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ETHOSOMAL NANOCARRIERS: A REVOLUTIONARY PATH BREAKING VESICULAR DRUG DELIVERY

Pharmacy

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ABSTRACT

The most flexible and broad method for administering both topical and systemic medications is through the skin. When administered topically, the stratum corneum, the skin's outermost layer, acts as the skin's strongest barrier to drug penetration, reducing the amount of bioavailability of the medication. Therefore, in order to get beyond the skin's natural barrier, it is essential to investigate and compare the different carriers required for systemic medicine administration. Transdermal drug delivery is a less intrusive method of administering medication that offers patient compliance, regulated drug distribution, and avoidance of first pass metabolism. It also requires less frequent dosage.

KEYWORDS

Ethosomes, ethanol, Composition, characterization, methods

INTRODUCTION

Drug delivery research entered a new era with the discovery of liposomes, and since then, several vesicular systems have been developed. In 1992, Cevc and Blume also developed transferosomes, which are elastic or pliable liposomes. After transferosomes, Touitou et al.'s groundbreaking research led to the identification of ethosomes, a distinct kind of lipid vesicular system[1]. Because of their smaller size, reduced entrapment effectiveness, and negative zeta potential, modified liposomes were developed. Ethosomes are novel modified lipid carriers composed of phospholipids, water, and ethanol. Ethosomes include relatively high concentrations of ethanol in addition to phospholipids and water, which have been proposed to have enhanced vesicular characteristics and skin penetration. Ethosomes, which are further classified as binary, classical, and transethosomes according to their contents, such as alcohol, have become a distinctive drug delivery method fairly fast[2].

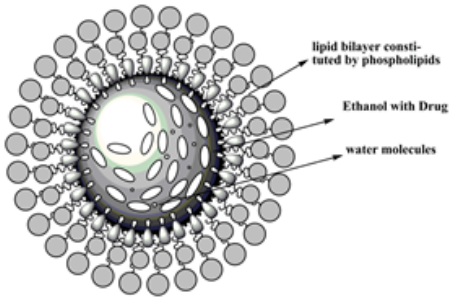

Figure 1 Structure of Ethosome

How Do Ethosomes Work?

In ethosome function, vesicles, ethanol, and skin lipids work in concert. Ethosome increase the dispersion of active substances over liposomes because they interact better with skin lipids. The transition temperature of the lipids in the stratum corneum is lowered when ethanol interacts with the lipid molecules in the polar head group area. They reduce the density of the lipid multilayer and increase fluidity, which allows the medicine to be absorbed into the skin's deeper layers. Moreover, ethanol gives vesicles a smoother, more flexible texture, which allows them to penetrate the epidermal layer more deeply[3].

Composition Of Ethosomes

The hydroalcoholic or hydro/alcoholic/glycolic phospholipid ethosomes are vesicular carriers with a relatively high concentration of alcohols or their combination. Propylene glycol (or other glycols), alcohol (ethanol or isopropyl alcohol), water, phosphatidylcholine (PC), hydrogenated PC, phosphatidic acid (PA), phosphatidylserine (PS), phosphatidylethanolamine (PE), phosphatidylglycerol (PPG), phosphatidylinositol (PI), hydrogenated PC, and other phospholipids are commonly found in ethosomes. A combination like this allows for the skin to absorb a high concentration of active substances. The ratio of alcohol to water or alcohol to polyol to water can be changed to control drug delivery. Soy-based phospholipids like Phospholipon 90 (PL-90) are among the favoured phospholipids. Typically, it is used in the range of 0.5–10% w/w[4].

Adding cholesterol to the mixture in amounts between 0.1 and 1% is another option. Isopropyl alcohol and ethanol are two types of alcohol that are useful. Propylene glycol and Transcutol are the two most often utilised glycols. Furthermore, PEG-alkyl ethers, which are non-ionic surfactants, can be mixed with phospholipids in these formulations. It is also possible to add cationic lipids such as cetrimide, dodecylamine, cocoamide, and POE alkyl amines. The finished product may have between 20 and 50% alcohol by content. The alcohol and glycol mixture that makes up the non-aqueous phase can have concentrations ranging from 22 to 70%[5].

Using ethanol to improve penetration Chemical penetration enhancers are substances that reversibly lower the stratum corneum's barrier resistance. One of the most popular permeation enhancers is ethanol. Many theories have been put out to explain how ethanol's permeation-enhancing properties work. Ethanol can be added to the formulation as a solvent to improve the drug's solubility. Because poorly soluble permeants are more likely to be depleted in the donor vehicle, this is especially crucial. At skin temperature, ethanol, a relatively volatile solvent, will quickly evaporate. Drug flux across the membrane may be impacted by supersaturation of the drug resulting from ethanol loss in a formulation. Furthermore, it is believed that ethanol modifies the stratum corneum's solubility characteristics, promoting better drug partitioning. In vitro, ethanol has been used to improve transdermal delivery of estradiol through human skin in vivo and of levonorgesterol, hydrocortisone, and 5-fluorouracil across rodent skin[6].

Megrab and associates observed that ethanol's enhancement effect depended on concentration. The authors looked into how ethanol affected the water content of the skin and found that formulations with high alcohol content could cause the skin to become dehydrated, which could account for ethanol's concentration-dependent effects[7].

Ethosomal Drug Delivery Benefits [8],[9],[10]

Ethosomal drug delivery systems are superior to other transdermal and dermal delivery systems in a number of ways. A few benefits include the following:

- Large molecules, such as peptides and protein molecules, can be delivered.
- The formulation uses non-toxic raw materials.
- Improved drug penetration through skin for transdermal administration.

- The ethosomal drug delivery system has broad applications in the veterinary, cosmetic, and pharmaceutical industries.
- High patient compliance: Because the ethosomal medication is administered in semisolid form (gel or cream), patient compliance is high.
- Ethosomal system is non-invasive, passive, and ready for immediate commercialization.
- Easy drug delivery method compared to Iontophoresis, Phonophoresis, and other complex methods.

Ethosomal Drug Delivery Drawbacks [9],[10]

They needed only strong molecules, those needing a daily dosage of 10 mg or less, may be given at high blood levels.

- Ethosomal administration is typically intended to provide slow, sustained drug delivery; it is not a method for achieving rapid bolus-type drug input.
- Sufficient solubility of the medication in aqueous and lipophilic environments to enter the systemic circulation and reach the dermal microcirculation.
- The drug's molecular size should be appropriate for transdermal absorption.
- Adhesive might not be cost-effective; it might not stick well to all skin types.
- Low yield
- Dermatitis or skin irritation brought on by enhancers and excipients used in drug delivery systems.
- When transferred into water, the ethosomes may coalesce and disintegrate if shell locking is ineffective.
- Product loss when switching from organic to water media.
- The enhanced drug permeation of ethosomes over liposomes is their primary benefit.

Benefits Of A High Alcohol Concentration

Etosomes contain a significant amount of ethanol (20–50%), a well-known and effective permeation enhancer. However, it was widely accepted that vesicles and high concentrations of ethanol could not coexist because of the interdigitation effect of ethanol on lipid bilayers. Ethamomes are lipid vesicular systems that Touitou identified, studied, and named to represent ethanol in comparatively high concentrations. The fundamental distinction between ethosomes and liposomes is found in their chemical makeup. Their superior skin penetration capacity was proposed to be primarily due to the synergistic effect of ethosomes' relatively high concentration of ethanol (20–50%) in vesicular form. The ethosomal formulation's high ethanol content (20–50%) may cause disruptions to the organisation of the skin's lipid bilayer[11]. Consequently, it might enable the vesicles to pass through the SC when integrated into a vesicle membrane. Moreover, the ethosomal lipid membrane had comparable stability to conventional vesicles but was packed less tightly because of the high ethanol concentration. This gave its membrane greater flexibility and stability by enabling a softer, more pliable structure that could fit through tiny gaps made in the disrupted SC lipids. Additionally, by altering the phospholipids' chemical structure and component ratio, it is possible to change the vesicular nature of ethosomal formulations. The reports of improved delivery of several medications, including acyclovir, minoxidil, triphexyphenidyl, testosterone, cannabidol, and zidovudine, demonstrate the adaptability of ethosomes for systemic delivery[12].

Table 1 Ethosomes as a vesicular medication delivery mechanism is being studied

Sr. No.	Researcher Name & Year	Title	Description	Publication
1.	Haij Muhammad Shoaib Khan et al. (2021)	Development, Characterization and Stability Evaluation of Topical Gel Loaded With Ethosomes Containing Achillea millefolium L. Extract	Drug: Achillea millefolium L. Extract MOP: Simple cold method Solvent: Ethanol Phospholipid: Propylene glycol	Frontiers in Pharmacology
2.	Naveed Akhtar et al. (2022)	Fabrication of Ethosomes Containing Tocopherol Acetate to Enhance Transdermal Permeation: In Vitro and Ex Vivo Characterizations	Drug: Tocopherol Acetate MOP: Modified cold method Solvent: Ethanol Phospholipid: Propylene glycol	Multidisciplinary Digital Publishing Institute
3.	Manuela Costanzo et al. (2021)	Formulative Study and Intracellular Fate Evaluation of Ethosomes and Transethosomes for Vitamin D3 Delivery	Drug: Vitamin D3 MOP: Cold Method Solvent: Ethanol Phospholipid: Phosphatidylcholine	International Journal of Molecular Science
4.	Ibrahim A. Mousa et al. (2022)	Formulation and Characterization of Metformin-Loaded Ethosomes for Topical Application to Experimentally Induced Skin Cancer in Mice	Drug: Metformin MOP: Cold Method Solvent: Ethanol and Isopropyl Alcohol Phospholipid: Lecithin	Multidisciplinary Digital Publishing Institute
5.	Francesca Ferrara et al. (2022)	Ethosomes and Transethosomes as Cutaneous Delivery Systems for Quercetin: A Preliminary Study on Melanoma Cells	Drug: Quercetin MOP: cold method Solvent: Ethanol, Distilled Water Phospholipid: Phosphatidylcholine	Multidisciplinary Digital Publishing Institute
6.	Sarvesh Paliwal et al. (2019)	Flurbiprofen loaded ethosomes - transdermal delivery of anti-inflammatory effect in rat model	Drug: Flurbiprofen MOP: Solvent: Ethanol Phospholipid: propylene glycol	Lipids in Health and Disease
7.	Nauman Rahim Khan et al. (2018)	5-Fluorouracil ethosomes – skin deposition and melanoma permeation synergism with microwave	Drug: 5-Fluorouracil MOP: Cold Method Solvent: Ethanol Phospholipid: Soya phosphotidylcholine	Artificial Cells, Nanomedicine, and Biotechnology An International Journal
8.	Yongtai Zhang et al. (2018)	CD44 Assists the Topical Anti-Psoriatic Efficacy of Curcumin-Loaded Hyaluronan-Modified Ethosomes: A New Strategy for Clustering Drug in Inflammatory Skin	Drug: Curcumin-Loaded Hyaluronan MOP: Hot Method Solvent: Phospholipid: Hydrogenated soybean Phospholipids	Ivyspring International Publisher
9.	Akhilesh Chandra et al. (2019)	Development of Topical Gel of Methotrexate Incorporated Ethosomes and Salicylic Acid for the Treatment of Psoriasis	Drug: Methotrexate MOP: Cold Method Solvent: Ethanol Phospholipid: Soya lecithin	Pharmaceutical Nanotechnology
10.	Yahua Cui et al. (2018)	Microneedle-Assisted Percutaneous Delivery of Paeoniflorin-Loaded Ethosomes	Drug: Paeoniflorin MOP: Solvent: Ethanol Phospholipid: P-glycoprotein	Multidisciplinary Digital Publishing Institute
11.	Hongdan Ma et al. (2018)	Paeonol-Loaded Ethosomes as Transdermal Delivery Carriers: Design, Preparation and Evaluation	Drug: Paeonol MOP: Cold Method Solvent: Ethanol Phospholipid: soybean phosphatidylcholine	Multidisciplinary Digital Publishing Institute
12.	Elsa Fitria Apriani et al. (2018)	Formulation, characterization, and in vitro testing of ethosome−based cream against	Drug: azelaic acid MOP: thin−layer hydration method Solvent: Ethanol Phospholipid:	Journal of Advanced Pharmaceutical Technolog

		Propionibacterium acnes for the treatment of acne	phospholipon 90 G	y & Research
13.	Xianglei Fu et al. (2019)	Ethosomal Gel for Improving Transdermal Delivery of Thymosin β-4	Drug: Thymosin β-4 MOP: Ethonal infusion Solvent: Ethanol Phospholipid: l-alpha-phosphatidylcholine	International Journal of Nanomedicine
14.	Vinny Wilson et al. (2017)	Development and evaluation of finasteride loaded ethosomes for targeting to the pilosebaceous unit	Drug: Finasteride MOP: Cold method Solvent: Ethanol Phospholipid: Soya phosphatidylcholine	Artificial Cells, Nanomedicine, and Biotechnology An International Journal

Drug Penetration Mechanism

There are two types of pathways in the stratum corneum: intercellular and transcellular. Drugs are applied topically via vesicles. The medication is absorbed into the skin's deepest layer by vesicles. The phospholipid and ethanol concentrations in ethosomes influence their size. Through the transcutaneous stratum corneum and open hair follicles, ethosomes enter the skin. The ethosome's ethanol increases the ethosome's penetration. Therapeutic agents can enter the skin thanks to the transcutaneous. Two effects form the basis of this phenomenon.

1. The effect of ethanol
2. The impact of ethosome

1. The ethanol effect: Ethanol acts as a penetration enhancer within the ethosome. It lowers the stratum corneum's barrier resistance. The lipids inside the cell are penetrated by ethanol. It improves the drug's solubility. Due to its volatility, ethanol causes formulation loss, which affects how well the medication passes through the membrane.

2. The ethosome effect: Because ethosomes pierce intracellular lipids, the density of the lipid multilayer in the cell membrane is reduced. The medication is being released into the skin's deep layer[13],[14].

Methods Of Preparation Of Ethosomes

Ethosomes can be prepared by two very simple and convenient methods that is;

1. Cold method
2. Hot method
3. Classic Mechanical Dispersion Method

1. The Cold Technique

This is the approach that is most frequently used to prepare ethosomal formulation. This method involves using a mixer to vigorously stir ethanol in a covered vessel at room temperature while dissolving phospholipid, medication, and other lipid materials. Stirring adds propylene glycol or another polyol. In a water bath, this mixture is heated to 30°C. After adding the water that has been heated to 30°C in a different vessel, the mixture is covered and stirred for five minutes. Using the sonication or extrusion methods, the ethosomal formulation's vesicle size can be reduced to the desired extent. The formulation is then refrigerated for storage.

2. Hot technique

This method involves heating phospholipid in a water bath at 40°C until a colloidal solution is produced. Ethanol and propylene glycol are combined and heated to 40°C in a different vessel. The aqueous phase is supplemented with the organic phase once both mixtures have reached 40°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.

3. Traditional Mechanical Dispersion Method

Soya phosphotidylcholine is dissolved in a mixture of ethanol and chloroform in a flask with a circular bottom. The organic solvent is eliminated using a rotary vacuum evaporator to leave a thin film on the flask wall. Leaving the contents overnight eliminates solvent traces. Drug-containing hydroethanolic mixtures at varying concentrations are hydrated at the right temperature[15],[16].

Table 2 Ethosome formulation Patents

Sr. No	Patent name and Patent year	Application	Name of Patent Inventor/s
1.	Composition for applying active substances to or through the skin (1998)	5176638	Elka Touitou, Jerusalem, Israel
2.	Tretinoin ethosomes gel and preparation method thereof (2015)	CN 104983675 A	Hu Chunmei, Liu Yan, Wang Jing, Li Rong
3.	Chines Medicinal ethosomes gel patch for treating herps zoster and preparation method (2014)	CN103536700 (A)	Bu Ping; Hu Rong; Chen Lin; Wei Rong; Wu Huanhuan; Huang Xiaoli
4.	Ethosome gel film-coating agent with multiple wound repair effects and preparation method of ethosome gel film-coating agent (2014)	Cn103893394 (A)	Chen Jie; Huang Chanping; Zheng Maoxin; Nie Kaipin
5.	Ethosome preparation of male hormone (2012)	CN102406605 (A)	Shu Meng; Jianxin Li; Yanmin Guan
6.	Paclitaxel ethosome gel and preparation method there of (2012)	CN102579323 (A)	Jinping tan; Lixin Jiang; Tanran Chang; Zhiwen Zhou
7.	Podophyllotoxin ethosomes and preparation method there of (2011)	Cn102144972 (A)	Nianping Feng; Yanyan Yu; Jihui Zhao; Haiting Weng; Xiaoqin Shi
8.	Acyclovir ethosome and preparation methods therefor of (2011)	Cn102133183 (A)	Xuewen Wu; Yan Xiong
9.	Terabinafine compositions for onychomycosis treatment (2011)	W020100 86723A1	E.Touitou
10.	Preparation method of ethosome/natural material/ polyvinyl alcohol composition hydrohel (2015)	CN 104706571 A	Yang Xingxing, Lynn, Chen Mengxia, Fanlin Peng
11.	Leflunomide ethosome composition and its preparation method (2014)	CN103800277 (A)	Zhang Tao, Ding Yanji, Deng Jie, Luo Jing, Zhong Xiaodong
12.	Chitosan-modified ethosome structure (2013)	EP 2810642 A1	Ching-Tung Lee, Po-Ling Chen
13.	Depomycin- modified ethosome preparation (2013)	CN103006562 (A)	Li Chon, Liu Xia, Yin Qikun, Wang Xiaoying, Chen Zhangbao
14.	Preparation method of lidocaine ethosome (2012)	Cn102688194 B	Liang Ju, Wu Wenlan, Li Mei, Miao Juan, Wei Xuefeng, Chen Shan, Wang Xiaotaro
15.	Bullataacin ethosome gel and preparation method thereof (2012)	CN102552147 (A)	Jianping Tan, Lixin Jiang, Tanran Chang, Zhiwen Zhou
16.	Lidocaine ethosome and preparation method thereof (2012)	CN102813624(A)	Zhao Xianying, Su Yongping, Gao Jining, Liu Yimin, Zhao Huawen, Xiao Xiang, Zhou Xiaoxia, Zhang Dinglin, Wu Liping
17.	Progesterone ethosomes, and preparation method and application thereof (2012)	CN102397255 (A)	Shu Zhang, Hong Deng, Huaqing Lin, Xiaoling Zhang

Characterization Of Ethosomes [17],[18],[19],[20],[21]

- **Vesicle structure:** Transmission electron microscopy (TEM) and scanning electron microscopy (SEM), which involve negative staining the formulation with an aqueous solution of agents like

phosphotungstic acid, etc., can reveal the vesicular morphology of ethosomal systems.

- **Size distribution and size of vesicles:** The size ranges of dynamic light scattering (DLS) between nanometers and microns, which are established by the formulation's composition, will determine the ethosomal framework's vesicular size.
- **Putative vesicular bilayer configuration:** Investigating the best bilayer formation is necessary because the ethosomal trapping system's effectiveness is dependent on its vesicle bilayer. Nuclear Magnetic Resonance (NMR) research can be used to accomplish this.
- **Efficiency of drug entrapment:** The effectiveness of drug entrapment Measuring the effectiveness of ethosomal trapping, which confers sustained release characteristics on the system, becomes the next crucial characterization parameter after the configuration of the vesicular bilayer of ethosomal systems has been thoroughly investigated and positively verified. Typically, two techniques are used, which are outlined below.
- **Ultracentrifugation:** The vesicle preparation of the first segment is divided into two steps, each of which is held overnight and ultracentrifuged for a specified amount of time and RPM. The second chapter uses every cutting-edge method to verify that the drugs are pure, such as High-performance liquid chromatography (HPLC). The entrapment efficiency is then determined by utilising the relationship that follows: v = wc - wc − wc × 100 Where Dt is the theoretical amount of drug added, Ds is the amount of drug detected only in the supernatant, and EE is the entrapment efficiency.
- **Dialysis:** To ensure full membrane wetting, bags containing polymers, such as cellulose acetate, were prepared one hour prior to dialysis and then filled with a measured quantity of drug-loaded vesicles or free drug in aqueous solution. The dialysis bag was then transferred to 500 millilitres of pH 7.0 phosphate buffer saline (PBS). The receiver mediums were stirred using a magnetic stirrer. To maintain ideal sink conditions, aliquots of the same amount were taken out of the receiver medium at predetermined intervals and replaced with equivalent amounts of PBS solution. HPLC techniques were used to further analyse the drug content samples.
- **Permeation distinctiveness:** It has long been believed that ethanol has qualities that improve permeation. However, the permeation enhancement from ethosomes was significantly greater than that predicted from ethanol alone, suggesting a synergistic mechanism involving ethanol, vesicles, and skin lipids that gives ethosomes adaptable properties that lead to increased penetration capabilities as a result of two findings. Two effects of ethanol evaporation are known as the "pressure effect": (A) an increase in thermodynamic activity; and (B) a decrease in the barrier properties of subcutaneous tissue, which leads to an increase in drug molecule penetration.
- **Physical stability:** The freeze-drying method might have guaranteed the stability of the ethosome suspension during long-term storage. It was discovered that cakes made of freeze-dried ethosomes were glassy, light, and distinguished by their quick rehydration. Nevertheless, the storage period, which revealed a drug leakage of roughly 10% following rehydration, had an impact on the percentage of drug encapsulation within ethosome. Ethamomes' lipid component is made from synthetic or naturally occurring phospholipid. It is well known that phospholipids containing unsaturated fatty acids can undergo oxidative reactions. The bilayer ethosomes' permeability may alter as a result of the reaction's products. By shielding the lipid preparation from light, antioxidants like α-tocopherol can reduce oxidative lipid degradation in general. In addition, lyso-PC is formed as a result of lipid hydrolysis. Because lyso-PC increases the permeability of ethosomes, its level must be kept to a minimum in a given preparation.
- **Transition temperature:** The temperature at which vesicular lipid transitions occur (T) can be measured twice using DSC in an aluminium pan with a constant nitrogen stream and a heating rate of 10 °C per minute.
- **Confocal laser scanning microscopy (CSLM):** The degree and purpose of the ethosomal preparation skin penetration can be investigated using CSLM. A confocal laser scanning microscope's z axis can be used to optically scan the skin's thickness at various increments.
- **Drug content:** The UV spectrophotometer can be used to determine the ethosome quality. A modified chromatographic high-performance liquid method can also be used to quantify it.
- **Surface tension measurement:** The ring process can be used in a Du Nouy ring tensiometer to measure the drug surface tension activity in an aqueous solution.
- **Phospholipid-ethanol interaction:** Using calorimetry differential scanning and 31P-NMR decoupled protons, the relationship between phospholipid and ethanol was investigated.
- **Degree of Degradability and turbidity:** The ethosomal preparation's degree of deformability can be assessed using the extrusion method, and a nemaline can be used to measure the preparation's turbidity.
- **Drug entrapment efficiency:** Anisotropy analysis of AVPC, a fluorescent analogue of phosphatidylcholine, and differential calorimetry scanning thermograms revealed that the bilayers in ethosomes were highly fluid and had a lower Tm than those in standard liposomes. This gave the vesicles a soft, amiable texture. Godin and Touitou showed that ethosomes are capable of effectively trapping both hydrophilic and hydrophobic fluorescent samples using confocal laser scanning microscopy (CLSM). When various drugs were tested for trapping using the ultracentrifugation method, similar results were obtained. Hydrophilic 6-carboxy fluorescein and hydrophobic Rhodamine 123 fluorescence markers have been used to successfully verify hydrophobic and hydrophilic medications. Because ethanol is present in the vesicles and ether has a high degree of lamellarity, ethersomes can effectively clog both hydrophilic and lipophilic drugs. Moreover, ethosomal formulations can trap more particles than liposomes. Trihexyphenidyl hydrochloride trapping efficiency rose from 36% for liposomes to 75% for ethosomes, as demonstrated by Dayan and Touitou.

Evaluation Of Ethosomes [22],[23],[24],[25],[26],[27],[28]

- **Vesicle skin interaction study:** Various visualisation techniques, such as evaluating the process of enhanced ethosomal formulation skin penetration. The techniques employed were laser microscopy (CSLM) confocal scanning, fluorescence microscopy, eosin-hematoxyl staining, and transmission electron microscopy. When combined, these visualisation techniques also improved knowledge of the modulation of the structure and vesicle penetration pathways. Traditional liposomes only penetrated the stratum corneum, the top layer of skin. Alcohol-free liposomes hardly penetrated the deep tissue. Comparatively, better distribution of 6-CF and Rhodamine 123 was observed when the ethosomal carrier was utilised, both in terms of quantity and depth (dermis-layer).
- **Filter membrane-vesicle interaction study by scanning Electron microscopy:** Filter membranes with a pore size of 50 nm must have vesicle suspension (0.2 ml) added to them before being placed in diffusion cells. While the lower side of the filter was in contact with the pH 6.5 phosphate buffer saline solution, the upper side of the filter was exposed to air.After an hour, the filters were taken out and ready for SEM research by being fixed for the next day at 4°C in Karnovsky's fixative and then being dehydrated using ethanol solutions varying in volume (30%, 50%, 70%, 90%, 95%, and 100% v/v in water).
- **Skin permeation studies:** The abdominal skin and underlying connective tissue were separated using a scalpel and a pair of scissors after the test animals' (rats') hair was clipped short (less than 2 mm). The skin that was removed was placed on aluminium foil and the dermal side was gently peeled off in order to remove any adhering fat and/or subcutaneous tissue. The volume permeation area of the receptor cell and effective difusion cell was 10 ml and 1.0 cm2, respectively. A temperature of 32 °C ± 1 °C was maintained. There was saline solution with phosphate buffer (10 ml pH 6.5) in the receptor compartment. It positioned the removed skin in between the receptor compartment and the donor. The skin's epidermal surface was treated with a 1.0 ml ethosomal formulation. Using the diffusion cell's sampling port, samples (0.5 ml) were taken at 1, 2, 4, 8, 12, 16, 20, and 24 hour intervals. A high-performance liquid chromatography assay was then used for analysis.
- **Stability study:** The vesicles were maintained at 4 °C ± 0.5 °C to ascertain their stability. Using the previously described method, the vesicle size, zeta potential, and trapping efficiency were determined 180 days later.
- **Drug uptake studies:** 100 µl of RPMI medium was applied to 24-well plates (Corning Inc.) containing 1,1106 cells per millilitre of drug. Cells were incubated with 100 µl of the drug solution in phosphate buffer saline solution (pH7.4), ethosomal formulation,

or advertised formulation. Drug absorption was then determined by HPLC assay analysis of the drug material.

- **HPLC assay:** During in vitro skin permeation experiments and in MT-2 cell, the amount of drug permeated in the receptor compartment was determined by HPLC assay using methanol: distilled water: acetonitrile mixture (70:20:10 v / v) as a mobile step.
- **Statistical analysis:** ANOVA was used to assess the statistical significance of all generated data, and then studied range testing was conducted. A confidence level of P<.05 was established for the results' interpretation using the PRISM programme.

Application Of Ethosomes [12),(29),(30),(31),(32),(33),(34),(35]

Since ethers, which are vesicles with a high ethanol content, can penetrate the skin's deeper layers, they are frequently used for transdermal drug delivery of hydrophilic and impermeable medications.

- **Hormone delivery:** Numerous problems, including high first-pass metabolism, low oral bioavailability, and a host of dose-dependent side effects, are associated with oral hormone delivery. Furthermore, oral hormonal preparations that significantly rely on patient adherence to these side effects. It is well known that every pill missed increases the chance of treatment failure. By conducting a comparative analysis of transdermal delivery of testosterone loaded ethosomes (Testosome) as compared to transdermal testosterone patch (Testoderm patch, Alza) through rabbit pinna skin, Touitou et al. demonstrated the ability of ethosomes in hormonal delivery. The results showed approximately 30-times higher skin permeation of testosterone from ethosomal formulation. The amount of drug deposited in the ethosomal formulation was significantly (p50.05) higher, measuring 130.76 ± 18.14 and 18.32 ± 4.05 mg for Testosome and Testoderm, respectively, after 7 hours. When Testosome was applied instead of Testoderm, the area under the curve (AUC) and Cmax of testosterone increased significantly. Thus, enhanced skin penetration and testosterone bioavailability from ethosomal formulation have been demonstrated in both in vitro and in vivo studies.
- **Transcellular delivery:** In ongoing clinical trials, ethosomes have demonstrated their efficacy as a carrier and penetration enhancer for the transcellular delivery of a variety of therapeutic agents. On the other hand, when integrated in a hydroethanolic solution or traditional liposomes, hardly any fluorescence was seen. All three of the tested probes were clearly intracellular after 3 minutes of incubation.
- **Pilosebaceous targeting:** Hair follicles and sebaceous glands are being acknowledged as potentially important components for percutaneous drug delivery. The use of pilosebaceous units as depots for localised therapy—particularly for the treatment of follicle-related disorders like alopecia or acne—has drawn attention. The use of the follicles as transportation shunts for systemic drug delivery has also received a lot of attention.
- **Delivery of anti-parkinsonism agent:** Trihexyphenidyl hydrochloride (THP) ethosomal formulations were prepared by Dayan and Touitou, who then compared their delivery to that of conventional liposomal formulations. THP is a muscarinic receptor antagonist that is used to treat Parkinson's disease. In comparison to liposome, phosphate buffer, and hydroethanol solution, the transdermal flux value of THP from ethosomes via the nude mouse skin was 87, 51, and 4.5 times higher, respectively. When ethosomes were applied, the amount of THP that was still in the skin after 18 hours was significantly higher than when liposomes or hydroethanolic (control) solution were applied. These results suggested that the ethosomal-THP formulation has a higher potential for skin penetration and may be used to treat Parkinson's disease.
- **Topical delivery of DNA:** Many environmental pathogens attempt to enter the body through the skin, which has evolved into an exceptional defensive barrier that is both gene-expressing and immunologically active. Based on the aforementioned information, one significant application of ethosomes is the topical delivery of DNA molecules to induce gene expression in skin cells. Ethosomes have been suggested as potential carriers for gene therapy applications requiring transient gene expression. The results also suggested that ethosomes could be used to successfully administer transdermal vaccinations. Therefore, the possibility of using these dosage types to deliver immunising agents is made possible by improved ethosomal skin permeation capacity.
- **Delivery of anti-arthritis drug:** Topical delivery of anti-arthritis medication solves issues related to traditional oral therapy and is a better option than site-specific delivery. A new medication candidate for the treatment of rheumatoid arthritis is cannabidol, or CBD. His oral administration is linked to several problems, including first pass metabolism, low bioavailability, and GIT degradation. Using a rat paw edoema model mediated by carrageenan, a significant increase in the biological anti-inflammatory activity of the CBD-ethosomal formulation was observed. It was therefore determined that the encapsulation of CBD in ethosomes significantly enhanced its biological activities, as well as its penetration and accumulation in the skin.
- **Delivery of antibiotics:** A safer way to increase the therapeutic efficacy of those medications is through topical administration of antibiotics. Many allergic reactions are caused by side effects from conventional oral therapy. The permeability of conventional outer formulations to subdermal tissues and deep skin layers is poor. This problem can be avoided by using etherosomes to deliver adequate antibiotics into the skin's deeper layers. Ethosomes are able to readily pass through the epidermis, deliver significant dosages of medication into the skin's deeper layers, and eradicate infections from their source. The results of this investigation demonstrated that antibiotic ethosomal formulation might be very successful in resolving issues related to conventional therapy.
- **Anti-viral drug delivery:** Zidovudine is a highly effective antiviral medication that targets the HIV virus. Quick side effects are associated with zidovudine taken orally. Therefore, to maintain the anticipated anti-AIDS effect, zidovudine must be administered appropriately at zero order. Numerous investigations led to the conclusion that ethosomes might improve transdermal flux, extend release, and present a desirable delivery method for continuous zidovudine. Another antiviral medication that is frequently applied topically to treat Herpes labialis is acyclovir. The conventional external formulation of commercially available acyclovir is linked to insufficient therapeutic efficacy due to low skin penetration of hydrophilic acyclovir to the dermal layer. Scientists have overcome the issue with conventional topical acyclovir preparation by developing the acyclovir ethosomal formulation for dermal delivery.
- **Delivery of problematic drug molecules:** Large biogenic molecules, like proteins or peptides, are difficult to transfer orally because the GI tract completely breaks them down. For the purpose of solving the oral delivery issues, non-invasive protein delivery is a more secure option. Researchers have been examining how ethosomal insulin delivery affects in vivo blood glucose reduction in both normal and diabetic SDI rats. The outcome demonstrated that both normal and diabetic rats receiving insulin from this patch experienced a significant (up to 60%) reduction in BGL. On the other hand, the BGL is not decreased by an insulin injection made from a control formulation.
- **Cosmaceutical application of Ethosomes:** Applying ethosomes to cosmetics has the advantage of improving transdermal permeation, especially in elastic types, as well as improving the stability of cosmetic chemicals and reducing skin irritation caused by them. In addition, the sizes and compositions of the vesicles are the main factors that must be taken into account in order to realise these advantages of elastic vesicles for cosmetics.

CONCLUSION

Ethosomes can significantly overcome the primary obstacle to transdermal medication distribution, the epidermal barrier. A possible substitute for oral medication distribution for systemic impact is the transdermal method. When contrasted with transdermal and dermal administration, ethosomes offer additional benefits. The non-invasive drug delivery vehicles known as ethers allow medications to enter the deep layers of the skin and eventually enter the bloodstream. Large molecules like peptides and protein molecules are delivered by it. When applied to different skin conditions, ethosomes offer benefits such enhanced skin penetration and targeting to deeper skin layers. High patient compliance due to its semisolid (gel or cream) administration and range of applications in the veterinary, cosmetic, and pharmaceutical fields.

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