Journal of Positive School Psychology http://journalppw.com
2022, Vol. 6, No. 8, 4099-4111

# Ethosomes: Novel Lipid Vesicular and Non-Invasive Delivery Carrier – A Review

**ROSHINI R, SARASWATHI T. S.*, DAMODHARAN N.**

*Department of Pharmaceutics, SRM College of Pharmacy, SRM Institute of Science and Technology, Kattankulathur, Tamil Nadu, India*
*Email: saraswas1@srmist.edu.in*

## Abstract

The skin is the body's most superficial and innately manageable organ. To administer medication through transdermal, the stratum corneum must be penetrated. There are a number of mechanisms that have been improved to further facilitate drug penetration. Ethosomes are vesicles that deliver drugs to the dermis and the bloodstream without causing any damage to the skin. Ethosomes are drug delivery vesicles that are both pliable and soft. As a well-known permeation enhancer, ethanol provides ethosomes with outstanding flexibility and deformability, allowing them to penetrate deeply and improving medicine penetration and deposition. Therapeutic medication delivery for a wide variety of skin conditions, including acne, psoriasis, alopecia, skin infections, and hormonal deficiencies, is much improved by the altered composition of ethosomes compared to that of conventional liposomes. Ethosomes enhance the efficiency of treatment, the well-being of patients, and the affordability of care.

**Keywords:** Ethosomes, liposomes, novel drug delivery, drug transporters, penetration enhancer, Percutaneous absorption

## INTRODUCTION

An efficient way to get around problems that might impair the absorption of drugs taken orally is through the use of transdermal drug delivery systems. It results in stable plasma drug concentrations, shorter drug dosing intervals, and improved patient compliance. The stratum corneum (SC) is the skin barrier that prevents drug absorption. Many approaches, such as using lipid vesicular-based systems, have been used to overcome such a barrier and achieve higher transdermal drug penetration. Liposomes have sparked considerable interest in skin treatment; however, their rigid structure and large vesicle size may limit drug permeation into deeper layers of skin, restricting it to the uppermost layer of the SC.[1]

As an improved type of liposome, flexible liposomes (transfersomes, ethosomes) have been developed over the last decade. Touitou developed lipid transdermal drug delivery systems that are soft malleable vesicular carriers composed primarily of phospholipids, ethanol (at relatively high concentrations), and water. Ethosomes increase drug permeability through the skin due to the synergistic effects of the phospholipid and ethanol combination, where ethanol increases lipid fluidity by decreasing the melting point of SC lipids by interacting with their polar head group. Furthermore, by fluidizing the phospholipid bilayers, ethanol increases the flexibility of ethosomes, allowing the resulting elastic vesicles to squeeze through skin pores that are much smaller than their diameters [2][3].

## NOVEL DRUG DELIVERY SYSTEM

Nanocarriers come in a variety of forms, including lipid-based, polymeric-based, and surfactant-based nanocarriers. Vesicular systems such as liposomes (composed of a phospholipid bilayer enclosing an aqueous cavity), niosomes (composed of non-ionic surfactants and amphipathic compounds that impart a neutral charge), ethosomes (composed of phospholipids and high concentrations of alcohol), transfersomes (containing an edge activator and double-chain lipids), and cubosomes (curved bicontinuous lipid) [4]. Furthermore, vesicular systems are extensively compatible with the skin because they are made of lipids, which are also found in the skin. Due to their beneficial qualities, including biodegradability, affordability, and an easy manufacturing process, liposomes had received a great deal of attention. However, it was well known that liposomes cannot easily pass through the skin layers and are therefore typically restricted to the SC or upper layers of the epidermis [5]. Ethosomes, on the other hand, are a vesicular carrier with enhanced skin delivery properties due to unique properties such as high deformability and flexibility. Ethosomes, like liposomes, have a phospholipid bilayer and excellent biocompatibility, but they also have a distinct feature: a relatively high concentration of ethanol (20-45 %). Because of its composition, ethanol gives ethosomes special properties like (1) small vesicular size, which can range from tens of nanometres to microns, (2) high deformability, (3) fluidity, and (4) stability. Thus, when compared to conventional hydro alcoholic solutions or classic liposomes, various studies indicate that ethosomes are more effective in enhancing the length and effectiveness of skin penetration [6].

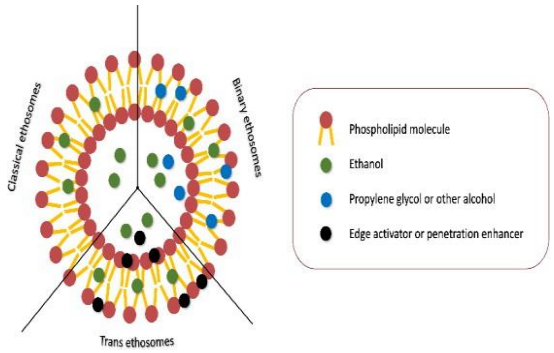
### Ethosomes

Ethosomes are liposomes that have been altered to include a little amount of a medication[7]. Ethosomes are non-invasive drug delivery systems that allow medications to penetrate deeply into the epidermal layers and/or the bloodstream. These flexible, soft vesicles are designed for improved active agent delivery. For many years, the vesicles significance in cellular communication and particle transportation has been well understood. Vesicles would also make it possible to manage the pace of drug release over an extended period of time, shield the medication from immune reaction or other removal mechanisms, and release the ideal amount of drug while maintaining a consistent concentration for longer periods of time. The discovery of a vesicle derivative known as an ethosome was one of the key developments in vesicle research [8].

## STRUCTURE AND ITS COMPOSITION

Ethosomes are vesicular carriers composed of hydroalcoholic with a relatively high concentration of alcohols or their combination. Phospholipids having diverse chemical structures, such as hydrogenated PC, phosphatidylcholine (PC), phosphatidic acid (PA), phosphatidylserine (PS), phosphatidy lethanolamine (PE), phosphatidylglycerol (PPG), phosphatidylinositol (PI), hydrogenated PC, alcohol (ethanol or isopropyl alcohol), water, and propylene glycol (or other glycols). Through the skin, a high concentration of active substances can be delivered by such a formulation. The ratio of alcohol to water or alcohol-polyol to water can be changed to control drug delivery. Soya phospholipids, such as Phospholipon 90, are some of the best phospholipids (PL-90). It isoften used in a range of 0.5-10% w/w. Cholesterol can be added to the mixture in amounts ranging from 0.1 to 1%. Ethanol and isopropyl alcohol are two examples of alcohols that can be employed. In these formulations, phospholipids may also be combined with non- ionic surfactants (PEG-alkyl ethers). Between 20 and 50 percent of alcohol may be present in the

finished product. The content of the non-aqueous phase (a mixture of alcohol and glycol) can range




from 22- 70% [9][10].

**Fig 1. Structure of Ethosomes**

### Advantages over Liposomes

Due to their numerous advantages, ethosomes offer a viable strategy for enhancing drugdelivery through the skin and have gained significant attention in recent years. The advantages of ethosomes over traditional liposomes and ultra-flexible liposomes for local drug delivery have been intensively studied during the past[11]. These analyses consider the following factors which include ethosomes transport active chemicals across the stratum corneum more efficiently than typical liposomes and then retain them in the skin layers. Ultra- flexible liposomes containing sodium cholate have greater barrier compatibility. Ethosomal systems were found to be superior to liposomes and hydroalcoholic solution in terms of both the breadth and depth of medication and fluorescent probe delivery over the skin. Unlike liposomes, ethosomes could carry drugs through the SC to deeper layers of skin and even into the bloodstream. Ethosomes, like liposomes or ethanolic drug solutions, are meant to increase thetransdermal perviousness of loaded drugs[12][13].

### Benefits of Ethosomal Drug Delivery

It is feasible to deliver big molecules (peptides, protein molecules), Its formulation uses non-toxic raw materials, improved medication penetration via skin for transdermal drug delivery. Patient compliance is high because the ethosomal medication is administered in semisolid form (gel or cream), patient compliance is good. Compared to Iontophoresis, Phonophoresis, and other complex procedures, this drug delivery approach is simple. The Ethosomal system may be immediately commercialized and is passive and non-intrusive [14][15].

### Drawbacks of Ethosomal Drug Delivery

Ethosomal administration is not intended to provide quick bolus drug input; rather, it is intended to provide steady, sustained drug delivery. Sufficient solubility in both lipophilic and aqueous conditions for the medicine to reach the dermal microcirculation and enter the systemic circulation. The drug's molecular size should be small enough to be absorbed percutaneously. Adhesive may not adhere properly to all skin types. Excipients and enhancers of medication delivery systems might cause skin irritation or dermatitis. If shell locking is inadequate, the ethosomes may coalescence and fall apart upon transfer into water [16-18].

## ETHANOL- AS PENETRATION ENHANCER

The compounds that reversibly lower the stratum corneum's barrier resistance are known as chemical penetration enhancers. Among these, ethanol is one of the penetration enhancers that was most frequently employed. A number of methods for ethanol's penetration-enhancing impact have been hypothesised. Ethanol can be used as a solvent in the formulation to increase the drug's solubility. This is crucial for poorly soluble permeants since they can easily deplete in the donor vehicle. Since ethanol is a somewhat volatile solvent, it will quickly evaporate at body

temperature. When ethanol is lost from a formulation, the medicine becomes oversaturated, which affects how much of it can pass through the membrane. Additionally, ethanol improves medication partitioning by modifying the stratum corneum's solubility characteristics. Ethyl alcohol was employed in-vitro and oestrogen was used in-vivo to support the percutaneous administration of levonorgestrel, corticosteroid, and 5-fluorouracil over chewing animal skin. According to reports, ethanol has a concentration-dependent enhancing impact. Since it has been established that ethyl alcohol may dehydrate skin, the concentration-dependent activity of ethanol can be explained by the fact that formulations with high alcohol content can do so
[19-21].

## MECHANISM OF SKIN PERMEATION

A number of processes most likely work together to provide the boosting effect, even if the precise mechanism of ethosomal drug transport is still up for debate. The stratum corneum lipid multilayer is densely packed and firmly conformationally organized at physiological temperature. The high concentration of ethanol in ethosomes allows them to penetrate the stratum corneum because it disrupts skin lipid bilayers.

Due to the high ethanol concentration, the lipid membrane is also less densely packed than conventional vesicles but is still stable, allowing for a more malicious form that allows it to fit through narrow passages like those made to rupture the corneum lipid stratum. By interacting with lipid molecules at the polar hard group, ethanol decreases the stiffness and increases the fluidity of the lipids in the corneum layer. The membrane will become more permeable as a result of the intercalation of ethanol into the polar head group's surroundings. The ethosome interacts with the stratum corneum barrier in addition to ethanol's action. Even though most of the drugs in classic liposomes stayed on the skin's surface, the ethosomal system was found to be a very effective way to increase drug delivery through the skin [22–25].

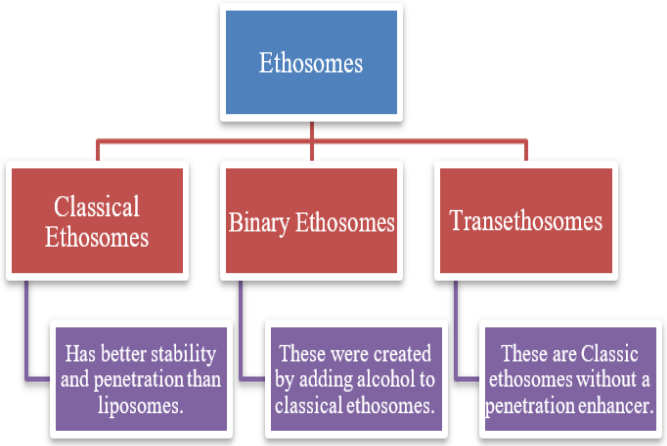



**Fig. 2 Classification of Ethosomes**

### Ethanol Effect

Alcohol acts as a penetration booster via the skin. Its increasing effect on penetration has a well-known mechanism. In addition to increasing the fluidity of cell membrane lipids and lowering the density of the lipid multilayer of the cell membrane, ethanol also penetrates into intercellular lipids.

### Ethosomes Effect

The ethosomal ethanol increases the lipid fluidity in the cell membrane, which increases the skin's permeability. Thus, the ethosomes rapidly enter the deep layers of the skin where they have merged with skin lipids and release the medications into the blood's deep layer [26–28].

## METHODS

Ethosomes can be prepared in three simple and convenient ways such as cold method, hot method, classic Method and mechanical dispersion method.

### Cold Method

Phospholipid, drug, and other lipid components were dissolved in ethanol in a covered jar at room temperature by vigorous stirring using a mixer. During the stirring process, propylene glycol or some other polyol was introduced. The mixture was heated in a water bath at 300 degrees Celsius. Water was heated to 300°C in a separate vessel before being added to the mixture, which was then agitated for 5 minutes in a closed vessel. Sonication or extrusion techniques can be used to shrink the vesicle size of the ethosomal formulation to the desired level. The final step was to refrigerate the mixture. [29][30]

### Hot method

In a water bath heated to 400°C, phospholipid was dispersed in water until a colloidal solution was formed. Propylene glycol and ethanol are mixed and boiled to 400°C in a separate vessel. At 400 degrees Celsius, the organic phase is introduced to the aqueous phase. Whether a medication was hydrophilic or hydrophobic determines whether it dissolves in water or ethanol. Using probing sonication or extrusion, the vesicle size of an ethosomal formulation can be decreased to the required degree.[31]

### Classic method

The medication and phospholipid were dissolved in ethanol and heated in a water bath to 30°C + 1°C. In a closed vessel, the lipid mixture was added to with double-distilled water in a thin stream while being constantly stirred at a speed of 700 rpm. Over three cycles of passing througha polycarbonate membrane using a manual extruder, the resulting vesicle solution was homogenised.[32–34]

### Mechanical dispersion method

In the round bottom flask, soy phosphatidylcholine has been dissolved in a solution of chloroform and methanol (RBF). To create a thin lipid coating on the RBF wall, the organic solvents are evaporated using a rotary vacuum evaporator above the lipid transition temperature. The deposited lipid film was then cleaned of any remaining solvent combination by placing the container's contents under vacuum for the night. By spinning the RBF at the appropriate temperature, hydration was carried out with various concentrations of a hydroethanolic mixture containing a medication.[35][36]

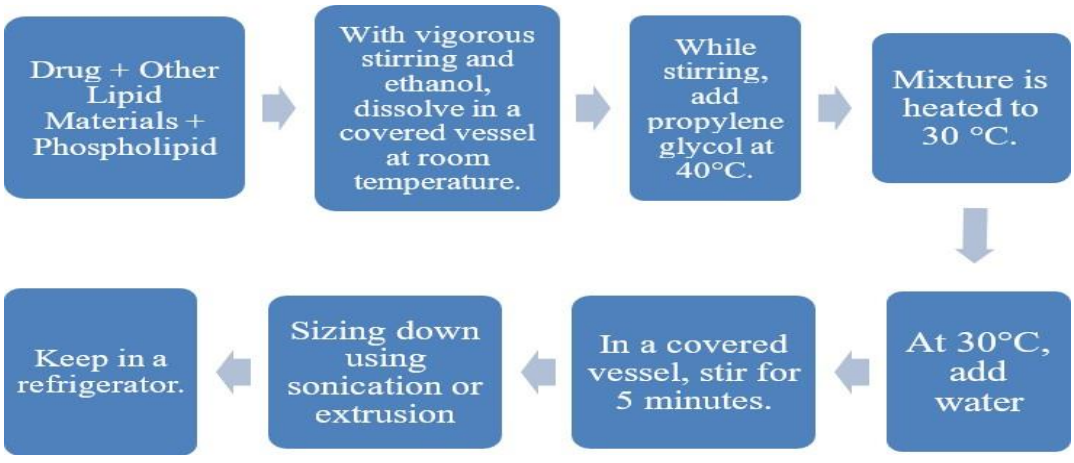



**Fig 3. General Preparation of Ethosomes**

## CHARACTERIZATION OF ETHOSOMES

### Vesicle shape

The surface morphology of the ethosomal vesicles is studied using Transmission electron

microscopy (TEM) and Scanning electron microscopy (SEM). The ethosomes should be mounted onto double-sided tape that has been coated with platinum, fastened to copper stubs, and then subjected to various magnifications of examination.[37]

### Vesicle size and Zeta potential

The two procedures used to examine the particle size and zeta potential of prepared Ethosomes are dynamic light scattering (DLS) utilising a computerised inspection system and photon correlation spectroscopy (PCS).[38]

### Entrapment Efficiency

The most often used approach for measuring the entrapment effectiveness of ethosomes using ultracentrifugation. The vesicles were separated in a high-speed cooling centrifuge for 90 minutes while the temperature is kept at 4°C, spinning at 20,000 rpm. To determine the amount of drug in the sediment, separate the liquid supernatant from the sediment by lysing the vesicles in methanol. Calculate the entrapment efficiency using the following equation,

$$\text{Entrapment efficiency} = \frac{\text{DE}}{\text{DT}} \times 100$$

Where, DE - Amount of drug in the ethosomal sediment DT - Theoretical amount of drug used to prepare the formulation (Equal to amount of drug in supernatant liquid and in the sediment)[39]

### Penetration and Permeation Studies

The depth of penetration from Ethosomes was determined using the Confocal laser scanning microscopy (CLSM) approach. The ethosomes have much higher skin deposition, which could be attributed to the combination impact of ethanol and phospholipid, offering a pathway for dermal and transdermal distribution.[40]

### Transition Temperature

DSC was used to measure the transition temperature (T) of vesicular lipids in duplicate in an aluminium pan at a heating rate of 10°C per minute in a continuous nitrogen stream. The ring method was used to figure out the activity of a drug's surface tension in an aqueous solution.[41]

### Vesicle Stability

The drug-retentive behaviour of ethosomal preparations can be tested by keeping them at different temperatures, such as 25±2°C (room temperature, RT), 37±2°C, and 45±2°C, for different amounts of time (1, 20, 40, 60, 80 and 120 days). After being flushed with nitrogen, the ethosomal preparations were kept in sealed 10 ml vials. The quantitative stability of ethosomes was also assessed by measuring the size and appearance of the vesicles using DLS and TEM.[42]

### Drug Content

Using a modified high performance liquid chromatographic approach, drugs can be measured.

## EVALUATION OF ETHOSOMES

### Vesicle skin interaction study

Various visualisation tools, for example, for evaluating the process of better skin permeability of ethosomal formulations. Transmissionelectron microscopy, eosin-hematoxyl staining, fluorescence microscopy, and laser scanning microscopy (CSLM) were all employed. Visualization approaches improved comprehension of modification of structure and vesicle penetration paths. The original liposome barely entered the upper layer of skin (stratum corneum). Liposomes free of alcohol had almost no deep penetration. Using an ethosomal carrier enhanced the depth and quantity of 6-CF and Rhodamine 123 dispersion (dermis-layer).[43]

### Filter membrane-vesicle interaction study by scanning Electron microscopy

This entails putting vesicle suspension (0.2 ml) to filter membranes with pore sizes of 50 nm and placing them in diffusion cells. The filter's upper side was exposed to air, while the lower side was in touch with phosphate buffer saline solution (having pH6.5). After 1 hour, the filters were prepared off and fixed overnight at 4°C in Karnovsky's fixative. The next day, they were dehydrated with ethanol solutions that were 30%, 50%, 70%, 90%, 95%, and 100% v/v in water.[44]

### Skin permeation studies

With a pair of scissors and a knife, the abdominal skin of test animals (rats) was meticulously peeled from the underlying connective tissue.The excised skin was carefully teased off the dermal side to remove any adhering fat and/or subcutaneous tissue. The volume permeation regions of the receptor cell and effective diffusion cell were 10 ml and 1.0 $cm^2$, respectively. It was maintained at 32°C, plus or minus 1°C. The receptor compartment was filled with phosphate-buffered saline (10 ml pH 6.5). Between the donor and the receptor compartment, it sandwiched removed skin. Ethosomal formulation was applied to the skin's epidermal layer (1.0 ml). Through the diffusion cell's sampling port, samples (0.5 ml) were collected at intervals of 1, 2, 4, 8, 12, 16, 20 and 24 hours, and they were then analysed using a high-performance liquid chromatography assay.[45]

### Stability study

The stability of the vesicles was determined by the vesicles being held at 4°C ± 0.5°C. The vesicle size, zeta potential, and trapping efficiency were calculated after 180 days using the method previously stated.[46]

### Drug uptake studies

In 24-well plates with 100µl of RPMI medium, the drug was taken up by MT-2 cells (1,1106 cells/ml). Cells were incubated with 100 µl of the drug solution in phosphate buffer saline solution (pH7.4), ethosomal formulation, or advertised formulation. The drug absorption was then calculated by analysing the drug material with an HPLC assay.[47]

### HPLC assay

The amount of drug that penetrated into the receptor compartment during in-vitro skin permeation tests and in the MT-2 cell was measured using an HPLC assay with methanol, distilled water, and acetonitrile as the mobile step.[48]

### Statistical analysis

ANOVA was used to assess the statistical significance of all the generated data, and then studentized range testing was performed. A confidence level of $P < 0.05$ was established for the results using the PRISM programme.[49]

## APPLICATIONS OF ETHOSOMES

Ethosomes offers an effective way to deliver a wide range of drug compounds through the skin. Some of the drugs formulated in the form of ethosomes were listed.[50–53]

| Targeted Delivery | Drugs | Applications |
|---|---|---|
| Viral and Fungal Infections | Acyclovir, Griseofulvin | Decreases dose-related side effects while increasing percutaneous penetration. |
| Hormone Delivery | Testosterone | Increased testosterone bioavailability by bypassing the hepatic first pass metabolism. |

| | | |
|---|---|---|
| For Parkinson Disease | Trihexphenidyl (THP) | The oral drug delivery method's recurring dosage and geriatric patient issues are avoided with topical THP administration. |
| For Arthritis | Cannabidol | The biological activity, accumulation, and skin permeation of the drug were all enhanced by the ethosomes. |
| Menopausal Syndrome | Buspirone hydrochloride (BH) | Enhance BH's skin permeability to increase bioavailability. |
| Pilosebaceous disorders | Minoxidil | Specifically targeting medicine distribution to hair follicles to treat pilosebaceous disease. |
| Anti-inflammatory | Diclofenac | Drug delivery to a targeted side for an extended period of time. |
| Anti-tumour | 5-flurouracil | In cancer models, tumour growth is significantly suppressed. |

**Table1. Applications of Ethosomes**

## MARKETED PRODUCTS OF ETHOSOMES

The first product made with ethosome technology that was sold to the public was in 2000, and most of the products that have been sold so far are cosmetics.[54 - 55]

| Marketed Product | Active Constituent | Manufacturer |
|---|---|---|
| Decorin cream | Glycosaminoglycan | Genome Cosmetics, Pennsylvania, US |
| Supravir cream | Acyclovir | Trima, Israel |
| Testoderm | Testosterone | Alza, Pakistan |
| Nanominox | Minoxidil (4%) | Sinere, Germany |

**Table2. Marketed product of ethosomes**

## CONCLUSION

The stratum corneum (SC) is the principal barrier for drug penetration into the skin. Ethosomes are a vesicular system that is specially designed to store a large amount of ethanol, rendering them flexible and dexterous enough to fluidize and penetrate the lipids of stratum corneum, resulting in efficient drug delivery into the deeper layer of skin. In dermatology and cosmetics, ethosomes have gained appeal because to their superior deformability, encapsulation efficiency, and skin penetrability. Always, skin permeation was achieved, but additional research is required to comprehend ethosomes for topical or systemic drug delivery. Future study should focus on clarifying the process of ethosome penetration, the therapeutic efficacy, and the cutaneous and systemic bioavailability of the encapsulated therapeutic active components agents in order to increase the commercialization of ethosomal-based formulations.

## REFERENCES


[1] H. F. Salem, S. F. El-Menshawe, R. A. Khallaf, and Y. K. Rabea, "Correction to: A novel transdermal nanoethosomal gel of lercanidipine HCl for treatment of hypertension: optimization using Box-Benkhen design, in vitro and in vivo characterization (Drug Delivery and Translational Research, (2020), 10, 1, (227-240), 10.1," Drug Delivery and Translational Research, vol. 10, no. 1, pp. 296–297, 2020, doi: 10.1007/s13346-019-00689-0.

[2] P. Verma and K. Pathak, "Nanosized ethanolic vesicles loaded with econazole nitrate for the treatment of deep fungal infections through topical gel formulation," Nanomedicine: Nanotechnology, Biology, and Medicine, vol. 8, no. 4, pp. 489–496, 2012, doi: 10.1016/j.nano.2011.07.004.

[3] U.S. Suma, S. Parthiban, and G. P. Senthil Kumar, "Formulation and evaluation of niosomal gel for transdermal delivery of lamivudine," World Journal of Pharmaceutical Research, vol. 5, no. 5, pp. 1332–1342, 2016.

[4] C. Sinico and A. M. Fadda, "Vesicular carriers for dermal drug delivery," Expert Opinion on Drug Delivery, vol. 6, no. 8, pp. 813–825, 2009, doi: 10.1517/17425240903071029.

[5] C. Bellefroid, A. Lechanteur, B. Evrard, D. Mottet, F. Debacq-Chainiaux, and G. Piel, "In vitro skin penetration enhancement techniques: A combined approach of ethosomes and microneedles," International Journal of Pharmaceutics, vol. 572, p. 118793, 2019, doi: 10.1016/j.ijpharm.2019.118793.

[6] P. Verma and K. Pathak, "Therapeutic and cosmeceutical potential of ethosomes: An overview," Journal of Advanced Pharmaceutical Technology and Research, vol. 1, no. 3, pp. 274–282, 2010, doi: 10.4103/0110-5558.72415.

[7] D. Mohanty, A. Mounika, V. Bakshi, M. Akiful Haque, and C. Keshari Sahoo, "Ethosomes: A Novel Approach For Transdermal Drug Delivery," International Journal of ChemTech Research, vol. 11, no. 8, pp. 219–226, 2018, doi: 10.20902/ijctr.2018.110826.

[8] A. Manosroi, P. Jantrawut, N. Khositsuntiwong, W. Manosroi, and J. Manosroi, "Novel elastic nanovesicles for cosmeceutical and pharmaceutical applications," Chiang Mai Journal of Science, vol. 36, no. 2, pp. 168–178, 2009.

[9] R. Valjakka-Koskela, M. Kirjavainen, J. Mönkkönen, A. Urtti, and J. Kiesvaara, "Enhancement of percutaneous absorption of naproxen byphospholipids," International Journal of Pharmaceutics, vol. 175, no. 2, pp. 225– 230, Dec. 1998, doi: 10.1016/S0378- 5173(98)00285-3.

[10] P. Patrekar, S. Inamdar, … S. M.-T. P., and undefined 2015, "Ethosomes as novel drug delivery system: A review," The Pharma Innovation Journal, vol. 4, no. 9, pp. 10–21, 2015, [Online]. Available: www.thepharmajournal.com

[11] I. M. Abdulbaqi, Y. Darwis, N. A. K. Khan, R. A. Assi, and A. A. Khan, "Ethosomal nanocarriers: The impact of constituents and formulation techniques on ethosomal properties, in vivo studies, and clinical trials," International Journal of Nanomedicine, vol. 11. Dove Medical Press Ltd., pp. 2279–2304, May 25, 2016. doi: 10.2147/IJN.S105016.

[12] J. Marto et al., "Ethosomes for enhanced skin delivery of griseofulvin," Colloids and Surfaces B: Biointerfaces, vol. 146, pp. 616–623, Oct. 2016, doi: 10.1016/j.colsurfb.2016.07.021.

[13] Y. S. Liu, C. F. Wen, and Y. M. Yang, "Development of ethosome-like catanionic vesicles for dermal drug delivery," J Taiwan Inst Chem Eng, vol. 43, no. 6, pp. 830–838, Nov. 2012, doi: 10.1016/j.jtice.2012.06.008.

[14] D. Aggarwal and U. Nautiyal, "Ethosomes: A review," International Journal of Pharmaceutical and Medicinal Research, vol. 4, no. 4, pp. 354–363, 2016, [Online]. Available: www.ijpmr.org

[15] S. Bansal, C. Prasad Kashyap, G. Aggarwal, and S. Harikumar, "Ijrpc 2012, 2(3) International Journal of Research in Pharmacy and Chemistry a Comparative Review on Vesicular Drug Delivery System and Stability Issues," Ijrpc, vol. 2, no. 3, pp. 704–713, 2012, [Online]. Available: www.ijrpc.com

[16] V. K. Shahwal, A. Samnani, B. K. Dubey, and M. Bhowmick, "ETHOSOMES: AN OVERVIEW," International Journal of Biomedical and Advance Research, vol. 2, no. 5, pp. 159–166, Oct. 2011, doi: 10.7439/ijbar.v2i5.31.

[17] S. R. Zahid, N. Upmanyu, S. Dangi, S. K. Ray, P. Jain, and G. Parkhe, "Ethosome: a novel vesicular carrier for transdermal drug delivery," Journal of Drug Delivery and Therapeutics, vol. 8, no. 6, pp. 318–326, Nov. 2018, doi: 10.22270/jddt.v8i6.2028.

[18] S. K. Rada and S. Yadav, "Ethosomes: A Novel Approach in Vesicular Drug Delivery Systems," Journal of Pharmaceutical Research International, pp. 62–71, Apr. 2022, doi: 10.9734/jpri/2022/v34i28b36036.

[19] R. Rakesh and K. R. Anoop, "Formulation and optimization of nano-sized ethosomes for enhanced transdermal delivery of cromolyn sodium," Journal of Pharmacy and Bioallied Sciences, vol. 4, no. 4, pp. 333–340, Oct. 2012, doi: 10.4103/0975-7406.103274.

[20] L. Yang, L. Wu, D. Wu, D. Shi, T. Wang, and X. Zhu, "Mechanism of transdermal permeation promotion of lipophilic drugs by ethosomes," International Journal of Nanomedicine, vol. 12, pp. 3357–3364, 2017, doi: 10.2147/IJN.S134708.

[21] F. Sherin, F. Saju, A. Jagadeesh, S. Syamasree, N. Paul, and A. Venugopal, "Ethosome: A novel approach to enhance drug permeation," International Journal of Pharmaceutical Sciences Review and Research, vol. 55, no. 1, pp. 18–22, 2019, [Online]. Available: http://globalresearchonline.net/journalcontents/v55-1/04.pdf%0Ahttp://ovidsp.ovid.com/ovidweb.cgi?T=JS&PAGE=reference&D=emed20&NEWS=N&AN=2001815215

[22] P. Carter, B. Narasimhan, and Q. Wang, "Biocompatible nanoparticles and vesicular systems in transdermal drug delivery for various skin diseases," International Journal of Pharmaceutics, vol. 555, pp. 49–62, Jan. 2019, doi: 10.1016/j.ijpharm.2018.11.032.

[23] M. A. Bolzinger, S. Briançon, J. Pelletier, and Y. Chevalier, "Penetration of drugs through skin, a complex rate-controlling membrane," Current Opinion in Colloid and Interface Science, vol. 17, no. 3. pp. 156–165, Jun. 2012. doi: 10.1016/j.cocis.2012.02.001.

[24] M. Sala, R. Diab, A. Elaissari, and H. Fessi, "Lipid nanocarriers as skin drug delivery systems: Properties, mechanisms of skin interactions and medical applications," International Journal of Pharmaceutics, vol. 535, no. 1–2. Elsevier B.V., pp. 1–17, Jan. 15, 2018. doi: 10.1016/j.ijpharm.2017.10.046.

[25] X. Wu and R. H. Guy, "Applications of nanoparticles in topical drug delivery and

in cosmetics," Journal of Drug Delivery Science and Technology, vol. 19, no. 6, pp. 371–384, 2009, doi: 10.1016/S1773-2247(09)50080-9.

[26] J. L. M. Heeremans et al., "The preparation of tissue-type plasminogen activator (t-PA) containing liposomes: Entrapment efficiency and ultracentrifugation damage," Journal of Drug Targeting, vol. 3, no. 4, pp. 301–310, 1995, doi: 10.3109/10611869509015959.

[27] E. Touitou, N. Dayan, L. Bergelson, B. Godin, and M. Eliaz, "Ethosomes - Novel vesicular carriers for enhanced delivery: Characterization and skin penetration properties," Journal of Controlled Release, vol. 65, no. 3, pp. 403–418, Apr. 2000, doi: 10.1016/S0168-3659(99)00222-9.

[28] A. C. Paiva-Santos et al., "Ethosomes as Nanocarriers for the Development of Skin Delivery Formulations," Pharmaceutical Research, 2021, doi: 10.1007/s11095-021-03053-5.

[29] "Ethosomes : A Novel Vesicular Carrier For Enhanced Transdermal Delivery Of An AntiHIV Agent | Abstract." https://www.ijpsonline.com/abstract/ethosomes--a-novel-vesicular-carrier-for-enhanced-transdermal-delivery-of-an-antihiv-agent-611.html (accessed Aug. 14, 2022).

[30] D. D. Verma and A. Fahr, "Synergistic penetration enhancement effect of ethanol and phospholipids on the topical delivery of cyclosporin a," Journal of Controlled Release, vol. 97, no. 1, pp. 55–66, May 2004, doi: 10.1016/j.jconrel.2004.02.028.

[31] "US5716638A - Composition for applying active substances to or through the skin - Google Patents." https://patents.google.com/patent/US5716638A/en (accessed Aug. 14, 2022).

[32] "Novel Elastic Nanovesicles for Cosmeceutical and Pharmaceutical Applications | Request PDF." https://www.researchgate.net/publication/282481313_Novel_Elastic_Nanovesicles_for_Cosmeceutical_and_Pharmaceutical_Applications (accessed Aug. 14, 2022).

[33] L. Tyagi, S. Kumar, S. S. Maurya, and M. L. Kori, "ETHOSOMES: NOVEL VESICULAR CARRIER FOR ENHANCED TRANSDERMAL DRUG DELIVERY SYSTEM," undefined, 2013.

[34] V. Dubey, D. Mishra, and N. K. Jain, "Melatonin loaded ethanolic liposomes: Physicochemical characterization and enhanced transdermal delivery," European Journal of Pharmaceutics and Biopharmaceutics, vol. 67, no. 2, pp. 398–405, Sep. 2007, doi: 10.1016/j.ejpb.2007.03.007.

[35] V. Dubey, D. Mishra, T. Dutta, M. Nahar, D. K. Saraf, and N. K. Jain, "Dermal and transdermal delivery of an anti-psoriatic agent via ethanolic liposomes," Journal of Controlled Release, vol. 123, no. 2, pp. 148–154, Nov. 2007, doi: 10.1016/j.jconrel.2007.08.005.

[36] S. Jain, A. K. Tiwary, B. Sapra, and N. K. Jain, "Formulation and evaluation of ethosomes for transdermal delivery of Lamivudine," AAPS PharmSciTech, vol. 8, no. 4, Dec. 2007, doi: 10.1208/pt0804111.

[37] A. Ahad et al., "Formulation and characterization of novel soft nanovesicles for enhanced transdermal delivery of eprosartan mesylate," Saudi Pharmaceutical Journal, vol. 25, no. 7, pp. 1040–1046, Nov. 2017, doi: 10.1016/j.jsps.2017.01.006.

[38] A. Ahad, M. Aqil, K. Kohli, Y. Sultana, and M. Mujeeb, "Enhanced transdermal delivery of an anti-hypertensive agent via nanoethosomes: Statistical optimization,

characterization and pharmacokinetic assessment," International Journal of Pharmaceutics, vol. 443, no. 1–2, pp. 26–38, Feb. 2013, doi: 10.1016/j.ijpharm.2013.01.011.

[39] K. Raza, B. Singh, N. Singla, P. Negi, P. Singal, and O. P. Katare, "Nano-lipoidal carriers of isotretinoin with anti-aging potential: Formulation, characterization and biochemical evaluation," Journal of Drug Targeting, vol. 21, no. 5, pp. 435–442, May 2013, doi: 10.3109/1061186X.2012.761224.

[40] I. M. Abdulbaqi, Y. Darwis, R. A. Assi, and N. A. K. Khan, "Transethosomal gels as carriers for the transdermal delivery of colchicine: Statistical optimization, characterization, and ex vivo evaluation," Drug Design, Development and Therapy, vol. 12, pp. 795–813, 2018, doi: 10.2147/DDDT.S158018.

[41] S. G. miyanda petty M, Priyanka S, "Ethosomes : A Potential Transdermal Drug Delivery System-In- Depth Review," vol. 11, no. 1, pp. 14–45, 2017, [Online]. Available: http://ijppr.humanjournals.com/wp-content/uploads/2018/01/2.Miyanda-Petty-M-Priyanka-S-Surya-Gautam.pdf

[42] A. C. Apolinário, L. Hauschke, J. R. Nunes, F. R. Lourenço, and L. B. Lopes, "Design of multifunctional ethosomes for topical fenretinide delivery and breast cancer chemoprevention," Colloids and Surfaces A: Physicochemical and Engineering Aspects, vol. 623, no. May, 2021, doi: 10.1016/j.colsurfa.2021.126745.

[43] C. Delivery, "Quality by Design for Development , Optimization and," 2021.

[44] D. Prasanthi and P. K. Lakshmi, "Development of ethosomes with taguchi robust design-based studies for transdermal delivery of alfuzosin hydrochloride," International Current Pharmaceutical Journal, vol. 1, no. 11, pp. 370–375, 2012, doi: 10.3329/icpj.v1i11.12063.

[45] M. R. Peram et al., "Factorial design based curcumin ethosomal nanocarriers for the skin cancer delivery: in vitro evaluation," Journal of Liposome Research, vol. 29, no. 3, pp. 291–311, 2019, doi: 10.1080/08982104.2018.1556292.

[46] N. Akhtar and K. Pathak, "Cavamax W7 composite ethosomal gel of clotrimazole for improved topical delivery: Development and comparison with ethosomal gel," AAPS PharmSciTech, vol. 13, no. 1, pp. 344–355, 2012, doi: 10.1208/s12249-012-9754-y.

[47] S. Ahmed, S. Sarim Imam, A. Zafar, A. Ali, M. Aqil, and A. Gull, "In vitro and preclinical assessment of factorial design based nanoethosomes transgelformulation of an opioid analgesic," Artificial Cells, Nanomedicine and Biotechnology, vol. 44, no. 8, pp. 1793– 1802, 2016, doi: 10.3109/21691401.2015.1102742.

[48] M. Sguizzato et al., "Ethosomes and transethosomes for mangiferin transdermal delivery," Antioxidants, vol. 10, no. 5, 2021, doi: 10.3390/antiox10050768.

[49] V. Garg et al., "Systematic Development of Transethosomal Gel System of Piroxicam: Formulation Optimization, In Vitro Evaluation, and Ex Vivo Assessment," AAPS PharmSciTech, vol. 18, no. 1, pp. 58–71, 2017, doi: 10.1208/s12249-016-0489-z.

[50] I. M. Abdulbaqi, Y. Darwis, N. A. K. Khan, R. A. Assi, and A. A. Khan, "Ethosomal nanocarriers: The impact of constituents and formulation techniques on ethosomal properties, in vivo studies,

and clinical trials," International Journal of Nanomedicine, vol. 11. Dove Medical Press Ltd., pp. 2279–2304, May 25, 2016. doi: 10.2147/IJN.S105016.

[51] G. W. Lu, K. S. Warner, and F. Wang, "Development of Pilosebaceous Unit-Targeted Drug Products," in Topical Drug Bioavailability, Bioequivalence, and Penetration, Springer New York, 2014,pp. 181–215. doi: 10.1007/978-1-4939-1289-6_12.

[52] A. S. Dobs, A. W. Meikle, S. Arver, S. W. Sanders, K. E. Caramelli, and N. A. Mazer, "Pharmacokinetics, Efficacy, and Safety of a Permeation-Enhanced Testosterone Transdermal System in Comparison with Bi-Weekly Injections of Testosterone Enanthate for the Treatment of Hypogonadal Men*," The Journal of Clinical Endocrinology & Metabolism, vol. 84, no. 10, pp. 3469–3478, Oct. 1999, doi: 10.1210/jcem.84.10.6078.

[53] M. I. Mohammed, A. M. A. Makky, M. H. M. Teaima, M. M. Abdellatif, M. A. Hamzawy, and M. A. F. Khalil, "Transdermal delivery of vancomycin hydrochloride using combination of nano-ethosomes and iontophoresis: in vitro and in vivo study," Drug Delivery, vol. 23, no. 5, pp. 1558–1564, Jun. 2016, doi: 10.3109/10717544.2015.1013200.

[54] C. C. Mbah, P. F. Builders, and A. A. Attama, "Nanovesicular carriers as alternative drug delivery systems: Ethosomes in focus," Expert Opinion on Drug Delivery, vol. 11, no. 1. Informa Healthcare, pp. 45–59, 2014. doi: 10.1517/17425247.2013.860130.

[55] K. Pavan Kumar, P. R. Radhika, and T. Sivakumar, "Ethosomes-a priority in transdermal drug delivery," International Journal of Advances in Pharmaceutical Sciences, vol. 1, no. 2. pp. 111–121, 2010. doi: 10.5138/ijaps.2010.0976.1055.01012.