ISSN 2521-0521

JOURNAL OF CONTEMPORARY PHARMACY

Volume 8: Issue 2: 2024

https://doi.org/10.56770/jcp2024828

A REVIEW ON ETHOSOMES AS NANOCARRIER FOR SKIN DELIVERY OF DRUGS

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Submitted: December 7, 2023; Revised: March 9, 2024; Accepted: May 20, 2024; Published: December 31, 2024

ABSTRACT

Ethosomes are novel lipid and ethanolic phospholipid vesicles that are primarily employed for medication administration through the skin. These nanocarriers are designed to carry therapeutic delivery of drugs with varying physicochemical qualities into skin layers. Ethosomes have a better penetration rate into the skin than liposomes, so they can be used to replace liposomes. Ethosomes' enhanced penetration is most likely due to their ethanolic component. Ethanol enhances the lipid fluidity of the cell membrane, resulting in greater ethosome skin penetrability. These ethosomes penetrate the skin, fusing with the cell membrane of lipids to release the medication. Since their discovery in 1996, ethosomes have undergone substantial investigation; additional substances were added to their basic formula, resulting in the development of various types of ethosomes such as classical, binary, and transethosomes. These are created using hot, cold and other procedures. Different types of materials are used for ethosomal formulation. Size, drug content, shape, zeta potential, and other properties are evaluated by using different techniques. Some ethosomal formulation are available in market like Supravir cream, Decorin cream etc. This review provides a thorough examination of the impacts of ethosomal system elements, production techniques, and their critical roles in defining the final characteristics of these nanocarriers.

Keywords: Ethosomes, Lipid-based vesicles, Ethanol, Transdermal.

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INTRODUCTION

Skin is the body’s largest and the most widely available organ and it can be used to provide drugs with systemic effects **[1]**. Skin comprises of distinctive multilayers, termed as the epidermis, the stratified epithelium which is regularly regenerates and the dermis, which is made up of connective tissues, are superimposed on the sub-cutis/hypothermis, or deepest layer, made up of fatty loose connective tissues **[2, 3]**. The epidermis is derived embryological form ectoderm and the dermis and hypodermis from mesoderm. The skin is the principal organ that covers the entire body’s surface and is connected to the mucous membrane that lines the body’s orifices. It accounts for around 15% of the total body weight of the adult **[4]** and the surface area of 1.5 -2m2 **[5]**. The skin protects against physical, chemical, and biological threats and helps in thermoregulation **[6]**. It has cells, fibers, and glands which are arranged in different layers **[7].**

Ethosomes are lipid-based nanovesicles with benefits for transdermal or dermal drug delivery because nanoparticles can easily penetrate the skin, release encapsulated drugs for extended periods of time while shielding them from degradation, behave as drug depots, and control the rate of confined transdermal delivery via their membrane **[8]**. Ethosomes are mostly used in cosmetic preparations to abduct antioxidants, vitamins, and other active substances available in gels, creams, and other forms of dispersion. They are rich in ethanol, which bestows malleability and softness on the vesicles and increases skin penetration, distinguishing them from other lipid-based vesicles such as liposomes **[9]**. Because ethanol prevents aggregation, ethosomes have more stable

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vesicles and negative charges. Drug-loaded ethosomes penetrate deep into the skin, allowing the drug to reach deeper into the skin layers and enhance drug efficacy **[10]**. Propylene glycol (PG) is a popular penetration enhancer used at concentrations ranging from 5% to 20% in ethosomal preparations **[11]**. The presence of PG and ethanol in ethosomes improve drug solubility, resulting in greater entrapment efficiency and better drug distribution through the vesicle **[12]**. PG improves ethosome stability by enhancing anti-hydrolysis property and viscosity **[13]**.

In general, the phospholipid concentration range in the formation of ethosomes is 0.5–5%. The formulation's concentration and phospholipid type are critical factors in the development of an ethosomal formulation because they affect the size, zeta-potential, stability, entrapment efficacy, and penetration characteristics of the vesicles **[14]**. Cholesterol is a steroid molecule that is rigid and is used to reduce vesicular permeability and fusion while preventing leakage. It is typically used at a concentration of 3% **[15]**, but in specific formulations, it has been used up to 70% of the overall phospholipid concentration for the ethosomal formulation **[16]**. Tween 80 is used in the ethosomal system at concentrations ranging from 10% to 50% of the cumulative phospholipid concentration. Tween-80 was expected to lower vesicular size and improve stability of the system and skin-permeation characteristics in ethosomal systems **[17]**.

TYPES OF ETHOSOMES

Classical Ethosomes

Classical ethosomes are a modified form of classical liposome that contains phospholipids, ethanol (high concentration up to 45 percent by weight) and water. Because they were smaller, had a negative-potential and had a better entrapment efficiency, classical ethosomes were found to be more significant than classical liposomes for transdermal drug administration. Furthermore, when compared to classical liposomes, classical ethosomes had better skin penetration and stability profiles **[18].** The entrapment of drug in classical ethosomes have molecular weights ranging from 130.077 Da to 24 kDa **[19]**.

Binary Ethosomes

Binary ethosomes were produced by mixing another sort of alcohol with the classical ethosomes **[20]**. Propylene glycol and isopropyl alcohol are the most widely used alcohol in binary ethosomes **[21]**.

Transethosomes

The latest generation of ethosomal system are known as transethosomes. This ethosomal system includes the essential constituents of classic ethosomes as well as an additional substance in their composition such as a penetration enhancer or a surfactant **[22, 23]**. Drugs having molecular weights in range of 130.077 Da t0 200-325 kDa have been reported to be entrapped by transethosomes **[24].**

METHODS OF PREPARATION OF ETHOSOMES

Ethosomes are prepared by following methods

1. The classical cold method
2. The ethanol injection-sonication method
3. The hot method
4. The thin-film hydration method
5. The reverse-phase evaporation method

Table 1: Comparison of different types of ethosomes.

Parameters	**Classical ethosomes**	**Binary ethosomes**	**Transethosomes**
Composition	Ethanol Phospholipid Water Charge inducer Stabilizer Drug	Ethanol Phospholipid Water Charge inducer Propylene glycol/ other alcohols Drug	Ethanol Phospholipid Water Charge inducer Edge activator or penetration enhancer Drug
Size	Less than classical liposomes	Smaller than or equal to classical ethosomes	Based on concentration and type of penetration enhancer used
Morphology	Spherical	Spherical	Spherical
Entrapment efficiency	More than classical liposomes	More than classical ethosomes	More than classical ethosomes
Stability	Stable than classical liposomes	Stable than classical ethosomes	No particular trend
Zeta-potential	Negatively charged	Negatively charged	Negatively or positively charged
Skin permeation	More than classical liposomes	More than and equal to classical ethosomes	More than classical ethosomes

The Classical Cold Method

This is the most basic and extensively used approach for preparing ethosomal systems and it can be conducted under nitrogen protection if needed. It entails preparing both the organic and aqueous phases separately. For the manufacturing of binary ethosomes at 25-30 °C, the organic phase is generated by dissolving the phospholipids (along with surfactants or transethosome penetration enhancers) in ethanol or a mixture of solvents (ethanol/PG). Water, buffer solution, or normal saline solution are employed as the aqueous phase. At a steady rate of 175 or 200 L/min, the aqueous phase is introduced to the organic phase in a fine stream, dropwise, or using a syringe pump. An overhead or magnetic stirrer is used to stir the mixture at a speed of 700–2,000 rpm. To obtain the requisite ethosomal suspension, the mixture is mixed for 5–30 minutes **[25]**.

The Hot Method

By using this approach, phospholipid is mixed in water and heated in a water bath at 400 °C until it forms a colloidal solution. In another vessel, ethanol and propylene glycol are thoroughly combined by heating it to 400 °C. The organic and aqueous phases are combined. Depending on the drug's solubility, dissolve it in water or ethanol. With the help of probe sonication or extrusion, the ethosomal formulation's vesicle size can be reduced to the desired extent **[26].**

The Ethanol Injection-Sonication Method

Using a syringe system, the organic phase containing the dissolved phospholipid in ethanol is injected into the aqueous phase at a flow rate of 200 L/min, then homogenized for 5 minutes with an ultrasonic probe **[27]**.

The Thin Film Hydration Method

This approach is an elaboration of the classic liposome synthesis method, however, the lipid film is hydrated by a hydro-ethanolic solution in this method. In a clear, sterile, round-bottom flask, dissolve the phospholipid in chloroform alone or a chloroform-methanol combination at a ratio of 3:1 or 2:1. A rotational vacuum evaporator at a temperature higher than the lipid-phase transition temperature removes organic solvents. The solvent traces are then removed from the deposited lipid coating overnight under a vacuum. The lipid film is then hydrated using a water-ethanol solution or a phosphate-buffered saline–ethanol solution. During the hydration process, the lipid film is spun and heated for 30 minutes, one hour, or six hours, depending on the phospholipid characteristic **[28]**.

The Reverse Phase Evaporation Method

This is the least commonly used approach and is specifically designed to generate huge unilamellar vesicles. The organic phase is formed by mixing the phospholipid in diethyl ether and then combining it with the aqueous phase in an ultrasonic bath at 0°C for 5 minutes to generate a water-in-oil emulsion. The organic solvent is extracted under reduced pressure to form a gel, which upon intense mechanical agitation transforms into a colloidal dispersion **[29]**

MECHANISM OF PENETRATION / PERMEATION OF DRUG

Ethosome components play a vital role in permeating ethosomes into the skin layers. Touitou et al. proposed a theoretical mechanism in which the inter-dependent impact of phospholipids and ethanol aids drug permeation into the systemic circulation and deeper skin layer.

The primary benefit of ethosomes is increased drug permeation over liposomes. The mechanism of drug absorption in ethosomes remains unknown. Drug absorption is most likely divided into two stages **[30]. 1.** Ethanol effect, and 2. Ethosome effect.

Ethanol Effect

Ethanol improves penetration through the skin. The mechanism underlying its penetration-enhancing effect is well understood. Ethanol penetrates the intercellular lipids, increasing the fluidity of the lipid cell membrane and decreasing the density of the cell membrane's lipid multilayer.

Ethosome Effect

The increased lipid fluidity of cell membranes caused by the ethanol of ethosomes results in increased skin permeability. As a result, the ethosomes permeate very easily into the deeper skin layers, where they fuse with the skin lipids and release the drugs.

MATERIALS MOST COMMONLY USED IN ETHOSOMAL FORMULATION

Ethanol

Ethanol is a powerful penetration enhancer **[31]** and it is important in ethosomal systems because it gives vesicles unique properties such as size, zeta-potential, entrapment efficacy, stability, and enhanced skin permeability **[18]**. Ethanol concentrations in ethosomal formulations have been observed to range between 10% and 50% **[32]**. Many researchers note that increasing the concentration of ethanol reduces the size of the ethosomes **[33]**. However, increasing the ethanol concentration above the optimum level causes the bilayer to leak, resulting in a minor increase in the vesicular size and a significant decrease in the entrapment efficacy, and further increasing the ethanol concentration solubilizes the vesicles. According to some studies, high ethanol concentrations cause inter-penetration of the ethanol hydro-carbon chain, which leads to a reduction of vesicular membrane thickness and thus vesicular size. Other scientists have suggested that ethanol modulates the net charge of the systems, resulting in steric stabilization and a decrease in the mean vesicle size **[34]**. The vesicular charge is a critical parameter that influences vesicular properties

like stability and vesicle-skin interaction. Because of the high ethanol concentration in ethosomes, the vesicular charge has shifted from positive to negative **[35]**. According to one study, the negative charge of empty ethosomes increases with the increasing ethanol concentration **[36]**. Ethanol plays the role of a negative charge provider for the ethosomes, preventing vesicular system aggregation due to electrostatic repulsion. Furthermore, ethanol has been reported to have stabilizing properties **[37]**. Ethanol has a significant effect on the ethosomal system and entrapment efficiency, and increasing ethanol concentration increases entrapment efficiency in general. This effect is applicable to molecules with varying lipophilicities, in which ethanol enhances the solubility of amphiphilic and lipophilic drugs, thereby increasing drug loading. With ethanol concentrations ranging from 20% to 40%, this relationship was discovered to be linear **[38]**. As a result, ethanol concentration should then be optimized during the formulation process, as entrapment efficacy will be minimal at low concentrations, and at very high concentrations, the ethosomal membrane becomes more permeable because the phospholipids can easily dissolve in ethanol, resulting in a substantial decrease in entrapment efficacy.

Cholesterol

Cholesterol is a rigid steroid molecule and its presence in ethosomal systems improves drug stability and entrapment efficiency. It reduces vesicular permeability and fusion while preventing leakage. It is typically used at a concentration of 3% **[15]**, but in some formulations, it has been used upto 70% of the overall phospholipid concentration [39]. Several studies have found that cholesterol increases the size of ethosomal vesicles **[40]**. When 25.87 mM of cholesterol was used in the formulation, researchers discovered that the ethosomal size increased from 136±42 nm to 230±27 nm **[16]**. Another study found that increasing cholesterol concentration from 0 to 0.15 percent w/w increased vesicular size from 102±13 nm to 152±12 nm **[40].** Even so, only one study showed that cholesterol had no effect on ethosome stabilization. In the formulation, the authors used 15% ethanol and phosphatidylethanolamine **[41]**. The inclusion of cholesterol in ethosomal formulation increased vesicular stability and rigidity, according to the researchers. However, in vitro studies using Franz diffusion cells and CLSM revealed that due to their higher rigidity, these multi-lamellar vesicles (MLVs) were unable to pass across the stratum corneum, making it more challenging for the drug to permeate across the skin. Other researchers reported that the addition of cholesterol increased the rigidity of the ethosomal vesicles.

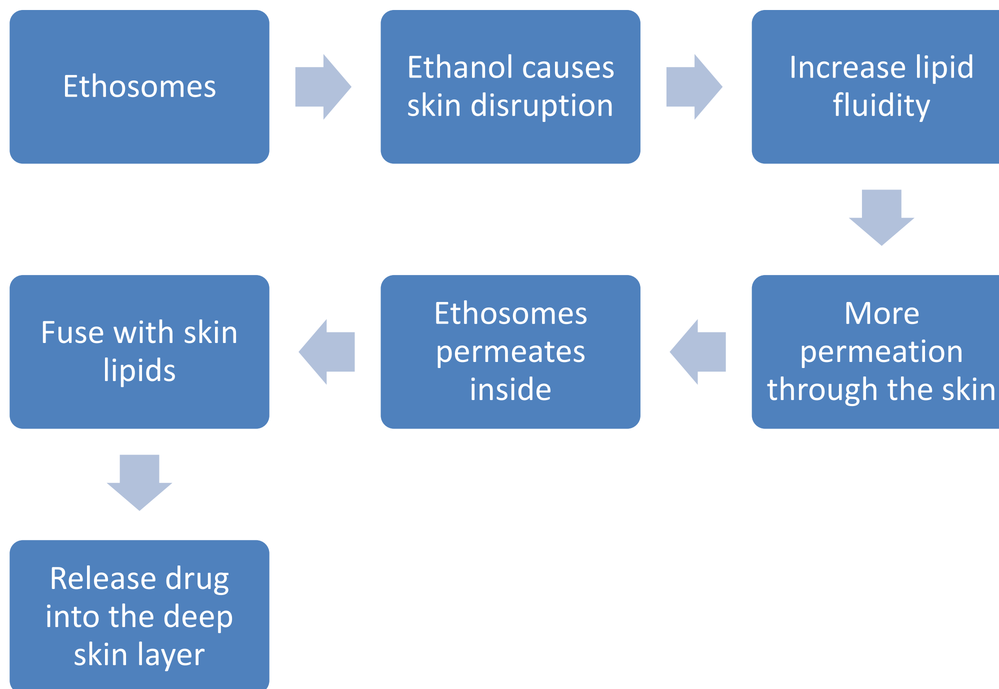

Figure 1: Mechanism of action of ethosomes.

Propylene Glycol (PG)

PG is a popular penetration enhancer and is used in the preparation of ethosomes at concentrations ranging from 5% to 20% and has been shown to influence ethosomal properties such as size, permeation, entrapment efficiency, and stability. When PG is added to ethosomal systems, particle size is reduced even further when compared to systems without using PG. When the PG concentration was changed from 0% to 20% v/v, the particle size decreased significantly from 103.7±0.9 to 76.3±0.5 nm. The researchers discovered that when PG was incorporated into the ethosomal system at a 1:1 ethanol-to-PG ratio, the entrapment efficiency of sophocarpine, sophoridine, lehmanine, and matrin extracted from Sophora alopecuroides increased significantly. Other researchers believe that the inclusion of ethanol and PG in ethosomes improves drug solubility, resulting in increased entrapment efficiency and better drug distribution all across the vesicle. In vitro drug-permeation investigations utilizing Franz diffusion cells revealed no significant difference in tacrolimus accumulation in the stratum corneum between classical and binary ethosomes. It was also found that raising the PG content in ethosomes from 0 to 20% v/v reduced tacrolimus accumulation in the epidermis from 2.23±0.10 g/m^2 to 1.48±0.04 g/m^2. Furthermore, vesicles containing PG at a concentration of 30% v/v still wouldn't significantly improve tacrolimus distribution in the epidermis. On the contrary, ethosomes containing just 30 percent v/v ethanol showed the maximum drug distribution in the epidermis. As a result, this data revealed that ethanol had more permeation-enhancing effects on the stratum corneum than PG. In another study researcher studied the effects of various PG to ethanol weight ratios of 10:0, 9:1, 5:5, 7:3, 3:7, 1:9, and 0:10 on the in-vitro skin deposition of terbinafine HCL ethosomes. In the ethosomal system, the overall amount of ethanol or PG was 45 percent v/w. They discovered that raising the PG fraction in the ethosomes reduced terbinafine hydrochloride skin deposition. This might be owing to the reduced ethanol content in ethosomes, which influenced phospholipid bilayer solubility. Terbinafine skin deposition was greatest in ethosomes containing ethanol: PG at a 7:3 ratio **[11]**. As a result, it can be stated that the ethanol: PG ratio in binary ethosomes should be improved to get better drug permeability. When kept at 4°C, binary ethosomes were shown to be more stable than classical ethosomes **[20]**. As a result, it is proposed that PG improves ethosome stability by enhancing viscosity and antihydrolysis characteristics.

Phospholipids Used in Ethosomes

In the formulation of the ethosomal system, phospholipids from various sources were used. The formulation's phospholipid type and concentration are critical factors in the development of an ethosomal system because they affect the size, zeta-potential, stability, entrapment efficacy, and penetration characteristics of the vesicles. In particular, the concentration of phospholipids in an ethosomal formulation ranges from 0.5 to 5%. Increasing phospholipid concentration increases vesicular size gently or moderately, but significantly increases entrapment efficiency. However, the relationship is only valid until a certain concentration is reached, after which further increases in phospholipid concentration have no impact on entrapment efficiency.

To provide photodynamic localized treatment in squamous cell carcinoma, ferrous chlorophyllin-infused ethosomes and chitosan-infused nanocarriers were formed and compared for entrapment efficiency, skin permeation in mouse skin, and ex vivo studies using HPLC. Ferrous chlorophyllin was used as a photosensitizer in this study. Ethosomes were made using the Touitou et al.[18] method with minor modifications. Nanocarriers were also encased with lipids to improve permeation. The results showed that the ethosome vesicles were larger in terms of size and the entrapment efficiency for the hydrophilic drugs, while ethosomes penetrated deeper into the mouse skin than nanocarriers, which remained confined to the epidermis. It was determined that, depending on the type of cancer, either formulation could be beneficial **[42]**.

The ethosome containing griseofulvin, a hydrophobic drug, was prepared using a biocompatible and biodegradable phospholipid (Phospholipon 90H), and the zeta potential, vesicle size, entrapment efficiency, and polydispersity index were measured. The drug penetration ability can be increased by using appropriate phospholipids because phospholipids line up with the lipid layer of the skin and are amphiphilic, allowing them to penetrate the skin. Five formulations were created, each with a different ethanol concentration. The drug, phospholipid, and cholesterol were first mixed with methanol: chloroform before being evaporated. Different ethanol concentrations were used to hydrate these vesicles. A significant rise in ethanol concentration decreased the particle size and increased drug and vesicle penetration through the skin. The findings supported the hypothesis that the ethosomes have the ability to transport BCS class 2 drugs and poorly water-soluble drugs **[43].**

Rosamarinic acid loaded ethosome and liposomal vesicles were ready by using soya phosphatidylcholine and dipalmitoyl phosphatidylcholine and dissolved in chloroform or methanol. The film was rehydrated with the ethanol by using the mechanical dispersion method. The particle size, polydispersity index, zeta potential, and SEM of these ethosomal and liposomal

vesicles were all measured. HPLC was used to evaluate the contents of in vitro release studies. The study found that the rosamarinic acid can be incorporated into both types of vesicles and that it has enzyme inhibition activity. Rosamarinic acid was directly incorporated into both vesicles, but ethosomes have greater permeation power than liposomes. The ethosomes were observed to be more effective in interpreting rosamarinic acid effects **[44]**.

There is a wide variety of phospholipids available for use in the preparation of ethosomes. Phospholipids that are commonly used include phospholipon 90 G, phospholipon 90 H, phospholipon 80 H, phosphatidyl ethanolamine, and many others. These phospholipids are essential in the formation of vesicles, but their relative concentration is critical.

Surfactants used in ethosomes

Selecting an appropriate surfactant is a crucial stage in the preparation of transethosomes because they have a major impact on the ethosomal system's properties. Surfactants and their effects on vesicle stability, dispersity, size, and permeability have been extensively studied in recent decades. It was discovered that the vesicular size and surfactant parameters (number and length of carbon chains, concentration, hydrophilicity, and HLB value) have an inverse relationship. Increase in any of the parameters result in a reduction in vesicle size. This is because the lipid and the surfactant compete for space. Surfactants influence vesicle Entrapment Efficiency (EE), but the effect of the drug is also important; for example, hydrophobic drugs are effectively entrapped when surfactants with low HLB values are used **[52].** In the case of hydrophilic drugs, surfactants with high HLB values enhance the entrapment of the hydrophilic drugs **[53]**.

Beside from these three parameters, carbon chain length, surfactant transition temperature, and gel-like surfactants were also discovered to improve vesicle EE. It was also assumed that increased membrane permeability led directly to less entrapment. Surfactants play a crucial role in vesicle stability by imparting increased charges on the vesicles **[54].**

In 2015 researchers investigated the permeation of the red ginseng using transferosomes containing ethosomes and surfactants without surfactants in comparison to conventional liposomes. The study found that transferosomes containing edge activators or surfactants (tween 80) had an irregular shape compared to liposomes and ethosomes. Similarly, liposomes and ethosomes had similar vesicle sizes, whereas transferosomes were smaller because of the result of surfactant. The negative charge on the ethosomes and the liposomes was anticipated based on the presence of permeation enhancers, whereas the negative charge on the transferosomes was anticipated based on the presence of the tween 80. Because surfactants increase vesicle flexibility, the permeability of transferosome formulations containing the tween 80 was increased **[49]**.

Liposomes, ethosomes, and transferosomes of lamivudine drugs were prepared for HIV treatment in a comparative study. In the liposomal formulation, no surfactant was used, whereas one transferosome formulation was prepared without using tween 80 (surfactant) and another with tween 80. In the situation of ethosomes, a mixture of tween 80 with varying amounts of less and more was prepared. The results showed that the concentration of surfactant or edge activator increases the efficiency of transferosomal vesicle entrapment. The formulation with an edge activator outperforms the other without using surfactants in terms of entrapment efficiency. It was concluded that an ethosomal formulation with a greater quantity of surfactants was more efficient in terms of entrapment efficiency, followed by transferosomes with less entrapment, and finally liposomes with poor entrapment efficiency due to vesicle inflexibility. Similarly, ethosome formulations with a high surfactant content and transferosome formulations with surfactants demonstrated better dissolution and in vitro release of surfactant-containing vesicles than others **[55]**.

In one investigation reseachers formed conventional liposomes (L-RSV), deformable liposomes (LD-RSV), ultra-deformable liposomes (LUD-RSV), and ethosomes (Etho-RSV) to improve resveratrol penetration and compared and characterized them for best performance. In conventional liposomes, phospholipid was mixed with chloroform, whereas in LD-RSV, tween was used in addition to chloroform and water. In addition to the phospholipid and the surfactant (tween 80), cholesterol was added to LUD-RSV. The results of the characterization revealed that the drug RSV can be effectively carried by LD-RSV due to the use of a rising surfactant ratio with the phospholipid in the ratio of 1:4 phospholipid: tween 80 respectively. The amount of surfactant improved the entrapment efficiency and also increased the size of deformable liposomes, while ideal characteristics were deemed due to the use of an appropriate amount of ethanol. Although ethanol was used in ethosomes, the lack of a surfactant affected the vesicles and made them less desirable **[56]**.

The surfactant review concludes that the use of an appropriate surfactant in sufficient amounts can make ethosomes a perfect carrier for the delivery drug.

Table 2: Different types of phospholipids that are used in preparation of ethosomes.

Phospholipids	**Composition &source**
Phospholipon 90H	Hydrogenated phosphatidylcholine from soya bean 90% **[45]**
Phospholipon 80H	Hydrogenated phospholipids from soya bean with phosphatidylcholine (70%) **[38]**
Phospholipon 90G	Phophatidylcholine from soya bean 90% **[46]**
Phospholipon 50	Lecithin from soy phosphatidylcholine, 45% concentration, rich in palmitic acid [38], linoleic acid (65%) and solid wax **[47]**
Lipoid E80	Phosphatidylcholine (81.7%) content from egg yolk **[48]**
Lipoid S75	Phophatidylcholine (68-73%) content from soya bean **[21]**
Lipoid S100	Phosphatidylcholine from soya bean **[19]**
Lipoid S75-3	Phosphatidylcholine (70%-75%) content from soya bean **[49]**
SPC50	Phosphatidylcholine (50.3%) content from soya bean **[48]**
NAT 8539	Contained phosphatidylcholine 73-75%, phosphatidic acid upto 6%, lysophosphatidylcholineupto 6%, and cephalin upto 4%, sterol and natural oil upto 6% and ethanol (23-27%) **[45]**
L-α-Phosphatidylcholine (PC)	1,2-diacyl-sn-glycero-3-phosphocholine (90%) from egg yolk and soya bean **[14]**
Dipalmitoylphosphatidylcholine (DPPC)	1.2-dipalmitoyl-rac-glycero-3-phosphocholine (99%) **[50]**
Phosphatidylethanolamine (PE)	3-sn-phosphatidylethanolamine (98%) from sheep brain or bovine **[51]**

Table 3: Different types of surfactants that are used in preparation of ethosomes.

Surfactants	Type	Concentration
Tween 20	Nonionic surfactant	15-20% of the total phospholipid concentration **[17]**
Tween 60	Nonionic surfactant	Upto 50% of the total phospholipid concentration **[48]**
Tween 80	Nonionic surfactant	10-50% of the total phospholipid concentration **[57]**
Span 20,40,60,80	Nonionic surfactant	Upto 50% of the total phospholipid concentration **[58]**
N-Decylmethyl sulfoxide	Nonionic surfactant	0.35-1% of the total ethosomal formulation **[59]**
Cremophor EL-35	Nonionic surfactant	0.5-1.5% of the total ethosomal formulation **[60]**
Cremphor RL-40	Nonionic surfactant	Upto 50% of the total phospholipid concentration **[48]**
Dimethyl sulfoxide	Penetration enhancer	10% of the total ethosomal formulation **[59]**
Oleic acid	Penetration enhancer	0.5-3% of the total ethosomal formulation **[61]**
L- Menthol	Penetration enhancer	5%of the total ethosomal formulation **[62]**
Polyethylene glycol 4000	Surfactant	Phosphatidylcholine: cholesterol: polyethylene glycol 4000 at molar ratios of 2:1:1 and 6:2:1 **[14]**
Sodium cholate	Anionic surfactant	0.66% of the total ethosomal formulation **[17]**
Sodium dodecyl sulfate	Anionic surfactant	0.8% of the total ethosomal formulation **[63]**
Sodium taurocholate	Anionic surfactant	0.53% of the total ethosomal formulation **[23]**
Sodium deoxycholate	Anionic surfactant	0.8% w/v of the total ethosomal formulation **[61]**
Sodium stearate	Anionic surfactant	Phosphatidylethanolamine: cholesterol: sodium stearate at a molar ratio of 2:1:2.5 **[41]**
Hexadecyltrimethyl ammonium bromide	Cationic surfactant	1% of the total ethosomal formulation **[60]**

Table 4: Parameters for the evaluation of ethosomes.

Sr.no.	**Parameters**	**Importance**	**Method**
1	Zeta-potential	Stability of vesicles	Zeta meter
2	Size and shape	Determine skin penetration	SEM, TEM, DLS
3	Skin permeation	Determines the rate at which drugs are transported through the skin.	CLSM
4	Drug content	Important in determining how much vesicle preparation to use.	UV, HPLC
5	Entrapment efficiency	Suitability of method	Ultracentrifugation
6	Stability studies	To ascertain the shelf life of a vesicle formulation	SEM, TEM, HPLC
7	In-vitro dissolution	To determine the vesicle drug release rate	Franz diffusion cell

Table 5: Ethosomal formulations that are commercially available.

Sr.no.	Product name	Drug	Importance	Manufacturer
1	Supravir cream	Acyclovir	For herpes infection treatment	Trima, Israel
2	Nanominox	Minoxidil 4%	Hair growth promotor	Sinere, Germany
3	Cellutight EF	Combination of ingredients	Topical cellulite cream and for increase breakdown and metabolism of fat	Hampden health, USA
4	Skin genuity	Combination of ingredients	Reduces orange peel, powerful cellulite buster	Physonics, Nottingham, UK
5	Decorin cream	Combination of ingredients	Antiaging and for hyperpigmentation	Genome cosmetics, Pennsylvania, US
6	Noicellex	Combination of ingredients	Topical anticellulite cream	Novel therapeutic technologies, Israel

EVALUATION OF ETHOSOMES

Vesical Size and Zeta-Potential

Photon correlation spectroscopy (PCS) and dynamic light scattering (DLS) can be used to determine the particle size of ethosomes. The formulation's zeta potential can be measured using a zeta meter **[64]**.

Vesicle Shape

Scanning electron microscopy (SEM) and transmission electron microscopy (TEM) can be used to easily visualize ethosomes **[65]**.

Stability Studies

The size and shape of the vesicles can be used to determine their stability. The evolution of vesicle structure DLS calculates the mean size. TEM detects structural changes.

Drug Content

A UV spectrophotometer can be used to determine the drug content of ethosomes. A modified high-performance liquid chromatographic (HPLC) method can also be used to quantify this.

Drug Entrapment

The ultracentrifugation technique can be used to assess the entrapment efficiency of the ethosomes **[66]**.

Skin Permeation Studies

Using CLSM (confocal laser scanning microscopy), the ability of the ethosomal formulation to penetrate into the deep skin layers can be determined **[67]**.

Transition Temperature

Differential scanning calorimetry can be used to determine the transition temperature of vesicular lipid systems **[60]**.

Surface Tension Measurement

The surface tension activity of a drug can be measured using the ring method in a Du-Nouy ring tensiometer in an aqueous solution **[36]**.

CONCLUSION

It has been nearly two decades since the identification of ethosomes, and in that time, these nano-carriers have demonstrated their unique capacity to carry therapeutic compounds with varying physicochemical qualities via the skin for both local and systemic usage. Continuous intensive study has resulted in the creation of a modern trend of ethosomal systems known as transethosomes, which have been discovered to offer superior qualities in terms of vesicular characteristics and skin-permeation capacities to traditional ethosomes. Transethosomes provide the formulator the most freedom in modifying the ethosomal characteristics to meet the study's needs by adjusting the edge activators (surfactants) and/or penetration enhancers. Ethosome use gives benefits such as increased penetration through the skin and targeting of the deeper skin layers for the various skin conditions. Using ethosomal encapsulation, several hydrophilic medicines may be conveniently delivered transdermally. The ethosomal technique provides a wide range of applications in medication delivery that has yet to be explored.

Author(s) Contribution: data collection, M. Khizra; data curation, A. K. Aleesha; review and editing, M. Afshan; proofreading, S. Humaira.

Funding: No funding involved.

***Ethical* Approval:** Not applicable

Conflict of Interest: Nil.

Consent for Publication: All authors approved the manuscript for publication.

REFERENCE

1. 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. International journal of nanomedicine. 2016 May 25:2279-304. https://doi.org/10.2147/IJN.S105016
2. Hendriks FM, Brokken D, Oomens CW, Bader DL, Baaijens FP. The relative contributions of different skin layers to the mechanical behavior of human skin in vivo using suction experiments. Medical engineering & physics. 2006 Apr 1;28(3):259-66.
3. Welin-Berger K. Formulations, Release and Skin Penetration of Topical Anesthetics (Doctoral dissertation, Acta Universitatis Upsaliensis).

4. Kanitakis J. Anatomy, histology and immunohistochemistry of normal human skin. European journal of dermatology. 2002 Jul 2;12(4):390-401.http://www.jle.com/en/revues/ejd/e-docs/anatomy_histology_and_immunohistochemistry_of_normal_human_skin_100285/article.phtml?tab=citer
5. Tobin DJ. Biochemistry of human skin—our brain on the outside. Chemical Society Reviews. 2006;35(1):52-67. DOI: 10.1039/b505793k)
6. Costin GE, Hearing VJ. Human skin pigmentation: melanocytes modulate skin color in response to stress. The FASEB journal. 2007 Apr;21(4):976-94. https://doi.org/10.1096/fj.06-6649rev
7. Marzulli FN. Barriers to skin penetration. Journal of Investigative Dermatology. 1962 Nov 1;39(5):387-93.
8. Godin B, Touitou E. Ethosomes: new prospects in transdermal delivery. Critical reviews™ in therapeutic drug carrier systems. 2003;20(1). DOI: 10.1615/CritRevTherDrugCarrierSyst.v20.i1.20
9. 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. International Journal of drug delivery. 2013 Jan 1;5(1):28.
10. Iizhar SA, Syed IA, Satar R, Ansari SA. In vitro assessment of pharmaceutical potential of ethosomes entrapped with terbinafine hydrochloride. Journal of advanced research. 2016 May 1;7(3):453-61. https://doi.org/10.1016/j.jare.2016.03.003
11. Zhang JP, Wei YH, Zhou Y, Li YQ, Wu XA. Ethosomes, binary ethosomes and transfersomes of terbinafine hydrochloride: a comparative study. Archives of pharmacal research. 2012 Jan;35:109-17.
12. Akhtar N, Pathak K. Cavamax W7 composite ethosomal gel of clotrimazole for improved topical delivery: development and comparison with ethosomal gel. AAPS pharmscitech. 2012 Mar;13:344-55.
13. Li G, Fan Y, Fan C, Li X, Wang X, Li M, Liu Y. Tacrolimus-loaded ethosomes: physicochemical characterization and in vivo evaluation. European journal of pharmaceutics and biopharmaceutics. 2012 Sep 1;82(1):49-57. https://doi.org/10.1016/j.ejpb.2012.05.011
14. Limsuwan T, Amnuaikit T. Development of ethosomes containing mycophenolic acid. Procedia chemistry. 2012 Jan 1;4:328-35. https://doi.org/10.1016/j.proche.2012.06.046
15. Zhu X, Li F, Peng X, Zeng K. Formulation and evaluation of lidocaine base ethosomes for transdermal delivery. Anesthesia & analgesia. 2013 Aug 1;117(2):352-7. DOI: 10.1213/ANE.0b013e3182937b74
16. Lopez-Pinto JM, Gonzalez-Rodriguez ML, Rabasco AM. Effect of cholesterol and ethanol on dermal delivery from DPPC liposomes. International journal of pharmaceutics. 2005 Jul 14;298(1):1-2. https://doi.org/10.1016/j.ijpharm.2005.02.021
17. Bragagni M, Mennini N, Maestrelli F, Cirri M, Mura P. Comparative study of liposomes, transfersomes and ethosomes as carriers for improving topical delivery of celecoxib. Drug delivery. 2012 Oct 1;19(7):354-61. https://doi.org/10.3109/10717544.2012.724472
18. Touitou E, Dayan N, Bergelson L, Godin B, Eliaz M. Ethosomes—novel vesicular carriers for enhanced delivery: characterization and skin penetration properties. Journal of controlled release. 2000 Apr 3;65(3):403-18. https://doi.org/10.1016/S0168-3659(99)00222-9
19. Zhang Z, Wo Y, Zhang Y, Wang D, He R, Chen H, Cui D. In vitro study of ethosome penetration in human skin and hypertrophic scar tissue. Nanomedicine: Nanotechnology, Biology and Medicine. 2012 Aug 1;8(6):1026-33. https://doi.org/10.1016/j.nano.2011.10.006
20. Zhou Y, Wei Y, Liu H, Zhang G, Wu XA. Preparation and in vitro evaluation of ethosomal total alkaloids of Sopho0ra alopecuroides loaded by a transmembrane pH-gradient method. Aaps Pharmscitech. 2010 Sep;11:1350-8.
21. Shen L-N, Zhang Y-T, Wang Q, Xu L, Feng N-P. Enhanced in vitro and in vivo skin deposition of apigenin delivered using ethosomes. International Journal of Pharmaceutics. 2014;460(1):280-8; https://doi.org/10.1016/j.ijpharm.2013.11.017.
22. Garg V, Singh H, Bimbrawh S, Kumar Singh S, Gulati M, Vaidya Y, et al. Ethosomes and Transfersomes: Principles, Perspectives and Practices. Current Drug Delivery. 2017;14(5):613-33. DOI: https://doi.org/10.2174/1567201813666160520114436
23. Song CK, Balakrishnan P, Shim C-K, Chung S-J, Chong S, Kim D-D. A novel vesicular carrier, transethosome, for enhanced skin delivery of voriconazole: Characterization and in vitro/in vivo evaluation. Colloids and Surfaces B: Biointerfaces. 2012;92:299-304.; https://doi.org/10.1016/j.colsurfb.2011.12.004
24. Chen M, Gupta V, Anselmo AC, Muraski JA, Mitragotri S. Topical delivery of hyaluronic acid into skin using SPACE-peptide carriers. Journal of controlled release. 2014;173:67-74.; https://doi.org/10.1016/j.jconrel.2013.10.007
25. Paolino D, Lucania G, Mardente D, Alhaique F, Fresta M. Ethosomes for skin delivery of ammonium glycyrrhizinate: In vitro percutaneous permeation through human skin and in vivo anti-inflammatory activity on human volunteers. Journal of Controlled Release.2005;106(1):99-110.; https://doi.org/10.1016/j.jconrel.2005.04.007
26. Satyam G, Shivani S, Garima G. Ethosomes: A novel tool for drug delivery through the skin. J Pharm Res. 2010;3(4):688-91.
27. Zhaowu Z, Xiaoli W, Yangde Z, Nianfeng L. Preparation of matrine ethosome, its percutaneous permeation in vitro and anti-inflammatory activity in vivo in rats. Journal of liposome research. 2009;19(2):155-62. https://doi.org/10.1080/08982100902722381
28. Mishra D, Mishra PK, Dubey V, Nahar M, Dabadghao S, Jain NK. Systemic and mucosal immune response induced by transcutaneous immunization using Hepatitis B surface antigen-loaded modified liposomes. European Journal of Pharmaceutical Sciences.2008;33(4):424-33.; https://doi.org/10.1016/j.ejps.2008.01.015
29. Maestrelli F, Capasso G, González-Rodríguez ML, Rabasco AM, Ghelardini C, Mura P. Effect of preparation technique on the properties and in vivo efficacy of benzocaine-loaded ethosomes. Journal of liposome research. 2009;19(4):253-60. https://doi.org/10.3109/08982100902788408
30. Niu X-Q, Zhang D-P, Bian Q, Feng X-F, Li H, Rao Y-F, et al. Mechanism investigation of ethosomes transdermal permeation. International Journal of Pharmaceutics: X. 2019;1:100027.; https://doi.org/10.1016/j.ijpx.2019.100027
31. Finnin BC, Morgan TM. Transdermal penetration enhancers: Applications, limitations, and potential. Journal of Pharmaceutical Sciences. 1999;88(10):955-8.; https://doi.org/10.1021/js990154g
32. Touitou E, Dayan N, Bergelson L, Godin B, Eliaz M. Ethosomes — novel vesicular carriers for enhanced delivery: characterization and skin penetration properties. Journal of Controlled Release. 2000;65(3):403-18. ; https://doi.org/10.1016/S0168-3659(99)00222-9
33. Puri R, Jain S. Ethogel topical formulation for increasing the local bioavailability of 5-fluorouracil: a mechanistic study. Anti-Cancer Drugs. 2012;23(9):923-34. ; DOI: 10.1097/CAD.0b013e3283534051
34. Verma P, Pathak K. Nanosized ethanolic vesicles loaded with econazole nitrate for the treatment of deep fungal infections through topical gel formulation. Nanomedicine: Nanotechnology, Biology and Medicine. 2012;8(4):489-96.; https://doi.org/10.1016/j.nano.2011.07.004
35. Rao Y, Zheng F, Zhang X, Gao J, Liang W. In Vitro Percutaneous Permeation and Skin Accumulation of Finasteride Using Vesicular Ethosomal Carriers. AAPS PharmSciTech. 2008;9(3):860-5. DOI https://doi.org/10.1208/s12249-008-9124-y
36. Dayan N, Touitou E. Carriers for skin delivery of trihexyphenidyl HCl: ethosomes vs. liposomes. Biomaterials. 2000;21(18):1879-85.; https://doi.org/10.1016/S0142-9612(00)00063-6
37. Dubey V, Mishra D, Jain NK. Melatonin loaded ethanolic liposomes: Physicochemical characterization and enhanced transdermal delivery. European Journal of Pharmaceutics and

Biopharmaceutics. 2007;67(2):398-405.; https://doi.org/10.1016/j.ejpb.2007.03.007
38. Prasanthi D, Lakshmi PK. Development of ethosomes with taguchi robust design-based studies for transdermal delivery of alfuzosin hydrochloride. International Current Pharmaceutical Journal. 2012;1(11):370-5.
39. López-Pinto JM, González-Rodríguez ML, Rabasco AM. Effect of cholesterol and ethanol on dermal delivery from DPPC liposomes. International Journal of Pharmaceutics. 2005;298(1):1-12. https://doi.org/10.1016/j.ijpharm.2005.02.021
40. Jain S, Tiwary AK, Sapra B, Jain NK. Formulation and evaluation of ethosomes for transdermal delivery of lamivudine. AAPS PharmSciTech. 2007;8(4):249. DOIhttps://doi.org/10.1208/pt0804111
41. Fang Y-P, Tsai Y-H, Wu P-C, Huang Y-B. Comparison of 5-aminolevulinic acid-encapsulated liposome versus ethosome for skin delivery for photodynamic therapy. International Journal of Pharmaceutics.2008;356(1):144-52. https://doi.org/10.1016/j.ijpharm.2008.01.020
42. Nasr S, Rady M, Gomaa I, Syrovets T, Simmet T, Fayad W, et al. Ethosomes and lipid-coated chitosan nanocarriers for skin delivery of a chlorophyll derivative: A potential treatment of squamous cell carcinoma by photodynamic therapy. International Journal of Pharmaceutics. 2019;568:118528.; https://doi.org/10.1016/j.ijpharm.2019.118528
43. Mbah CC, Builders PF, Agubata CO, Attama AA. Development of ethosomal vesicular carrier for topical application of griseofulvin: effect of ethanol concentration. Journal of Pharmaceutical Investigation.2019;49(1):27-36.; DOIhttps://doi.org/10.1007/s40005-017-0367-z
44. Yücel Ç, Şeker Karatoprak G, Değim İT. Anti-aging formulation of rosmarinic acid-loaded ethosomes and liposomes. Journal of Microencapsulation. 2019;36(2):180-91. https://doi.org/10.1080/02652048.2019.1617363
45. Verma DD, Fahr A. Synergistic penetration enhancement effect of ethanol and phospholipids on the topical delivery of cyclosporin A. Journal of Controlled Release. 2004;97(1):55-66. https://doi.org/10.1016/j.jconrel.2004.02.028
46. Paolino D, Celia C, Trapasso E, Cilurzo F, Fresta M. Paclitaxel-loaded ethosomes®: Potential treatment of squamous cell carcinoma, a malignant transformation of actinic keratoses. European Journal of Pharmaceutics and Biopharmaceutics. 2012;81(1):102-12. https://doi.org/10.1016/j.ejpb.2012.02.008
47. Caddeo C, Sales OD, Valenti D, Saurí AR, Fadda AM, Manconi M. Inhibition of skin inflammation in mice by diclofenac in vesicular carriers: Liposomes, ethosomes and PEVs. International Journal of Pharmaceutics. 2013;443(1):128-36. https://doi.org/10.1016/j.ijpharm.2012.12.041
48. 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;10:4239-53. http://dx.doi.org/10.2147/IJN.S83402
49. Choi J-H, Cho S-H, Yun J-J, Yu Y-B, Cho C-W. Ethosomes and Transfersomes for Topical Delivery of Ginsenoside Rh1 from Red Ginseng: Characterization and In Vitro Evaluation. Journal of Nanoscience and Nanotechnology. 2015;15(8):5660-2. https://doi.org/10.1166/jnn.2015.10462
50. Bodade SS, Shaikh KS, Kamble MS, Chaudhari PD. A study on ethosomes as mode for transdermal delivery of an antidiabetic drug. Drug Delivery. 2013;20(1):40-6. https://doi.org/10.3109/10717544.2012.752420
51. Fang Y-P, Huang Y-B, Wu P-C, Tsai Y-H. Topical delivery of 5-aminolevulinic acid-encapsulated ethosomes in a hyperproliferative skin animal model using the CLSM technique to evaluate the penetration behavior. European Journal of Pharmaceutics and Biopharmaceutics. 2009;73(3):391-8. https://doi.org/10.1016/j.ejpb.2009.07.011
52. Bayindir ZS, Yuksel N. Characterization of niosomes prepared with various nonionic surfactants for paclitaxel oral delivery. Journal of Pharmaceutical Sciences. 2010;99(4):2049-60. https://doi.org/10.1002/jps.21944
53. Mahale NB, Thakkar PD, Mali RG, Walunj DR, Chaudhari SR. Niosomes: Novel sustained release nonionic stable vesicular systems — An overview. Advances in Colloid and Interface Science. 2012;183-184:46-54. https://doi.org/10.1016/j.cis.2012.08.002
54. Uchegbu IF, Florence AT. Non-ionic surfactant vesicles (niosomes): Physical and pharmaceutical chemistry. Advances in Colloid and Interface Science. 1995;58(1):1-55. https://doi.org/10.1016/0001-8686(95)00242-I
55. Sudhakar K, Mishra V, Jain S, Rompicherla NC, Malviya N, Tambuwala MM. Development and evaluation of the effect of ethanol and surfactant in vesicular carriers on Lamivudine permeation through the skin. International Journal of Pharmaceutics. 2021;610:121226. https://doi.org/10.1016/j.ijpharm.2021.121226
56. Tosato MG, Maya Girón JV, Martin AA, Krishna Tippavajhala V, Fernández Lorenzo de Mele M, Dicelio L. Comparative study of transdermal drug delivery systems of resveratrol: High efficiency of deformable liposomes. Materials Science and Engineering: C. 2018;90:356-64. https://doi.org/10.1016/j.msec.2018.04.073
57. Zhao Y-Z, Lu C-T, Zhang Y, Xiao J, Zhao Y-P, Tian J-L, et al. Selection of high efficient transdermal lipid vesicle for curcumin skin delivery. International Journal of Pharmaceutics. 2013;454(1):302-9. https://doi.org/10.1016/j.ijpharm.2013.06.052
58. Ascenso A, Raposo S, Batista C, Cardoso P, Mendes T, Praça FG, et al. Development, characterization, and skin delivery studies of related ultradeformable vesicles: transfersomes, ethosomes, and transethosomes. Int J Nanomedicine. 2015;10:5837-51. http://dx.doi.org/10.2147/IJN.S86186
59. Ainbinder D, Touitou E. A new approach for skin tumor treatment: from delivery system characterization to in vivo evaluation. Drug Delivery and Translational Research. 2011;1(1):53-65. DOIhttps://doi.org/10.1007/s13346-010-0006-y
60. Meng S, Chen Z, Yang L, Zhang W, Liu D, Guo J, et al. Enhanced transdermal bioavailability of testosterone propionate via surfactant-modified ethosomes. Int J Nanomedicine. 2013;8:3051-60. http://dx.doi.org/10.2147/IJN.S46748
61.Ma M, Wang J, Guo F, Lei M, Tan F, Li N. Development of nanovesicular systems for dermal imiquimod delivery: physicochemical characterization and in vitro/in vivo evaluation. Journal of Materials Science: Materials in Medicine. 2015;26(6):192. DOIhttps://doi.org/10.1007/s10856-015-5524-1
62. Nainwal N, Jawla S, Singh R, Saharan VA. Transdermal applications of ethosomes – a detailed review. Journal of Liposome Research. 2019;29(2):103-13. https://doi.org/10.1080/08982104.2018.1517160
63. Guo F, Wang J, Ma M, Tan F, Li N. Skin targeted lipid vesicles as novel nano-carrier of ketoconazole: characterization, in vitro and in vivo evaluation. Journal of Materials Science: Materials in Medicine. 2015;26(4):175. DOI https://doi.org/10.1007/s10856-015-5487-2
64. Rakesh R, Anoop KR. Formulation and optimization of nano-sized ethosomes for enhanced transdermal delivery of cromolyn sodium. Journal of pharmacy & bioallied sciences. 2012;4(4):333-40. DOI: 10.4103/0975-7406.103274
65. Verma P, Pathak K. Therapeutic and cosmeceutical potential of ethosomes: An overview. Journal of advanced pharmaceutical technology & research. 2010;1(3):274-82. DOI: 10.4103/0110-5558.72415
66. Elsayed MMA, Abdallah OY, Naggar VF, Khalafallah NM. Deformable liposomes and ethosomes: Mechanism of enhanced skin delivery. International Journal of Pharmaceutics. 2006;322(1):60-6. https://doi.org/10.1016/j.ijpharm.2006.05.027
67. Toll R, Jacobi U, Richter H, Lademann J, Schaefer H, Blume-Peytavi U. Penetration Profile of Microspheres in Follicular Targeting of Terminal Hair Follicles. Journal of Investigative Dermatology. 2004;123(1):168-76. https://doi.org/10.1111/j.0022-202X.2004.22717.x