ISSN: 2357-0547 (Print)
ISSN: 2357-0539 (Online)

Review Article / JAPR / Sec. C
Shetty and Miranda, 2024, 8 (4), 205-217

Journal of Advanced Pharmacy Research

Section C: Drug Design, Delivery and Targeting

Ethosomes: The Upgraded Liposomes – A Review

Bhuvankumar Manjunath Shetty*, Fmith Celvia Miranda
Department of Pharmaceutics, Srinivas College of Pharmacy, Mangalore, Karnataka, India- 574143

***Corresponding author:** Bhuvankumar Manjunath Shetty, Department of Pharmaceutics, Srinivas College of Pharmacy, Mangalore, Karnataka, India- 574143 Tel.: +919611743278*

E-mail address: bhuvankumar7673@gmail.com

Submitted on: 05-06-2024; Revised on: 01-08-2024; Accepted on: 23-08-2024

To cite this article: Bhuvankumar Manjunath Shetty, B. M.; Miranda, C. M. Ethosomes: The Upgraded Liposomes – A Review. *J. Adv. Pharm. Res.* **2024**, *8* (4), 205-217. DOI: 10.21608/aprh.2024.295469.1274

ABSTRACT

The shortcomings of the conventional drug delivery systems necessitate the need to use other routes. On account of its merits over other routes, the transdermal approach has drawn the interest of investigators globally. Transdermal drug delivery systems aim to achieve therapeutic concentrations of the drug through skin. Ethosomal systems are unique lipid vesicular transporters that contain comparatively high ethanol content. The efficient distribution of drugs with various physicochemical characteristics into deep skin layers and across the skin is the specific purpose of these nanocarriers. Since their invention in 1996, a great deal of study has been done on ethosomes; new compounds have been introduced to their core formula, resulting in the development of new types of ethosomal systems. Various methods of preparation are employed for developing these innovative carriers. This review offers an in-depth investigation of ethosomal systems, classifying them into three categories: classical ethosomes, binary ethosomes, and transethosomes, based on the components of each system. The formulation, size, zeta potential, entrapment efficiency, skin-permeation properties, stability, and other distinctions between these systems are studied. The effects of ethosomal system components, preparation techniques, and their important roles in defining the final properties of these nanocarriers are reviewed in detail in this work.

Keywords: *Ethosomes, Transdermal delivery, Vesicular carrier, Ethanol*

INTRODUCTION

Compared to conventional medication delivery techniques, such as oral and parenteral drug administration methods, transdermal drug delivery has numerous benefits. The oral route is the most widely used method of medication delivery, however it has limitations such first-pass metabolism, frequent dosage, and high cost.[1,2] Oral medication administered conventionally is frequently linked to various kinds of allergic responses, adverse effects, and low therapeutic efficacy. Furthermore, continuous intravenous infusion is acknowledged as the best method of drug administration for parenteral drug delivery systems since it avoids hepatic first-pass metabolism and keeps the drug level in the body stable for an extended period of time. Nevertheless, there are hazards associated with this kind of drug delivery, necessitating hospitalisation of the patients and close medical care during administration.[3,4,5]

A novel drug delivery method that enhances the therapeutic efficacy, safety, and stability of medications is required to get over all of these challenges. 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. Stoughton R. B. [6] originally proposed the idea of percutaneous absorption of pharmacological compounds in 1965, which helped to establish the transdermal drug delivery system (TDDS).[2,7]

The potential advantages of transdermal medication administration are extensively documented in the literatures. In recent times, it has been evident that the advantages of intravenous medication infusion can be nearly replicated without the risks involved by administering drugs transdermally into the systemic arteries through the skin.[3,8]

SKIN AND DRUG PERMEATION

It is crucial to examine the structural and biochemical aspects of human skin, as well as the characteristics that affect medication absorption through the skin and the barrier function, in order to fully understand the idea of transdermal drug delivery systems. One of the human body's most prominently smooth and all-around organs, the skin can deliver drugs with a number of benefits over more conventional drug delivery methods, such as minimal changes in plasma drug levels, protection from gastrointestinal issues and drug metabolism at the first pass, and high compliance rates.[3,9]

For an average adult one of the largest organs is the skin, which covers an area of approximately 2m². The skin serves as a barrier against microbial, chemical, and physical attacks, regulates blood pressure, protects against UV radiation from the sun, and serves as a thermostat to regulate body temperature. It also isolates the basal blood circulation system from the external environment.[3]

The epidermis, dermis, and hypodermis are the three main tissue layers that collectively make up the skin's anatomy, while there are many more layers beneath the surface. The horny layer, also known as the stratum corneum, is the rate-limiting barrier that prevents chemicals from moving both inward and outward. Numerous studies have reported on a variety of mechanisms to get past the stratum corneum barrier, such as the use of encapsulation technologies like liposomes, niosomes, transferosomes, and ethosomes, or chemical or physical enhancers like sonophoresis, iontophoresis, surfactants, permeation enhancers, and lipid-based systems.[3,9,10]

TRANSDERMAL DRUG DELIVERY SYSTEM:

Recently, transdermal medication delivery systems have been designed with the purpose of systemic medicine applied topically to intact skin surface. The definition of a transdermal therapeutic system is a discrete, self-contained dose forms that allows the medication to be delivered via the skin at a regulated rate to the systemic circulation.[11]

Only lipophilic drugs with a molecular weight less than 500 Da can pass through the stratum corneum, which is a barrier that the transdermal drug delivery system confronts. Transdermal drug delivery (TDD) has some other therapeutic benefits, such as sustained drug delivery to provide a steady state plasma profile and hence reduced systemic side effects, and the bypass of first pass metabolism effect for drug with poor oral bioavailability. Drugs having a short biological half-life period can be delivered via the transdermal route to keep a steady drug concentration within the therapeutic range. All of this promotes better patient compliance, particularly in situations when long-term care is needed, such as in hormonal therapy, pain management, hypertension and smoking cessation therapy.[11,12]

The topical delivery of triamcinolone using liposomes brought forth a new age of research in this area, leading to the development of numerous unique lipid-based vesicular systems. The most common lipid dosage forms for transdermal distribution are liposomes and its derivatives, including transferosomes, niosomes, and ethosomes. Because of their excellent transdermal penetration rate, high entrapment efficiency, and high deformability, ethosomes have gained significant attention as a novel liposome carrier in recent years.[4,12,13]

ETHOSOMES:

Touitou et al. 1996 made the discovery and developed ethosomes. Ethosomes are ethanolic liposomes. They can be characterised as innovative, non-invasive delivery systems that facilitate the passage of medications through and into deep skin layers and/or the systemic circulation. These pliable, soft vesicles are made to improve the distribution of active substances. Since ethanol and lipid concentrations in ethosomes are higher, it is essential to understand how these entities affect the skin. It has been observed that the formulation's ethanol content enhanced drug solubilization and the formation of lipid structures that were easily flexible enough to pass between skin corneocytes, improving medication penetration and skin retention.[13,14,15]

Ethosomes are vesicles with a size range of 30 nm to several microns which are soft and pliable. According to reports, when made using the same procedure without employing size-reduction step, ethosomes are smaller than liposomes in size. The size is reduced because of the high alcohol level, and it gets smaller as the concentration of ethanol rises nearly to 20–45%. The net negative charge of the ethosomes comes from ethanol, which minimizes their size. The addition of 30% ethanol caused the charge of the vesicles to change from positive to negative.[16] The zeta potential of ethosomes, which is a measure of the surface charge, can influence their interaction with the skin. Although a high negative charge might suggest repulsion, the

dynamic nature of the skin's lipid environment and the ethosome's ability to deform can facilitate their penetration despite this charge.[17] The high concentration of ethanol in ethosomes enhances their flexibility and deformability, allowing them to squeeze through the intercellular spaces of the stratum corneum, which is the outermost layer of the skin. This flexibility is crucial for overcoming the skin barrier without causing drug leakage during the process.[18,19]

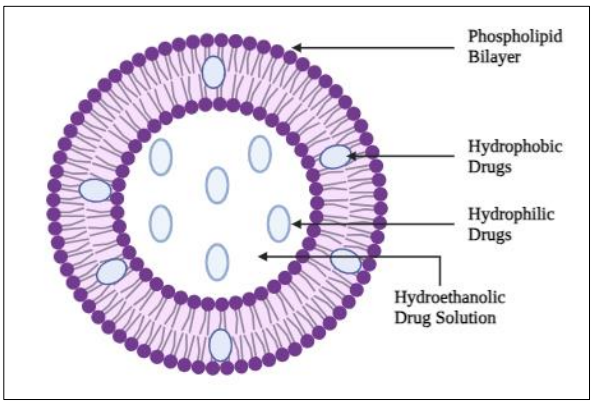

Figure 1. Structure of Ethosome

Advantages of Ethosomes

1. The ethosome improves the permeation of drugs through skin corneocytes in an efficient manner due to the complex interplay of ethanol's effects on lipid fluidity, the flexible nature of the vesicles.
2. The ethosome provides drug solubility in both lipophilic and hydrophilic phase to achieve microcirculation of the dermal and to obtain access to circulation.
3. Achieve delivery of various molecules with different physicochemical properties, hydrophilic and lipophilic molecules, peptides, proteins and other macromolecules.
4. The composition of the ethosomes is generally accepted as safe, non-toxic and approved for pharmaceutical, biotechnology, veterinary, cosmetic & nutraceutical fields.
5. The ethosomal system is passive, non-invasive, and suitable for quick commercialization.
6. High patient compliance: The ethosomal drug is delivered in a semi-solid form (gel or cream) with high patient adherence.
7. Simple and economical methods for drug delivery over iontophoresis, sonophoresis and other complicated methods.
8. Ease of industrial scale-up: comparatively easy to produce without any expensive technical investments required for production.
9. Higher entrapment efficiencies of drugs are reported compared to liposomes for both hydrophilic and hydrophobic drugs.
10. It exhibits excellent stability over prolonged periods.
11. Alcohol in the ethosomes acts as a natural preservative, and hence there is no necessity to incorporate any other preservative.
12. Drug delivery through the skin is independent of concentration [8,13].

Disadvantages of Ethosomes:

1. There are possibilities of allergic reactions if the patients are allergic to ethanol or any of the ethosomal components.
2. In contrast to other carriers (solid lipid nanoparticles, polymeric nanoparticles, etc.), which re suitable for multiple routes, ethosomal carriers are significantly only for transdermal use.
3. Considering the fact that ethanol is inflammable, the utmost care should be taken during planning, application, transport and storage.
4. Very poor yield, so may not be cost effective.
5. Probability of product loss when transferring from the organic phase to the aqueous phase.
6. It is confined to potent drug molecules, either long- or short-acting, that need a daily dosage.
7. The drug's molecular size should be appropriate for it to be absorbed percutaneously.
8. Excipients and penetration enhancers in drug delivery systems have the potential to cause dermatitis or skin irritation.[8,13]

ETHOSOMAL SYSTEM TYPES[13,17,20]

1. Classical Ethosomes

Classical ethosomes consist of phospholipids, water, and a high concentration of ethanol up to 45% w/w. They are a modification of classical liposomes. For transdermal drug delivery, classical ethosomes were found to be more effective than classical liposomes due to their smaller size, negative zeta potential, and increased entrapment efficiency. The high negative charge helps stabilize the ethosomal system by preventing aggregation of vesicles through electrostatic repulsion. It also helps in the entrapment of drugs, especially lipophilic ones, within the ethosomal vesicles.[21,22] Moreover, compared to classical liposomes, classical ethosomes show superior skin penetration and stability profiles. Drugs included in classical ethosomes have had molecular weights ranging from 130.077 Da to 24 kDa.

2. Binary Ethosomes

Zhou et al.[23] reported binary ethosomes which produced by adding an alternate type of alcohol to the classical ethosomes. Propylene glycol (PG) and isopropyl alcohol (IPA) are the two alcohols that are most frequently used in binary ethosomes.

Table 1. Comparison of classical ethosomes, binary ethosomes, and transethosomes in their initial suspension form[17]

Parameter	Classical ethosomes	Binary ethosomes	Transethosomes
Composition	1. Phospholipids 2. Ethanol 3. Stabilizer 4. Charge inducer 5. Water 6. Drug/agent	1. Phospholipids 2. Ethanol 3. Propylene glycol (PG) or other alcohol 4. Charge inducer 5. Water 6. Drug/agent	1. Phospholipids 2. Ethanol 3. Edge activator (surfactant) or penetration enhancer 4. Charge inducer 5. Water 6. Drug/agent
Morphology	Spherical	Spherical	Regular or irregular spherical shapes
Size	Smaller than the classical liposomes	Equal to or smaller than classical ethosomes	Size based on type and concentration of penetration enhancer or edge activator used
Zeta Potential	Negatively charged	Negatively charged	Positively or negatively charged
Entrapment efficiency	Higher than classical liposomes	Typically, higher than classical ethosomes	Typically, higher than classical ethosomes
Skin permeation	Typically, higher than classical liposomes	Typically, equal to or higher than classical ethosomes	Typically, higher than classical ethosomes
Stability	Stabler than classical liposomes	Stabler than classical ethosome	No particular trend determined

3. Transethosomes:

Song et al.[24] initially introduced transethosomes, the foreseeable generation of ethosomal systems, in 2012. The main components of classical ethosomes with an extra substance, like an edge activator (surfactant) or penetration enhancer, constitute this ethosomal system. In an effort to create transethosomes, these unique vesicles combined the benefits of deformable liposomes (transfersomes) and classical ethosomes into a single formulation. Transethosomes have been shown by numerous researches to have better qualities than classical ethosomes. Various kinds of penetration enhancers and edge activators have been tried to create ethosomal systems with improved properties. It has been observed that medicines with molecular weights ranging from 130.077 Da to 200–325 kDa are entrapped by transethosomes.

COMPOSITION OF ETHOSOMES:

Ethanol

Ethanol is a potent penetration enhancer. It contributes significantly to ethosomal formulation by giving the vesicles unique features such as improved skin permeability, stability, zeta potential, entrapment efficacy, and size. According to reports, the ethanol concentrations in ethosomal systems are within the range of 10% to 50%. Many researchers stated that, the vesicle size of the ethosomes would decrease when the concentration of ethanol was increased.[3,25-28] However, if the ethanol concentration were raised over the optimal level, the bilayer would become leaky, which would cause the vesicles to become more soluble in ethanol due to a slight increase in size and a large drop in entrapment efficiency.[17]

In their evaluation of tocotrienol ethosomes for transdermal delivery, *Nair RS et al.*[29] observed that when the amount of ethanol increased from 30 to 40%, the vesicle size decreased from 79.6 ± 3.9nm to 64.9 ± 2.2nm respectively. However, further increasing the ethanol concentration as high as 50% produced large vesicles. Ethanol concentrations above a certain threshold may soften the lipid bilayer within the ethosome, which compromises structural integrity and encapsulation efficiency. The encapsulation efficiencies of the ethosomes were 66.8 ± 1.9% and 68.5 ± 1.2% correspond to 30 and 40% of ethanol.

A significant factor that can affect vesicular characteristics like stability and vesicle–skin interaction is vesicular charge. The vesicular charge has changed from positive to negative due to the increased ethanol content in ethosomes. Because ethanol gives the ethosomal system's surface a negative charge, it keeps the vesicular system from aggregating as a result of electrostatic repulsion. Furthermore, it was discovered that ethanol had stabilising properties. The entrapment efficiency was shown to have a linear relationship with ethanol concentrations between 20% and 40%. Optimising the ethanol concentration throughout the formulation process is crucial because of this.[13,17]

Phospholipids

The ethosomal system has been developed using phospholipids from various sources. The selection of phospholipid type and concentration is a crucial step in the formulation of the ethosomal system, as it affects

the vesicle's size, stability, zeta potential, entrapment efficacy, and penetrating abilities.[13,14,17]

Vijayakumar KS. et al.[30] developed ethosomal systems of Gliclazide using varying percentages of soya lecithin (1%, 2%, 3%, and 4%). It was discovered that the phospholipid concentrations significantly impacted the vesicle nature, stability, drug content, entrapment efficiency, and size.

Phospholipid concentrations in ethosomal formulations typically range from 0.5% to 5%. Vesicular size will slightly or moderately increase with an increase in phospholipid concentration, while entrapment efficiency will increase significantly.[26,27,31] The link holds true, though, only up to a certain concentration, after which further increases in the concentration of phospholipids will not impact the efficiency of entrapment. This can be explained by the fact that after a certain phospholipid concentration, the vesicles become saturated with the drug. Any additional phospholipids will not be able to entrap more drug, hence the entrapment efficiency plateaus.[32] Conversely, larger vesicles were produced by higher phospholipid levels, most likely as a result of the vesicular structural thickening. Thus, the lowest possible vesicle size is achieved when phospholipid concentrations are low and ethanol levels are high.[33]

Phospholipids can be derived from a variety of sources, including natural, semisynthetic, and synthetic materials. Numerous phospholipids have been employed in the past ethosomal formulations, include Phospholipon®90, Phosphatidylcholine (PC), phosphatidylethanolamine (PE), dipalmitoyl phosphatidylcholine (DPPC), and Lipoid S100. The phospholipid that is used for skin permeation also important since it will interact with the lipid bilayer of the skin to enable the vesicle to make tiny holes in the stratum corneum (SC). As a result, selection and concentration of phospholipid during the pharmaceutical manufacturing process is essential to the ethosome's ability to successfully penetrate the skin.[14,17,33]

Cholesterol

Since cholesterol is a rigid steroid molecule, adding it to ethosomal systems increases the stability and effectiveness of active ingredient entrapment. In addition to lowering vesicular fusion and permeability, it prevents leaks. Cholesterol reduces the flexibility and permeability of membranes while controlling their fluidity. Ethosomes become more rigid when added to the mixture, so limiting the amount of the active substance that can permeate. On the other hand, because cholesterol has stabilising properties, high cholesterol concentrations improve medication entrapment efficiency.[14,17,33]

In general, it is used at a concentration of <3%. *Mousa, I.A. et al.*[26] found that an increment in the concentration of cholesterol led to an increased entrapment efficiency (EE%) and vesicle size of the metformin as well. Conversely, the percentage of drug release (%DR) and zeta potential decrease as the concentration of cholesterol increases.

Dicetyl phosphate

Dicetyl phosphate is indeed a charge inducer used in ethosomes, but its role is somewhat nuanced. While Dicetyl phosphate is incorporated into the formulation to enhance stability and prevent aggregation of ethosomes, it has minimal influence on the overall charge characteristics of the vesicles.[34,35] In the ethosomal formulation, it is utilized at concentrations ranging from 8% to 20% of the total phospholipid concentration. Dicetyl phosphate's impacts on other ethosomal system features, however, remain unknown.[13,17]

Other alcohols

In addition to ethanol, other alcohols are also utilised in the production of binary ethosomes, including PG and IPA.

Propylene glycol (PG)

The penetration enhancer PG is extensively utilised. It has been observed to affect the ethosomal features of size, entrapment efficiency, permeability, and stability when utilised in the production of binary ethosomes at concentrations between 5% and 20%. Particle size will decrease significantly in ethosomal systems when PG is added as compared to non-PG systems. Better drug solubility, higher entrapment efficiency, and improved drug distribution throughout the vesicle are the results of ethanol and PG incorporation in ethosomes.[11,15]

Zhang JP et al.[20] discovered that increasing the PG concentration from 0% to 20% v/v resulted in a significant decrease in particle size, from 103.7±0.9 nm to 76.3±0.5 nm.

Isopropyl alcohol (IPA)

Another penetration enhancer that is used in the ethosome preparation process is IPA. *Dave V et al.*[36] investigated how IPA affected an ethosomal system loaded with diclofenac's entrapment efficiency and skin penetration. Three different formulations were made: a vesicular system with 40% IPA, binary ethosomes with 20% IPA and 20% ethanol, and classical ethosomes with 40% ethanol. The one vesicular system with 40% IPA was shown to have a higher entrapment efficiency (95%) compared to the binary ethosomes (83.8%). Nevertheless, in comparison to binary ethosomes (85.4%) and classical ethosomes (93%), this vesicular system showed the lowest in vitro drug release in 8 hours (83.2%). It was determined that whereas IPA had a

significant impact on drug release, it had less of an impact on entrapment efficiency. The impact of IPA or other alcohols on other ethosomal system features needs to be investigated further.[13,17]

Edge activators and Penetration enhancers

A crucial step in the formulation of transethosomes is selecting an appropriate edge activator or penetration enhancer, as these components have a significant impact on the properties of the ethosomal system. While preparing transethosomes, a variety of edge activators and penetration enhancers are employed, with tweens and spans being the most often utilised.[13,17,37]

Tweens and Spans

In the ethosomal system, tween 80 is employed at concentrations ranging from 10% to 50% of the total phospholipid concentration. *Shen et al.*[38] observed that tween 80 in lipid vesicles could increase the fluidity of the membrane structure and soften the membrane structure. With an increase in fluidity, particle size and EE will naturally decrease. Unstable vesicles were formed when tween 60 and tween 20 were added to ethosomal systems. It was not possible to produce homogenous and stable transethosomes with spans 80, 60, and 40.[13,17]

METHODS OF PREPARATION OF ETHOSOMES:

Classical Cold Method [17,39-41]

This is the easiest and widely used technique for formulating ethosomal systems, and it can be carried out in nitrogen-protected conditions if necessary. It was first presented by Touitou in 1996 and entails preparing the aqueous and organic phases independently. The phospholipids are dissolved in ethanol or a combination of solvents (ethanol/PG) at 30°C, together with surfactants or penetration enhancers for transethosomes, to obtain the organic phase for the synthesis of binary ethosomes. Either water, buffer solution, or normal saline solution is employed as the aqueous phase. At a consistent rate of 175 or 200 µL/min, the aqueous phase is introduced to the organic phase in a fine stream, dropwise, or using a syringe pump. A magnetic stirrer is used to agitate the mixture at a rate of 700–2,000 rpm. To achieve the necessary ethosomal suspension, the mixing process is carried out for 20–30 minutes. The drug that is to be incorporated into the ethosomal system will dissolve in either the organic or aqueous phase, depending on its physicochemical characteristics. The appropriate degree of vesicle size reduction for the ethosomal formulation can be achieved by the use of sonication or extrusion techniques. The formulation is then refrigerated for storage.

Iizhar S.A. et al.,[42] investigate the entrapment of terbinafine hydrochloride (TH) in ethosomal vesicles by the classical cold method via unsonication and sonication method. Carbopol 934P was incorporated in the best formulation, F6, obtained by the sonication method. Drug entrapment efficiency (DEE) was ranked from 55.33 ± 1.32% to 69.11 ± 2.11%. The highest DEE was seen with the F6 ethosomal formulation, with a vesicle size of 248 ± 1.02 nm. *Ex-vivo* results suggested that drug diffusion observed after 12 h from F6 and marketed cream (MR) formulations was 74.01 ± 0.62% and 61.45 ± 0.86%, respectively. This study disclosed that F6 resides at the targeted site for a relatively longer period of time, thereby signifying improved patient compliance.

Hot Method[12,17,39,40]

This method was first described by the inventor of ethosomes in 1996. The hot method is a unique method to formulate stable ethosomes. In this method, the required quantity of ethanol and propylene glycol (organic phase) are taken in a separate vessel, maintaining the temperature at 40°C. In another beaker, the phospholipid is dispersed in water, and then it is kept in a water bath maintaining the temperature at 40°C, until a colloidal suspension formulation (aqueous phase) is obtained. Then the organic phase is slowly added to the aqueous phase with continued stirring with a magnetic stirrer. Based on the hydrophilic/hydrophobic properties of the drug, it is dissolved in either the organic or aqueous phase. The final formulation was subjected to sonication or extrusion method to get evenly dispersed ethosomal vesicles.

Yadav KK et al.,[43] successfully developed ethosomal vesicles of Mefenamic acid through the hot method under stirring and sonicated. In the stability study the drug content was found to be 97.85%, 98.69% and 98.43% as respectively. In vitro drug release was 94.509%, 95.957% and 88.068% respectively, as per formulation (MET1, MET2, and MET3). The entrapment efficiency of drug in vesicles was found to be 57.98%, 67.26% and 33.19% respectively.

Thin-Film Hydration Method [13,17,40,41]

This procedure is an expansion of the conventional liposome preparation technique, with the exception that a hydroethanolic solution is used to hydrate the lipid film. In a clean, dry, round-bottom flask, the phospholipid is first dissolved in either chloroform by itself or in a mixture of chloroform and methanol at ratios of 3:1 or 2:1. A rotary vacuum evaporator removes the organic solvents at a temperature higher than the lipid-phase transition temperature. Subsequently, the solvent residue is eliminated from the lipid film by vacuum for an entire night. A water-ethanol solution or a phosphate buffered saline-ethanol solution

is subsequently used to hydrate the lipid film. The phospholipid property determines the necessary temperature at which it is necessary to rotate and heat the lipid film throughout the hydration process.

Limsuwan T et al.,[27] developed the ethosome formulations containing phenylethyl resorcinol (PR) using a thin-film hydration method. The formulation was developed from 0.5% w/v PR, 0.5% w/v cholesterol from lanolin, 3% w/v L-*α*-phosphatidylcholine from soybean, 30% v/v absolute ethanol, and water up to 100% v/v. It was characterized by a vesicular size of 389 nm, a low polydispersity index of 0.266, a zeta potential of −34.19 ± 0.44 mV, a high PR entrapment efficiency of 71.43 ± 0.77%, and good stability on storage at 4°C and 30°C at 75% RH for 4 months.

Ethanol Injection–Sonication Method:[13,17]

Using a syringe system, the organic phase containing the phospholipid that has been dissolved in ethanol is injected into the aqueous phase at a rate of 200 µL/min. The mixture is then homogenised for five minutes using an ultrasonic probe.

Ma H et al.,[44] produced Paeonol-loaded ethosomal formulation using a slightly modified ethanol injection technique. The ideal paeonol-loaded ethosomes had a negative charge of −16.8 ± 0.36 mV, a vesicle size of 120.2 ± 1.3 nm, an EE of 84.33 ± 1.34%, and a PDI of 0.131 ± 0.006. The results showed that the skin retention and in-vitro transdermal absorption of paeonol from paeonol-loaded ethosomes were 52.60 ± 7.90 µg/cm2 and 138.58 ± 9.60 µg/cm2, respectively. Ethosomes have a significant potential for transdermal administration of paeonol due to their skin permeation.

Transmembrane pH-Gradient Method [17,40,41]

The transmembrane pH-gradient approach "actively" loads the drug based on the pH gradient difference between the acidic interior of the internal phase and the basic exterior of the external phase of the ethosomal system. Only water-soluble drugs with protonizable amine functionalities can be used with this technique. The three steps of this approach are production of the blank ethosomal system, active loading of the drug, and incubation.

The preparation of empty binary ethosomes and the active loading of the drug molecule are two distinct processes in this procedure. Initially, an alcoholic phase made up of PG and ethanol dissolves the phospholipid (for instance, PC). With continual stirring at 700rpm, a citrate buffer solution is progressively added to the initial solution. Throughout this procedure, the system is maintained at a temperature of around 30° C before being cooled to ambient temperature. The generation of blank binary ethosomes is now complete. In order to efficiently disperse and dissolve the drug, it is then actively incorporated into the ethosomes and the system is continually stirred at 700 rpm. This process involves two distinct steps: the formation of an empty ethosomal system's internal phase (acid) and exterior portion (alkaline), which can be separated by a pH gradient that can be generated by adjusting by introducing a 0.5 M sodium hydroxide (NaOH) solution to the pH outside. The system is then incubated in a favourable moment and climate, enabling the union. Drugs actively pass through the lipid bilayer of ethosomes and get entrapped in the vesicles. Ethosomes can effectively incorporate hydrophilic drugs into their aqueous core due to the high ethanol content. The ethanol contributes to increasing the fluidity of the lipids in the ethosomal bilayer, allowing for better encapsulation of hydrophilic compounds.[19]

Zhou et al.,[20] used transmembrane pH-gradient method in the development of ethosomal systems of total alkaloid extracts of *Sophora alopecuroides.*

SKIN PERMEATION MECHANISM OF ETHOSOMES:

Ethosomes provide substitute pathways for drugs to reach the underlying tissues. The unique structure of ethosomes can improve the permeation of drugs through the skin, using different pathways results in improved efficacy.[41] In ethosomal formulations, phospholipids and ethanol are said to work in unison to improve the active ingredients ability to penetrate the skin. Ethanol concurrently fluidizes the stratum corneum and ethosomal vesicles lipid bilayers, changing their organisation and decreasing the density of skin lipids. As a result, an ethosomal system's extremely soft and flexible vesicles will penetrate the stratum corneum's modified structure and create a passage through the skin. The fusing of these vesicles with cell membranes in the skin's deeper layers results in the release of the therapeutic substance.[17]

This can be summarised into the main pathways

Ethanol effect

Ethanol effect is the first step for ethosome penetration, in which ethanol changes the conformation of stratum corneum lipid, which are extremely compacted. Ethanol interacts with the hydrophilic (polar) heads of the lipid layers, which results in increasing lipid fluidity and decreasing the consistency of the lipid layers, thus providing the vesicles with flexibility and allowing them to penetrate deeper layers.[7,14,41] Furthermore, ethanol gives vesicles a smoother, more flexible texture that allows them to penetrate the epidermal layer more deeply.[18]

Ethosomes effect

Because of the ethanol inethosomes, there is an increase in the lipid fluidity of cell membranes, which is linked to increased skin permeability. The improved skin attachment to the vesicles in the case of drugs

encapsulated in ethosomes can be attributed to the drug's higher positive zeta potential. Thus, the ethosomes go extremely easily through the layers of deep skin, where they combine with skin lipids to release the medications into the tissues under the skin's surface.[7,14,45]

Transethosomes are said to have better skin-permeation abilities than classical ethosomes. The reason for this is because transethosomes consist of ethanol and an edge activator, also known as a penetration enhancer, which work together to promote vesicular malleability and skin-lipid perturbation. Nevertheless, there is still much to learn about the precise mechanisms underlying ethosomal system skin penetration.[17]

CHARACTERIZATION OF ETHOSOMAL SYSTEM:

Visualisation of Vesicles by TEM and SEM

Transmission electron microscopy (TEM) and scanning electron microscopy (SEM) are used to observe the size and shape of the vesicles.[3,11,46]

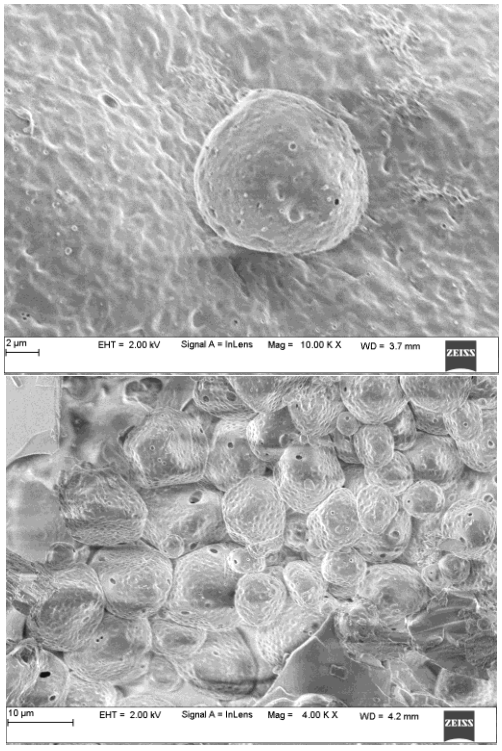

Figure 2. Scanning electron microscope image of ethosomes.

Particle Size, Zeta Potential and PDI

All the ethosomal formulations will be analyzed for particle size, polydispersity index (PDI) and zeta potential wherein the principle of dynamic light scattering will be utilized using the suitable device.[3,47]

Entrapment Efficiency:

Lamsal et al.[48] determined the entrapment efficiency of gliclazide ethosomes by the ultra-centrifugation technique. 15 ml of ethosomal formulation was taken in a centrifuge tube and allowed to centrifuged at 12000 rpm for 2 hours. Then total volume of supernatant was measured. Then amount of drug in the supernatant layer was determined by using UV spectrophotometer. The entrapment efficiency will be calculated by using the following formula:[13,49]

Entrapment Efficiency = $[\langle Q_t - Q_s \rangle \div Q_t] \times 100$

Where,

***Q_t** is the amount of drug added*

***Q_s** is the amount of drug detected in the supernatant.*

This is an indirect method of determining the entrapment efficiency by calculating the unentrapped drug by taking supernatant layer. In the direct method the sediment layer of the ethosomes are taken after the centrifugation.

Barupal AK et al.[50] separated the sediment and supernatant liquids; amount of drug in the sediment was determined by treating the sediment with 1 ml of 0.1% Triton X 100 to lyse the vesicles and then diluted to 100 ml with methanol. The vesicles were broken to release the drug and then estimated for the drug content by using UV spectrophotometer. The percent entrapment was calculated using the formula,[51]

% entrapment= amount of drug in sediment/amount of drug added ×100

pH Measurement

The pH measurement of the formulation was carried out using a calibrated pH meter for accurate measurement. The pH electrode should be rinsed with distilled water and then immersed in the ethosome sample. Dip the pH electrode into the ethosome formulation, ensuring that it is fully submerged and that the electrode is adequately covered by the vesicular solution. Allow the reading to stabilize before recording the pH value. Once the reading stabilizes, note the pH value displayed on the meter.[27,52]

Transition Temperature

By using DSC in an aluminium pan with a continuous nitrogen stream and a heating rate of 10°C per minute, the transition temperature (T) of vesicular lipids can be determined in duplicate. The transition temperature provides insight into the fluidity of the ethosomal membranes. A lower transition temperature indicates that the vesicles are more fluid, which enhances their ability to penetrate biological membranes.[3,13,45,53]

Drug Content

A UV spectrophotometer can be used to determine the drug content of the ethosomes. A modified high-performance liquid chromatographic method can also be used to quantify this.[3,11,54]

Surface Tension Measurement

The ring method in a Du Nouy ring tensiometer can be used for measuring the surface tension activity of drugs in aqueous solutions.[3,11,13]

Phospholipid-Ethanol Interaction

Differential Scanning Calorimetry (DSC) and proton-decoupled 31P NMR were employed to investigate the phospholipid-ethanol interaction. The interaction between phospholipids and ethanol is fundamental to the performance of ethosomes. Phospholipid-Ethanol Interaction test is integral to understanding and optimizing ethosomal formulations. Ethanol not only enhances the permeability of ethosomes but also influences their physical characteristics and stability, ultimately leading to improved drug delivery through the skin.[13,14,32,55]

Degree of Deformability and Turbidity

Degree of Deformability

The degree of deformability of ethosomes is assessed using a modified extrusion method. In this process, a sample of the ethosome formulation is forced through a filter with a known pore size (typically 200 nm) under controlled pressure. The amount of ethosome extruded, along with the size of the vesicles before and after filtration, is measured. The deformability (D) is calculated using the formula:

$$D=J\,(r/v_r)\,p^2$$

where:

- D is the deformability of the vesicle membrane,
- J is the volume of ethosome extruded,
- r is the size of the vesicles after passing through the filter,
- v_r is the pore size of the filter, and
- p is the pressure applied during extrusion.[27]

The results typically show that ethosomes possess a high degree of deformability, allowing them to pass through smaller pores compared to their size. This is attributed to the presence of ethanol in the formulation, which reduces the interfacial tension and enhances the elasticity of the vesicle membrane.[17,56]

Turbidity

Turbidity in ethosome formulations is measured using a nephalometer, which assesses the cloudiness or haziness of the solution. This parameter is essential as it can indicate the size and distribution of the vesicles, as well as their stability in the formulation. Increased turbidity often correlates with larger vesicle sizes or aggregation, which can affect the efficacy of drug delivery.[13,56]

***In-Vitro* Drug Release Study and Drug Deposition Study**

Franz diffusion cells with artificial or biological membranes and dialysis bag diffusion can be used for in vitro drug release studies and drug deposition of ethosomal preparation.[3,13,57]

Stability Studies

The stability studies will be done on the most satisfactory formulation at two different temperatures, i.e. refrigeration temperature (4º ± 2ºC) and room temperature (25º ± 2ºC) for 180 days (as per ICH guidelines).[11,13,48]

Stability of Ethosomes

Ethosomes are composed of ethanol, phospholipids, and water, which contribute to their unique stability profile. The presence of ethanol not only enhances the fluidity of the membrane but also imparts a net negative charge, which helps prevent aggregation through electrostatic repulsion. This characteristic makes ethosomes significantly more stable than traditional liposomes, which are prone to fusion and leakage over time due to a lack of such stabilization mechanisms.[18,58]

Key Stability Features of Ethosomes:

- **Entrapment Efficiency**: Ethosomes exhibit high entrapment efficiency for both hydrophilic and lipophilic drugs, which is superior to that of liposomes. The fluidizing effect of ethanol allows for better incorporation of drugs within the vesicular structure.[18,59]
- **Resistance to Aggregation**: Ethosomes maintain their size and structural integrity better than liposomes, which can aggregate and grow into larger vesicles upon storage, leading to drug leakage.[58,59]
- **Impact of Ethanol Concentration**: The stability of ethosomes is influenced by the concentration of ethanol. While increasing ethanol concentration can enhance entrapment efficiency up to a point (around 30-45%), excessive ethanol can destabilize the vesicles by making the membrane too permeable.[52,58]

Comparison with Other Vesicular Carriers

Liposomes

- **Stability Issues**: Liposomes face significant stability challenges, including aggregation, fusion, and leakage of encapsulated drugs. These issues arise from their neutral surface charge, which does

not provide the same level of repulsion as ethosomes.[58,59]

Niosomes

- **Physical Stability**: Niosomes, which are non-ionic surfactant vesicles, also encounter stability problems such as fusion and sedimentation. While they are considered superior to liposomes in some aspects, they still do not match the stability of ethosomes.[59]

Transferosomes

- **Deformability vs. Stability**: Transferosomes are highly deformable and can penetrate deeper into the skin layers, but they may not provide the same level of stability as ethosomes. Their ability to deform does not compensate for the aggregation and leakage issues that can arise during storage.[18,59]

APPLICATIONS OF ETHOSOMES IN DRUG DELIVERY

In the recent two decades, ethosomes have been widely used for transdermal/topical drug delivery applications. Due to the amphiphilic nature of ethosomes, they are frequently used as a popular nano-carrier for drug and protein/peptide delivery, cosmeceutical applications, etc. The numerous literature surveys reported that novel ethosomes have to gain great importance in advanced drug delivery systems, especially delivery through skin membranes.[32]

Hormone Delivery

Ainbinder D. et al.,[60] formulated testosterone ethosomes for enhanced transdermal delivery. Physiological decrease in testosterone levels in men with age causes various changes with clinical significance. Recent testosterone replacement therapy is based mainly on transdermal non-patch delivery systems. These products have the limitation of being applied to extremely large areas to achieve the required hormone blood levels. The objective of the present study was to design and test a testosterone non-patch formulation using ethosomes for enhanced transdermal absorption. This work shows that the ethosomal formulation could enhance testosterone systemic absorption and also be used for designing new products that could solve the weaknesses of the current testosterone replacement therapies.

Delivery of Anti-arthritis Drug

Abdelbary G.A. et al.,[61] improved the transdermal delivery of Mometasone Furoate (MF) by developing an optimal ethosomal system for targeting inflammatory cases. The skin flux of the optimal MF formula (OMF) was 2.33 and 3.53 folds of liposomes, hydroalcoholic dispersion; respectively. Moreover, confocal laser scanning microscopy (CLSM) confirmed the penetration potential of OMF in comparison to other systems. The arthritis degree was significantly improved after daily application of ethosomal gel for ten days, while normal joint histological structures were observed after twenty days. Therefore, MF-loaded ethosomes represent a promising therapeutic approach for the treatment of arthritis.

Antiviral Drug Delivery

Sicurella M. et al.,[62] investigated an ethosomal gel for topical administration of dimethyl fumarate in the treatment of HSV-1 infections. The infections caused by the HSV-1 virus induce lesions on the lips, mouth, face, and eye. The antiviral effect of dimethyl fumarate loaded in ethosome gel was demonstrated by a reduction in viral growth both 1 h and 4 h post-infection. Moreover, the patch test demonstrated the safety of the ethosomal gel applied to the skin.

Cosmetic Applications of Ethosomes

Abu-Huwaij R. et al.,[41] reviewed about the potential of cosmetic dermal delivery with ethosomes. The unique structure and composition of ethosomes make them suitable for the delivery of active cosmetic ingredients for the treatment of hair loss, acne, and skin whitening. such as vitamins, antioxidants, and skin-lightening agents, through the skin. Their ability to enhance the penetration of drugs through the skin makes them a versatile and effective alternative to traditional transdermal drug delivery systems. The use of ethanol in their composition allows for increased stability, enhanced drug-loading capacity, and targeted drug delivery.

Acne Treatment

Kausar H. et al.,[63] developed and optimized ethosome formulations for topical delivery of thymoquinone (THQ) for improved therapy in skin acne. The resulting formulations presented lipid-based vesicles in a nanosized range, high entrapment efficiency, and better flux across rat skin. Further studies like antimicrobial studies, anti-inflammatory studies, and anti-acne studies further support the conclusion that the developed THQ ethosome formulation can be used as an effective treatment option for acne vulgaris and various skin disorders.

Antidiabetic Drug Delivery

Hussain SR. et al.,[57] formulated and evaluated ethosomal gels containing saxagliptin for antidiabetic drug delivery. The transdermal flux of ethosomal gel formulation was calculated, and the results showed that ethosomal gel formulation shows significantly higher permeability as compared to conventional gel formulation. Finally, it can be concluded that a formulated ethosome gel formulation

loaded with saxagliptin can be prepared with an appropriate size, maximum drug entrapment efficiency and enhanced transdermal flux. The efficient skin permeability makes ethosomal gel formulations a potential and effective transdermal drug delivery system for saxagliptin.

Management of Parkinsonism

Navaneethan S. et al.,[64] developed of nanoethosomes containing rasagiline mesylate for effective treatment of Parkinson's disease. This study of rasagiline mesylate-loaded nanoethosomes revealed amelioration in the encapsulation efficiency upon increasing the amount of ethanol and phospholipids to a certain extent in preparation. Further, the nanoethosomes formulations showed controlled release of the drug for 12 hours. Analysis of the drug release mechanism showed that the drug release followed the first-order kinetic model. These results confirmed that nanoethosomes containing rasagiline mesylate have the potential for the treatment of Parkinson's disease.

CONCLUSION

Since ethosomes were first developed over three decades ago, these nanocarriers have demonstrated their exceptional capacity to carry therapeutic compounds with various physicochemical characteristics through the skin for both local and systemic application. The ethosomes are promising options for topical applications due to their higher stability and penetrating abilities than liposomes. It has also been demonstrated that ethosomes make promising drug and cosmetic delivery systems. Improved skin penetration and improved therapeutic outcomes can be achieved by incorporating ethosomal systems into appropriate vehicles, such as gels, patches, and creams.

Funding Acknowledgments

None.

Conflict of interest

The author declares that there isn't any conflict of interest regarding the publication of this paper.

REFERENCES

1. Tiwari RK, Chauhan NS, Yogesh HS. Ethosomes: A potential carries for transdermal drug delivery. Int J Drug Dev Res. 2010; 2(2): 448-52.
2. Jain A, Jain P, Kurmi J, Jain D, Jain R, Chandel S, *et al.,* Novel strategies for effective transdermal drug delivery: a review. Critical Reviews™ in Therapeutic Drug Carrier Systems. 2014; 31(3): 219-72.
3. Patrekar PV, Inamdar SJ, Mali SS, Mujib MT, Ahir AA, Hosmani AH. Ethosomes as novel drug delivery system: A review. Pharm Innov. 2015; 4(9): 10-21.
4. Razavi H, Janfaza S. Ethosome: a nanocarrier for transdermal drug delivery. Arch Adv Biosci. 2015; 6(2): 38-43.
5. Alva SH, Shripathy D, Shabaraya AR. Nanoparticle drug delivery system: an overview. World J Pharm Med Res. 2023; 9(6): 95-103.
6. Stoughton RB. Percutaneous absorption of drugs. Ann Review Pharmacol Toxicol. 1989; 29(1): 55-69.
7. Roshini R, Saraswathi TS, Damodharan M. Ethosomes: novel lipid vesicular and non-invasive delivery carrier–a review. J Posit School Psychol. 2022; 6(8): 4099-111.
8. Zahid SR, Upmanyu N, Dangi S, Ray SK, Jain P, Parkhe G. Ethosome: a novel vesicular carrier for transdermal drug delivery. J drug deliv ther. 2018; 8(6): 318-26.
9. Tyagi S, Chaurasia L, Islam M, Sagar B, Tyagi A. A review on novel approach ethosome as a nanocarrier for transdermal drug delivery. Biomed Res. 2022; 33(3): 67-73.
10. Harshitha K, Shabaraya AR, Vinayak K. A review on ethosomes: a promising novel vesicular carrier for transdermaldrug delivery. World J Pharmacy Pharm Sci. 2021; 10(8): 634-45.
11. Kumar N, Dubey A, Mishra A, Tiwari P. Ethosomes: A Novel Approach in Transdermal Drug Delivery System. Int J pharm life sci. 2020; 11(5): 6598-08.
12. Mohanty D, Babu MN, Ankitha K, Gunde S, Bakshi V, Jat RM. Ethosome: a recent optimized technology for transdermal drug penetration. Eur J Biomed Pharm sci. 2016; 3(12): 232-40.
13. Jadhav PK, Kapadnis KA, Shinkar DM, Pathan VT, Jadhav AG. Ethosomes as Novel Drug Delivery System: A Review. Int J Pharm Sci Rev Res. 2020; 62(1): 173-82.
14. Miyanda PM, Priyanka SS. Ethosomes: A potential transdermal drug delivery system-in- depth review. Int J Pharm Res. 2017; 11(1): 14-55.
15. Prajwal KC, Shabaraya AR, Vineetha K. Ethosomes: A unique nanocarrier for drug delivery. World J Pharmacy pharm Sci., 2021; 10(5): 588-98.
16. Nainwal N, Jawla S, Singh R, Saharan VA. Transdermal applications of ethosomes–a detailed review. J Liposome Res. 2019; 29(2): 103-13.
17. Abdulbaqi IM, Darwis Y, Khan NA, Assi RA, Khan AA. Ethosomal nanocarriers: the impact of constituents and formulation techniques on ethosomal properties, *in vivo* studies, and clinical trials. Int J nanomedicine. 2016; 11(1): 2279-304.

18. Chauhan N, Vasava P, Khan SL, Siddiqui FA, Islam F, Chopra H, et al., Ethosomes: A novel drug carrier. Ann Med Surg. 2022; 82(1): 104595-98.
19. Emanet M, Ciofani G. Ethosomes as Promising Transdermal Delivery Systems of Natural-Derived Active Compounds. Adv NanoBiomed Res. 2023; 3(10): 2300020-31.
20. Zhang JP, Wei YH, Zhou Y, Li YQ, Wu XA. Ethosomes, binary ethosomes and transfersomes of terbinafine hydrochloride: a comparative study. Arch Pharm Res. 2012; 35(1): 109-17.
21. Huanbutta K, Rattanachitthawat N, Luangpraditkun K, Sriamornsak P, Puri V, Singh I, *et al*. Development and evaluation of ethosomes loaded with Zingiber zerumbet Linn rhizome extract for antifungal skin infection in deep layer skin. Pharmaceutics. 2022; 14(12): 2765-76.
22. Nasri S, Ebrahimi-Hosseinzadeh B, Rahaie M, Hatamian-Zarmi A, Sahraeian R. Thymoquinone-loaded ethosome with breast cancer potential: optimization, in vitro and biological assessment. J Nanostructure Chem. 2020; 10: 19-31.
23. Zhou Y, Wei Y, Liu H, Zhang G, Wu XA. Preparation and *in vitro* evaluation of ethosomal total alkaloids of *Sophora alopecuroides* loaded by a transmembrane pH-gradient method. AAPS Pharm sci tech. 2010; 11(1): 1350-58.
24. Song CK, Balakrishnan P, Shim CK, Chung SJ, Chong S, Kim DD. A novel vesicular carrier, transethosome, for enhanced skin delivery of voriconazole: characterization and *in vitro/in vivo* evaluation. Colloids Surf B Biointerfaces. 2012; 92(1): 299-304.
25. Ramakrishna GA, Manohar SD, Bhanudas SR. Ethosomes: carrier for enhanced transdermal drug delivery system. J Adv Pharm Edu Res. 2014; 4(4): 380-87.
26. Mousa IA, Hammady TM, Gad S, Zaitone SA, El-Sherbiny M, Sayed OM. Formulation and characterization of metformin-loaded ethosomes for topical application to experimentally induced skin cancer in mice. Pharmaceuticals. 2022; 15(6): 657-82.
27. Limsuwan T, Boonme P, Khongkow P, Amnuaikit T. Ethosomes of phenylethyl resorcinol as vesicular delivery system for skin lightening applications. Biomed Res Int. 2017; 1(1): 01-12.
28. Fathalla D, Youssef EM, Soliman GM. Liposomal and ethosomal gels for the topical delivery of anthralin: preparation, comparative evaluation and clinical assessment in psoriatic patients. Pharmaceutics. 2020; 12(5): 446-69.
29. Nair RS, Billa N, Leong CO, Morris AP. An evaluation of tocotrienol ethosomes for transdermal delivery using Strat-M® membrane and excised human skin. Pharm Dev Technol. 2021; 26(2): 243-51.
30. Vijayakumar KS, Parthiban S, Senthilkumar GP, Vikneswari A. Effect of soyalecithin concentration on formulation of ethosomes. Int J Res Pharm Nano Sci. 2014; 3(5): 450- 60.
31. Paliwal S, Tilak A, Sharma J, Dave V, Sharma S, Yadav R, *et al*., Flurbiprofen loaded ethosomes-transdermal delivery of anti-inflammatory effect in rat model. Lipids Health Dis. 2019; 18(1): 133-47.
32. Shitole M, Nangare S, Patil U, Jadhav NR. Review on drug delivery applications of ethosomes: Current developments and prospects. Thai J Pharma Sci. 2022; 46(3): 251-65.
33. Paiva-Santos AC, Silva AL, Guerra C, Peixoto D, Pereira-Silva M, Zeinali M, *et al*., Ethosomes as nanocarriers for the development of skin delivery formulations. Pharm Res. 2021; 38(6): 947-70.
34. Devaki J, Pavuluri S, Suma N. Ethosomes: a vesicular carrier as a novel tool for transdermal drug delivery system. J Drug Deliv Ther. 2023; 13(4): 159-64.
35. Abdelkader H, Alani AW, Alany RG. Recent advances in non-ionic surfactant vesicles (niosomes): self-assembly, fabrication, characterization, drug delivery applications and limitations. Drug Deliv. 2014; 21(2): 87-100.
36. Dave V, Kumar D, Lewis S, Paliwal S. Ethosome for enhanced transdermal drug delivery of aceclofenac. Int J Drug Deliv. 2010; 2(1): 81–92.
37. Vindhya VS, Kamath KK, Jain SK, Shabaraya AR. Spanlastics: a modern formulation approach in drug delivery. Eur J Pharm Med Res. 2023; 10(4): 96-102.
38. Shen S, Liu SZ, Zhang YS, Du MB, Liang AH, Song LH, *et al*., Compound antimalarial ethosomal cataplasm: preparation, evaluation, and mechanism of penetration enhancement. Int J Nanomedicine. 2015; 10(1): 4239-53.
39. Pandey V, Golhani D, Shukla R. Ethosomes: versatile vesicular carriers for efficient transdermal delivery of therapeutic agents. Drug Deliv. 2015; 22(8): 988-1002.
40. Shinde P, Page A, Bhattacharya S. Ethosomes and their monotonous effects on skin cancer disruption. Front Nanotechnol. 2023; 5(1): 1087413-33.
41. Abu-Huwaij R, Zidan AN. Unlocking the potential of cosmetic dermal delivery with ethosomes: A comprehensive review. J Cosmet Dermatol. 2024; 23(1): 17-26.
42. Iizhar SA, Syed IA, Satar R, Ansari SA. In vitro assessment of pharmaceutical potential of ethosomes entrapped with terbinafine hydrochloride. J Adv Res. 2016; 7(3): 453-61.
43. Yadav KK, Verma NK. Formulation and evaluation of ethosome of mefenamic acid using

hot method. J Chem Pharm Res. 2018; 10(5): 04-15.
44. Ma H, Guo D, Fan Y, Wang J, Cheng J, Zhang X. Paeonol-loaded ethosomes as transdermal delivery carriers: design, preparation and evaluation. Molecules. 2018; 23(7): 1756-70.
45. Sheel S, Biswas P, Karmakar V, Khanam S. Ethosome as a potential transdermal drug delivery system. J Pharm Biol Sci. 2022; 10(2): 72-78.
46. Touitou E, Dayan N, Bergelson L, Godin B, Eliaz M. Ethosomes—novel vesicular carriers for enhanced delivery: characterization and skin penetration properties. J controlled release. 2000; 65(3): 403-18.
47. Dharwadkar K, Gude R, Naik K, Navti P. Systematic development of binary ethosomal nsaid based gel. Int J Pharm Sci Res. 2020; 11(7): 3181-96.
48. Lamsal R, Geethalakshmi A, Gubbala S. Formulation and evaluation of gliclazide ethosomes as a novel drug carrier. Int J Pharm Sci Res. 2015; 6(5): 2072-80.
49. Rai S, Bhavyashree T, Kamath KK, Shabaraya AR. Ethosomes: a novel vesicular carrier for transdermal drug delivery. Eur J Pharm Med Res. 2023; 10(6): 232-37.
50. Barupal AK, Gupta V, Ramteke S. Preparation and characterization of ethosomes for topical delivery of aceclofenac. Indian J Pharm Sci. 2010; 72(5): 582-86.
51. Mishra AD, Khunt DM, Ghayal AH, Patel CN, Shah DR. Formulation and optimization of ethosomes for transdermal delivery of felodipine. Res J Pharm Technol. 2012; 5(12): 1509-17.
52. David SR, Hui MS, Pin CF, Ci FY, Rajabalaya R. Formulation and in vitro evaluation of ethosomes as vesicular carrier for enhanced topical delivery of isotretinoin. Int J Drug Deliv. 2013; 5(1): 28-34.
53. Nandure HP, Puranik P, Giram P, Lone V. Ethosome: A Novel Drug Carrier. Int J Pharm Res Allied sci. 2013; 2(3): 18-30.
54. Avasarala H, Dinakaran S, Boddeda B, Dasari SP, Jayanthi VR, Swaroopa P. Ethosomal gel: a novel choice for topical delivery of the antipsychotic drug Ziprasidone Hydrochloride. Braz J Pharm Sci. 2022; 58: 01-12.
55. Maurya SD, Prajapati S, Gupta A, Saxena G, Dhakar RC. Formulation development and evaluation of ethosome of stavudine. Int J Pharm Edu Res. 2010; 13(01): 16-22.
56. Jadhav PU, Gujare SG, Shende MA. Ethosomes: A novel tool for vesicular drug delivery. Asian J Pharm Res. 2024;14(1):45-52.
57. Hussain SR, Kumar P, Alam P. Formulation and *In-vitro* Evaluation of Ethosomal Gels Containing Saxagliptin As the Model Drug. Indo Am J Pharm Sci. 2021; 08(04): 01-20.
58. Verma P, Pathak K. Therapeutic and cosmeceutical potential of ethosomes: An overview. J Adv Pharm Technol Res. 2010; 1(3): 274-82.
59. Bansal S, Kashyap CP, Aggarwal G, Harikumar SL. A comparative review on vesicular drug delivery system and stability issues. Int J Res Pharm Chem. 2012; 2(3): 704-13.
60. Ainbinder D, Touitou E. Testosterone ethosomes for enhanced transdermal delivery. Drug Deliv. 2005; 12(5): 297-303.
61. Abdelbary GA, Khowessah OM, Bakr AH, Abu-Elyazid SK. Potential treatment of arthritis with an optimized Mometasone Furoate loaded-ethosomal gel in carrageenan-induced rat joint arthritis. J Drug Deliv Sci Technol. 2020; 57(1): 101771-82.
62. Sicurella M, Pula W, Musiał K, Cieślik-Boczula K, Sguizzato M, Bondi A, *et al*., Ethosomal gel for topical administration of dimethyl fumarate in the treatment of HSV-1 infections. Int J Mol Sci. 2023; 24(4): 4133-54.
63. Kausar H, Mujeeb M, Ahad A, Moolakkadath T, Aqil M, Ahmad A, *et al*., Optimization of ethosomes for topical thymoquinone delivery for the treatment of skin acne. J Drug Deliv Sci Technol. 2019; 49(1): 177-87.
64. Navaneethan S, Somashekhar CN, Harshitha VY. Formulation and Evaluation of Nano Ethosomes Contaning Rasagiline Mesylate for the Effective Management of Parkinsonism. Int J Pharm Res Appl. 2022; 7(3): 2112-28.