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REVIEW ARTICLE

ETHOSOMES: A NOVEL APPROACH IN TRANSDERMAL DELIVERY OF THE DRUGS

* [1]Srujan Kumar M, [1]Samitha Kumari, [1]Keerthi Reddy, [1]Bharath Kumar G, [2]Prof. Satyanand Tyagi, [3]Patel Chirag J

[1]Samskruti College of Pharmacy, Ghatkesar, Ranga Reddy District, Hyderabad, Andhra Pradesh, India-501301.

[2]President & Founder, Tyagi Pharmacy Association (TPA) & Scientific Writer (Pharmacy), Chattarpur, New Delhi, India-110074.

[3]Editor-in-Chief, Tyagi Pharmacy Association (TPA) & Scientific Writer (Pharmacy), Chattarpur, New Delhi, India-110074.

ABSTRACT

Skin acts as a major target as well as a principal barrier for topical/transdermal drug delivery. Despite the many advantages of this system, the major obstacle is the low diffusion rate of drugs across the stratum corneum. Several methods have been tried to increase the permeation rate of drugs temporarily. One simple and convenient approach is application of drugs in formulation with elastic vesicles or skin enhancers. Vesicular system is one of the most convenient methods for transdermal delivery of active substances and in that ethosomes are most useful vesicular systems. Ethosomal carriers are systems containing soft vesicles, composed of hydroalcoholic or hydro/glycolic phospholipid in which the concentration of alcohols is relatively high. The high concentration of ethanol brings increase in fluidity of lipids hence increase in permeability of the skin and improves the drug penetration. Ethosomal formulation may contain many drugs such as acyclovir, salbutamol, Insulin, cyclosporine, fluconazole, minodixil, etc. These are prepared by hot method and cold methods. The size of Ethosomal formulation can be decreased by sonication and extrusion method. The high concentration of ethanol makes the ethosomes unique and useful for transcellular delivery, delivery of hormones, anti-arthritis, anti-HIV etc.

Thus, it can be a logical conclusion that ethosomal formulation possesses promising future in effective dermal/transdermal delivery of bioactive agents.

KEY WORDS: Stratum Corneum (SC), Liposome, Classic Liposomes, Ethosomes, Ethanol, Phospholipid, Vesicles, Transdermal Drug Delivery.

INTRODUCTION:

Transdermal administration of drugs is generally limited by the barrier function of the skin. Vesicular systems are one of the most controversial methods for transdermal delivery of active substances[1]. The interest in designing transdermal delivery systems was relaunched after the discovery of elastic vesicles: Ethosomes. Ethosomes are novel carrier system used for delivery of drugs having low penetration through the biological membrane mainly skin. Ethosomes are the slight modification of well-established drug carrier liposomes[2].

ADVANTAGES OF ETHOSOMAL DRUG DELIVERY[3, 4, 5] IN COMPARISON TO OTHER TRANSDERMAL & DERMAL DELIVERY SYSTEM:

- Ethosomes have enhanced permeation of drug through skin for transdermal drug delivery.
- The delivery of large molecules (peptides, protein molecule) is possible.
- It contains non-toxic raw material in formulation.
- High patient compliance- The ethosomal drug is administrated in semisolid form (gel or cream) hence producing high patient compliance.
- The Ethosomal system is passive, non-invasive and is available for immediate commercialization.
- Ethosomal drug delivery system can be applied widely in Pharmaceutical, Veterinary, Cosmetic fields.
- Simple method for drug delivery in comparison to Iontophoresis and Phonophoresis and other complicated methods.
- Low risk profile- the technology has no large-scale drug development risk since the toxicological profiles of the ethosomal components are well documented in the scientific literature.
- High market attractiveness for products with proprietary technology.
- Relatively simple to manufacture with no complicated technical investments required for production of Ethosomes.
- The Ethosomal drug delivery system offers a passive, non-invasive approach and it is readily available for the immediate commercialization

*Corresponding author: Srujan Kumar M,

STRUCTURE OF SKIN:

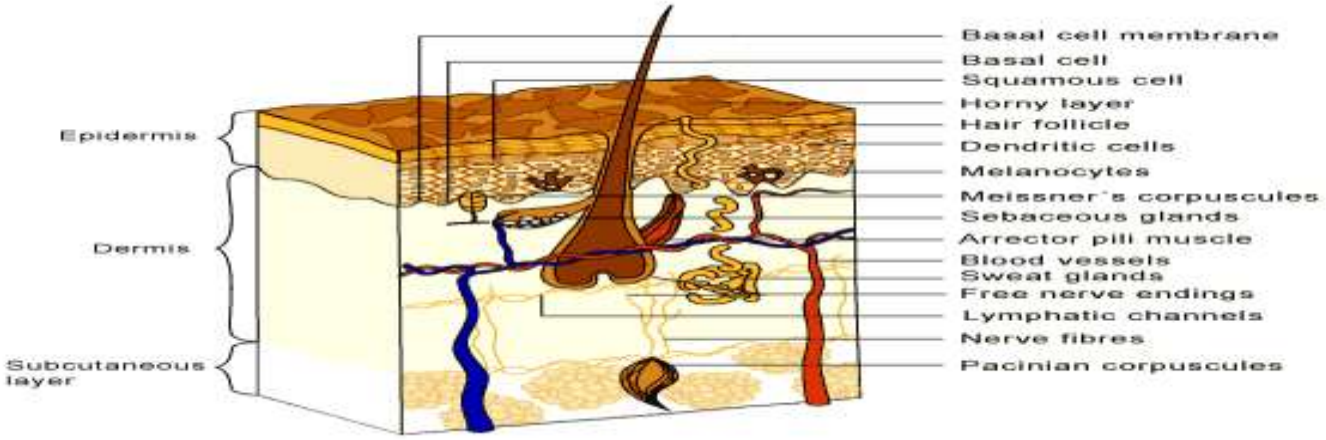


Figure 1: Structure of skin

Stratum corneum is the outermost layer of the epidermis. It consists of 10 to 25 layers of dead, elongated, fully keratinized corneocytes, which are embedded in a matrix of lipid bilayers[6, 7]. It has been shown that the stratum corneum is the main barrier to penetration through the skin. When a topical formulation is placed on the skin, the active drug is required to penetrate through the stratum corneum into the viable tissue.

The limiting factor for these processes is the slow diffusion through the dead horny layer of skin.7-10 Stratum corneum behaves as a hydrophobic membrane. The rates of permeation of skin by low and high molecular weight organic non-electrolytes are mostly determined within the stratum corneum[8, 9].

The molecular structures and appearance of the molecules can be examined using molecular modeling computer programs.

MAIN ROUTES OF PENETRATION:

Under normal conditions, the main route is observed through the intercellular spaces or lipid bilayers[10, 11]. The diffusional path length is therefore much longer than simple thickness of the stratum corneum (20-30 mm). The penetration through skin is also affected by several biological factors such as skin age, body site, skin condition and diseases, water content of the skin or hydration. The intercellular spaces contain structured lipids/proteins and a diffusing molecule has to cross a variety of lipophilic and hydrophilic domains before reaching to the stratum corneum and viable epidermis junction. Although the nature of the barrier is very heterogeneous, the diffusion through the skin can be described by simple Fick's laws[12].

To overcome the stratum corneum barrier, various mechanisms have been investigated, including use of chemical or physical enhancers such as iontophoresis, sonophoresis, etc. Liposomes, niosomes, transferosomes and ethosomes also have the potential of overcoming the skin barrier and have been reported to enhance permeability of drug through the stratum corneum barrier. The non-invasive approaches for providing transdermal drug delivery of various therapeutic substances are:

DRUG AND VEHICLE INTERACTIONS:

- Selection of correct drug or prodrug
- Chemical potential adjustment
- Ion pairs and complex coacervates
- Eutectic systems

STRATUM CORNEUM MODIFICATION:

- Hydration
- Chemical penetration enhancers

STRATUM CORNEUM BYPASSED OR REMOVED:

- Micro needle array
- Stratum corneum ablated
- Follicular delivery

ETHOSOMES:

Ethosomes are lipid vesicles containing phospholipids, alcohol (ethanol and isopropyl alcohol) in relatively high concentration and water[13]. Ethosomes are soft vesicles made of phospholipids and ethanol (in higher quantity) and water.

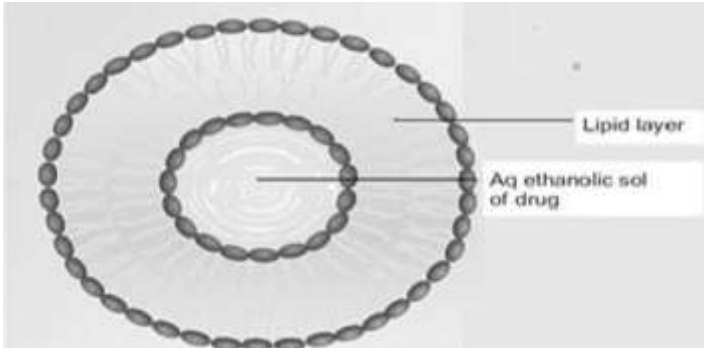
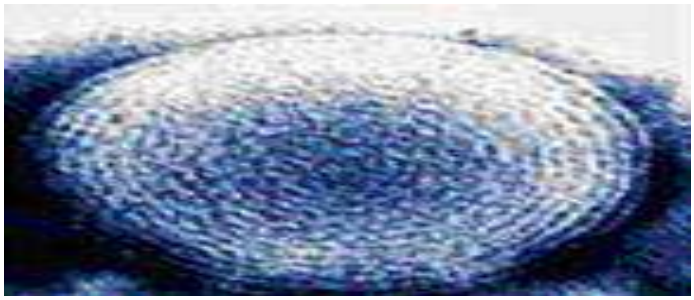


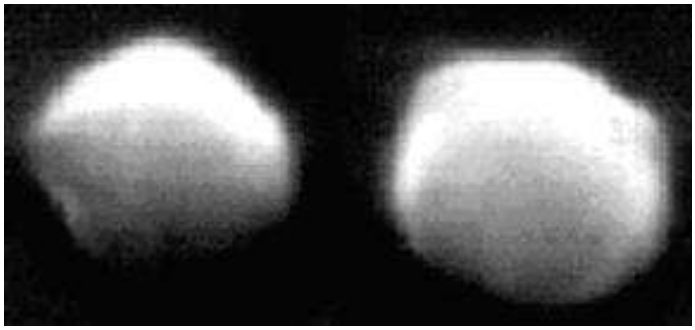
Figure 2: Structure of Ethosomes

The size range of ethosomes may vary from tens of nanometers to microns (μ) [14]. Ethosomes permeate

through the skin layers more rapidly and possess significantly higher transdermal flux in comparison to conventional liposomes[14, 15]. Visualization of ethosomes is shown in Figure 2. Although, the exact mechanism for better permeation into deeper skin layers from ethosomes is still not clear. The synergistic effects of combination of phospholipids and high concentration of ethanol in vesicular formulations have been suggested to be responsible for deeper distribution and penetration in the skin lipid bi-layers.



Visualization of ethosome (TEM Magnification: 315000)



Visualization of ethosome (SEM X 100, 000)

Figure 3: Visualization of ethosomal vesicles

The ethosomes are vesicular carrier comprise of hydroalcoholic or hydro/alcoholic/glycolic phospholipid in which the concentration of alcohols or their combination is relatively high. Typically, ethosomes may contain phospholipids with various chemical structures like phosphatidylcholine (PC), hydrogenated PC, phosphatidic acid (PA), phosphatidylserine (PS), phosphatidylethanolamine (PE), phosphatidylglycerol (PPG), phosphatidylinositol (PI), hydrogenated PC, alcohol (ethanol or isopropyl alcohol), water and propylene glycol (or other glycols)[16,17].

Such a composition enables delivery of high concentration of active ingredients through skin. Drug delivery can be modulated by altering alcohol: water or alcohol-polyol: water ratio. Some preferred phospholipids are soya phospholipids such as Phospholipon 90 (PL-90). It is usually employed in a range of 0.5-10% w/w. Cholesterol at concentrations ranging between 0.1-1 percent can also be added to the preparation1. Examples of alcohols, which can be used, include ethanol and isopropyl alcohol. Among glycols, propylene glycol and Transcutol are generally used. In addition, non-ionic surfactants (PEG-alkyl ethers) can be combined with the phospholipids in these preparations. Cationic lipids like cocoamide, POE alkyl amines, dodecylamine, cetrimide etc. can be added too. The concentration of alcohol in the final product may range from 20 to 50%. The concentration of the non-aqueous phase (alcohol and glycol combination) may range between 22 to 70% [18, 19]. Different additives used in the ethosomal formulation are presented in the table 1 below.

COMPOSITION:

Table 1: **Tabular form represents different additives used in the ethosomal formulation**

Class	Examples	Uses
Phospholopids	Soya phosphatidyl choline; Egg phosphatidyl choline; Diestearyl phopshatidyl choline	Vesicle forming components
Polyglcerol	Propylene glycol; Transcutol RTM	As a skin penetration enhancer
Alcohol	Ethanol; Isopropyl alcohol	For providing the softness for vesicle membrane; As a skin penetration enhancer
Cholesterol	Cholesterol	For providing the stability for vesicle membrane
Dyes	Rhodamine 123; Rhodamine red	For characterization studies
Vehicles	Carbopol D934	As a gel former

MECHANISM OF DRUG PENETRATION[20]:

The main advantage of ethosomes over liposomes is the increased permeation of the drug. The mechanism of the drug absorption from ethosomes is not clear. The drug absorption probably occurs in following two phases[21, 22].

1. Ethanol Effect: Ethanol acts as a penetration enhancer through the skin. The mechanism of its penetration enhancing effect is well known. Ethanol penetrates into intercellular lipids and increases the fluidity of cell membrane lipids and decrease the density of lipid multilayer of cell membrane.

2. Ethosomal Effect: Increased cell membrane lipid fluidity caused by the ethanol of ethosomes results increased skin permeability. So the ethosomes permeates very easily inside the deep skin layers, where it got fused with skin lipids and releases the drugs into deep layer of skin.

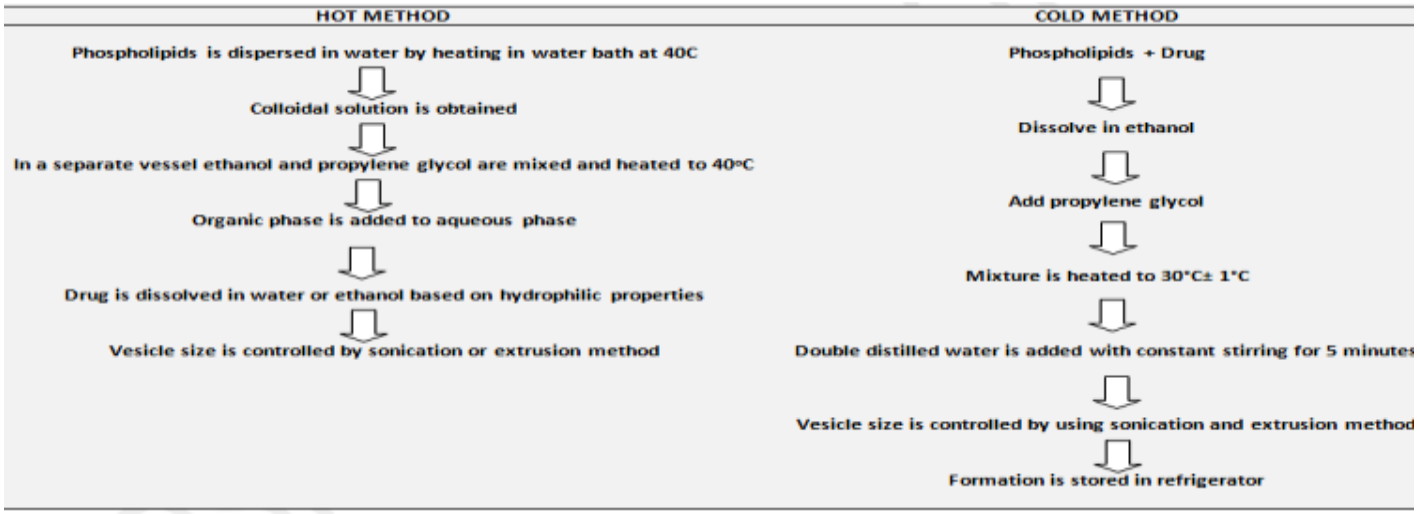
Methods of Preparation[18, 23]: There are two methods which can be used for the formulation and preparation of ethosomes. Both of the methods are very simple and convenient and do not involve any sophisticated instrument or complicated process.

Ethosomes can be formulated by following two methods: The formulation of ethosomes involves hot and cold method.

1. **Hot Method:** In this method disperse phospholipid in water by heating in a water bath at 400°C until a colloidal solution is obtained. In a separate vessel properly mix ethanol and propylene glycol and heat up to 400°C. Add the organic phase into the aqueous phase. Dissolve the drug in water or ethanol depending on its solubility. The vesicle size of ethosomal formulation can be decreased to the desire extent using probe sonication or extrusion method.

2. **Cold Method:** This is the most common and widely used method for the ethosomal preparation. Dissolve phospholipids, drug and other lipid materials in ethanol in a covered vessel at room temperature with vigorous stirring. Add propylene glycol or other polyglycol during stirring. Heat the mixture up to 300°C in a water bath. Heat the water up to 300°C in a separate vessel and add to the mixture and then stir it for 5 min in a covered vessel. The vesicle size of ethosomal formulation can be decreased to desire extend using sonication or extrusion method. Finally, the formulation should be properly stored under refrigeration.

FLOW CHART REPRESENTATION OF HOT METHOD AND COLD METHOD



EVALUATION:

The methods of evaluation for ethosomes are discussed below:

- **Vesicle Shape[24]:** Ethosomes can be easily visualized by using transmission electron microscopy (TEM) and by scanning electron microscopy (SEM).
- **Vesicle Size and Zeta Potential[23]:** Particle size of the ethosomes can be determined by dynamic light scattering (DLS) and photon correlation spectroscopy (PCS). Zeta potential of the formulation can be measured by Zeta meter.
- **Transition Temperature[22]:** The transition temperature of the vesicular lipid systems can be determined by using differential scanning calorimetry (DSC).
- **Drug Entrapment[25]:** The entrapment efficiency of ethosomes can be measured by the ultracentrifugation technique.
- **Drug Content[26]:** Drug content of the ethosomes can be determined using UV spectrophotometer. This can also be quantified by a modified high performance liquid chromatographic method.
- **Surface Tension Measurement[27]:** The surface tension activity of drug in aqueous solution can be measured by the ring method in a Du Nouy ring tensiometer.
- **Stability Studies[28]:** The stability of vesicles can be determined by assessing the size and structure of the vesicles over time. Mean size is measured by DLS and structure changes are observed by TEM.

A) Stability studies for Ethosomal cream and gel Percent entrapment

The optimized ethosomal formulation was kept in sealed vials (10 ml) at 5±3ºC and at 25±2ºC for 1, 2 and 3 months to study the effect of different storage conditions on percent entrapment.

B) Stability studies of ethosomal gel

1. Physical Appearance: Optimized gel was kept for 1, 2 and 3 months under 5ºC ± 3ºC as well as 25ºC ± 2 ºC

temperature conditions to study the effect of storage conditions on their physical appearance.

2. Content Uniformity of Gel: The uniformity of drug content in ethosomal gel formulation was evaluated in triplicate. For this investigation ethosomal gel (1.0g) was kept in a beaker containing 1000 ml of phosphate buffer pH (7.4) containing SLS 2.5%w/v for 48 h on magnetic stirrer. Solution was filtered and analyzed by UV spectrophotometer at λmax 290nm.

3. Skin Permeation Studies[21]: The ability of the ethosomal preparation to penetrate into the skin layers can be determined by using confocal laser scanning microscopy (CLSM).

Evaluation parameters and instrument/methods used in Ethosomes are shown in the below table 2.

Table 2: Evaluation parameters and instrument/methods used in ethosomes

PARAMETERS	INSTRUMENTS/METHODS USED	IMPORTANCE
Vesicle Shape	Transmission Electron Microscopy (TEM) Scanning Electron Microscopy (SEM)	Determines skin penetration
Vesicle Size and Zeta Potential	Dynamic Light Scattering (DLS), Photon Correlation Spectroscopy (PCS) and Zeta Meter	Determines skin penetration and stability of vesicles
Transition Temperature	Differential Scanning Calorimetry (DSC)	Determines transition temperature of lipid vesicles
Drug Entrapment	Ultracentrifugation Technique	Suitability of method
Drug Content	UV Spectrophotometer, High Performance Liquid Chromatographic Method (HPLC)	Important in deciding the amount of vesicle preparation to be used
Surface Tension Measurement	Ring Method in a Du Nouy ring tensiometer	Determines surface tension activity of drug in aqueous solution
Stability Studies	Dynamic Light Scattering (DLS), Transmission Electron Microscopy (TEM)	To determine the shelf-life of vesicle formulation
Skin Permeation Studies	Confocal Laser Scanning Microscopy (CLSM)	Determines rate of drug transport through skin
In-vitro dissolution	Franz diffusion cell	Determines the drug release rate from vesicle

THERAPEUTIC APPLICATION OF ETHOSOMES:

1. In the treatment herpetic infection-

5% acyclovir ethosomal preparation compared to the 5 % acyclovir cream showed significant improvements in treatment of herpetic infections.

2. Transcellular Delivery- **Ethosomes** as compared to the marketed formulation suggested ethosomes to be an attractive clinical alternative for anti-HIV therapy.

3. Ethosomes are used in pilosabeceous targeting[31, 32]. Ethosomes, the high ethanol containing vesicles are able to penetrate the deeper layers of the skin and hence appear to be vesicles of choice for transdermal drug delivery of hydrophilic and impermeable drugs through the skin.

4. Transdermal Delivery of Hormones. Oral administration of hormones is associated with problems like high first pass metabolism, low oral bioavailability and several dose dependent side effects. The risk of failure of treatment is known to increase with each pill missed.

5. Delivery of Anti-Arthritis Drug Topical delivery of anti-arthritis drug is a better option for its site-specific delivery and overcomes the problem associated with conventional oral therapy.

6. Topical Delivery of DNA: Another important application of ethosomes is their use for topical delivery of DNA molecules. Touitou *et al* demonstrated that better intracellular uptake of DNA, better delivery and expression of genes in skin cells can be achieved by ethosomal formulation[30]. Hence was concluded that ethosomes can be used carrier for gene therapy application that require transient expression of genes.

7. Delivery of Antibiotics: Conventional oral therapy of antibiotics is usually associated with several allergic reactions along with side effects and low therapeutic efficacy. Topical delivery of antibiotics is a better choice to increase therapeutic efficacy, but conventional topical preparation possess low permeability to deep skin layers and sub dermal tissues. Ethosomes formulation of antibiotics could be highly efficient and overcome the problems associated with conventional therapy since they

penetrate rapidly into deeper layer of skin and suppress infection at their root[31, 33, 34].

8. Delivery of HIV Drugs[29]: An effective antiretroviral therapy is required on a long term basis and is associated with strong side effects. Adequate zero order delivery of zidovudine,

Lamivudine a potent antiviral agent is required to maintain expected anti – AIDS effect.

Subheet Jain et al reported that ethosomal formulation of the above drugs prolong the release with increased Transdermal flux. Conventional topical preparation acyclovir an topically used antiviral drug for treatment of herpes labials show low therapeutic efficiency due to poor permeation through skin as replication of virus take places at the basal dermis. Ethosomal formulation of acyclovir show high therapeutic efficiency with shorter healing time and higher percentage of abortive lesions.

9. Delivery of Problematic Drug Molecules: Oral delivery of large biogenic molecules such as peptides or proteins and insulin is difficult because they are completely degraded in the

GIT tract hence transdermal delivery is a better alternative. But conventional transdermal formulation of biogenic molecules such as peptides or protein and insulin has poor permeation. Formulating these above molecules into ethosomes significantly increase permeation and therapeutic efficacy.

ETHOSOMES AS A DRUG CARRIER:

Ethosomes can be used for many purposes in drug delivery. Ethosomes are mainly used as replacement of liposomes. Ethosomes can be used for transdermal delivery of hydrophilic and impermeable drugs through the skin. Following drugs have been used with ethosomal carrier.

FUTURE PERSPECTIVE:

For transdermal delivery of drugs, stratum corneum is the main barrier layer for penetration of drug. Introduction of ethosomes has initiated a new area in vesicular research. Ethosomes has shown promising result and potential for delivery of various agents more effectively. Better control over drug release, non – invasive delivery of small, medium and large size drug molecules can be achieved by ethosomes. Ethosomes can be the promising tool for dermal/transdermal delivery of various agents and can be a alternate formulation for problematic drugs.

CONCLUSION:

Ethosomes has initiated a new area in vesicular research for transdermal drug delivery. Ethosomes are soft, malleable vesicles and possible carrier for transportation of drugs. Ethosomes are characterized by simplicity in their preparation, safety and efficacy and can be tailored for enhanced skin permeation of active drugs. Ethosomes have been found to be much more efficient at delivering drug to the skin, than either liposomes or hydro-alcoholic solution. Ethosomes have been tested to encapsulate hydrophilic drugs, cationic drugs, proteins and peptides. Ethosomal carrier opens new challenges and opportunities for the development of novel improved therapies.

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