of the clinical parameters that were tested significantly improved after the application of the ethosomal acyclovir system. When compared to Zovirax cream, the parallel arm indicated that 80% of lesions crusted after treatment with the ethosomal drug system on the third day following the start of the herpetic episode, compared to just 10% in the Zovirax group. In the EA group, crust formation took place after 1.6 days as opposed to 4.3 and 4.8 days in the ZC and V groups, respectively. Additionally, compared to only 10% in the ZC group, 33% of the lesions in the EA group were recurrent (Fig. 4). On day 2 of the crossover arm, the EA group's days to crust loss dramatically decreased from 4.2 to 5.9 compared to the ZC group. Compared to the 15% of lesions treated with ZC, 60% of lesions in the EA group crusted. The Supra-Vir topical acyclovir cream was developed in response to the results of this clinical investigation, which showed increased clinical efficacy of ethosomal acyclovir compared to ZC (Trima, Israel).

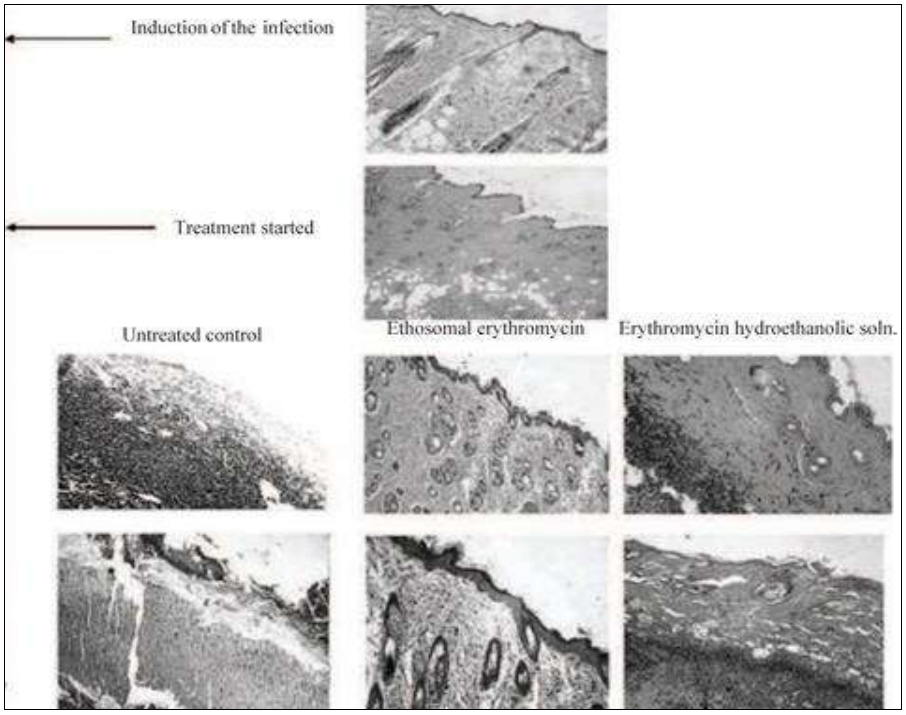
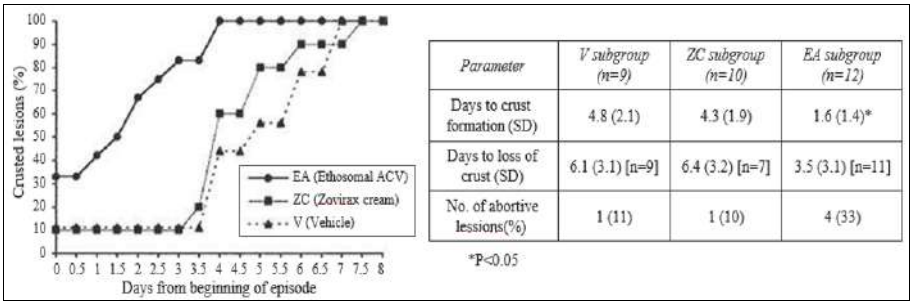


Fig 3: Histological images taken from skin of mice intradermally inoculated with 0.1 mL × 10^8 cfu/mL (10^7cfu/mouse)



Parameter	*V subgroup (n=9)*	*ZC subgroup (n=10)*	*EA subgroup (n=12)*
Days to crust formation (SD)	4.8 (2.1)	4.3 (1.9)	1.6 (1.4)*
Days to loss of crust (SD)	6.1 (3.1) [n=9]	6.4 (3.2) [n=7]	3.5 (3.1) [n=11]
No. of abortive lessions(%)	1 (11)	1 (10)	4 (33)

*P<0.05

Fig 4: Days to crust formation: parallel arm. Day 0: 33% of lesions aborted in EA subgroup, 10% in ZC subgroup, 11% in V subgroup. Day 3: 80% of lesions crusted in EA subgroup, 10% in ZC subgroup, 11% in V subgroup. Day 4: 100% of lesions crusted in EA subgroup, 60% in ZC subgroup, 44% in V subgroup. Time to crusting of all lesions: 4 days in EA subgroup, 7 days in ZC subgroup, 7.5 days in V subgroup. Reprinted with permission from, E.

Conclusion and Potential Futures

The majority of medicinal agents and medications cannot pass through the skin's stratum corneum barrier. Ethosomes are specially crafted vesicles with high ethanol concentrations that are extra malleable and capable of retaliating to peripheral irritation by fluidizing and upsetting the rigid stratum corneum lipid system, ultimately leading to the successful delivery of therapeutic agents deeply across the skin. These methods not only provide a better chance for the non-invasive delivery of tiny, medium, and big drug molecules, but they also make patient compliance easier and reduce the cost of care. This view is supported by the findings of the initial clinical trial of the acyclovir-ethosomal formulation. However, more research on the safety of ethosomes in specific clinical circumstances is necessary. For example, the irritating impact of ethanol applied to open areas of dermatitis cannot be disregarded as a potential shortcoming of ethosomes. Therefore, greater study in this field will enable improved control over medication release *in vivo*, enabling medical professionals to improve therapy. Given the interest that researchers have shown in these ethanol-based vesicles, it is clear that these systems have great promise for the future due to factors including their simplicity in manufacturing, breadth of drug administration, and therapeutic efficacy. Ethosomes are a promising carrier in the transport of bioactive substances due to all these factors.

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