Modernize Devising Method of Ethosomes

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Abstract: Ethosomes are noninvasive drug delivery system that enables release of drug to deep skin layers or systemic circulation. Ethosomes are the ethanolic phospholipid vesicles which are used mainly for transdermal delivery of drugs. Over the year it has showed promising result in comparison to oral drug delivery system as it eliminates gastrointestinal interferences and first pass metabolism of the drug. Ethosomes have higher penetration rate through the skin as compared to liposomes hence these can be used widely in place of liposomes. Ethosomes, noninvasive delivery carriers that enable drugs to reach deep into the skin layers or the systemic circulation made up of phospholipids, high concentration of ethanol and water. This review article summarizes structure, advantages, disadvantages, composition and mechanism of drug penetration method of preparation, evaluation and applications of ethosomes.

Keywords: Transdermal Skin, Phospholipid, ethanol, Ethosomes

1. Introduction

Transdermal drug delivery system (TDDS) showed promising result in comparison to oral drug delivery system as it eliminates gastrointestinal interferences and first pass metabolism of the drug but the main drawback of TDDS is it encounters the barrier properties of the Stratum Corneum

To improve the permeation of drugs through the skin various mechanisms have been investigated, including use of chemical or physical enhancers, such as iontophoresis, sonophoresis, etc

Ethosomes also have been reported to enhance permeability of drug through the stratum corneum barrier. Permeation enhancers increase the permeability of the skin, so that the drugs can cross through the skin easily.

Ethosomes

Ethosomes are mainly used for the delivery of drugs through transdermal route. The transdermal delivery is one of the most important routes of drug administration. The high concentration of ethanol makes the ethosomes unique, as ethanol is known for its disturbance of skin lipid bilayer organization; therefore, when integrated into a vesicle membrane, it gives that vesicle the ability to penetrate the stratum corneum. Ethosomes are the slight modification of well established drug carrier liposome. Ethosomes are lipid vesicles containing phospholipids, alcohol (ethanol and isopropyl alcohol) in relatively high concentration and water. Ethosomes are soft vesicles made of phospholipids and ethanol (in higher quantity) and water. The size range of ethosomes may vary from tens of nanometers (nm) to microns (µ) ethosomes permeate through the skin layers more rapidly and possess significantly higher transdermal flux

Figure 1: Structure of Ethosomes
Advantages of Ethosomal Drug Delivery
In comparison to other transdermal & dermal delivery systems, Ethosomal drug delivery systems contain several advantages. Few advantages are:
1) Delivery of large molecules (peptides, protein molecules) is possible.
2) It contains non-toxic raw material in formulation.
3) Enhanced permeation of drug through skin for transdermal drug delivery.
4) Ethosomal drug delivery system can be applied widely in Pharmaceutical, Veterinary, Cosmetic fields.
5) High patient compliance: The ethosomal drug is administrated in semisolid form (gel or cream) hence producing high patient compliance.
6) Simple method for drug delivery in comparison to Iontophoresis and Phonophoresis and other complicated methods.
7) The Ethosomal system is passive, non-invasive and is available for immediate commercialization

Disadvantages of Ethosomal Drug Delivery
They required High blood levels cannot be administered – limited only to potent molecules, those requiring a daily dose of 10mg or less.
1) Poor yield.
2) Adequate solubility of the drug in both lipophilic and aqueous environments to reach dermal microcirculation and gain access to the systemic circulation.
3) The molecular size of the drug should be reasonable that it should be absorbed percutaneously.
4) Adhesive may not adhere well to all types of skin.
5) May not be economical.
6) Skin irritation or dermatitis due to excipients and enhancers of drug delivery systems

Composition of Ethosomes
Ethosomes are composed mainly of phospholipids, (phosphatidylcholine, phosphatidylserine, phosphatidic acid), high concentration of ethanol and water. The nonaqueous phase range between 22 % to 70 %. The alcohol may be ethanol or isoproply alcohol. The high concentration of ethanol makes the ethosomes unique, as ethanol is known for its disturbance of skin lipid bilayer organization; therefore, when integrated into a vesicle membrane, it gives that vesicle the ability to penetrate the stratum corneum. Also, because of their high ethanol concentration, the lipid membrane is packed less tightly than conventional vesicles but has equivalent stability, allowing a more malleable structure and improves drug distribution ability in stratum corneum lipids.

Advantage of high alcohol content
Ethanol is an established efficient permeation enhancer and is present in quite high concentration (20-50%) in ethosomes. However, due to the interdigitation effect of ethanol on lipid bilayers, it was commonly believed that vesicles could not coexist with high concentration of ethanol. Touitou [20] discovered and investigated lipid vesicular systems embodying ethanol in relatively high concentration and named them ethosomes. The basic difference between liposomes and ethosomes lies in their composition. The synergistic effect of combination of relatively high concentration of ethanol (20- 50%) in vesicular form in ethosomes was suggested to be the main reason for their better skin permeation ability. The high concentration of ethanol (20-50%) in ethosomal formulation could disturb the skin lipid bilayer organization. Therefore, when integrated into a vesicle membrane, it could give an ability to the vesicles to penetrate the stratum corneum. Furthermore, due to high ethanol concentration the ethosomal lipid membrane was packed less tightly than conventional vesicles but possessed equivalent stability. This allowed a softer and malleable structure giving more freedom and stability to its membrane, which could squeeze through small openings created in the disturbed SC lipids. In addition, the vesicular nature of ethosomal formulations could be modified by varying the ratio of components and chemical structure of the phospholipids. The versatility of ethosomes for systemic delivery is evident from the reports of enhanced delivery of quite a few drugs like acyclovir, minoxidil, trihexyphenidyl, testosterone, cannabidol and zidovudine.

Skin
The skin is the largest organ of the body and has surface area about 1.5-2cm2 in adult. There are 2 important layer: a) Epidermis b. Dermis The epidermis is most superficial layer of the skin and is composed of stratified keratinized squamous epithelium.

The epidermis is composed of 4-5 layers depending on the region of skin being considered. The layers area) –

a) Cornified layer (stratum corneum)

b) Translucent layer (stratum granulose)

c) Spinous layer (stratum spinosum)

d) Germinal layer (stratum basale)

a) Stratum corneum
It is composed of 10-30 layers of polyhedral anucleated corneocytes, with the palms and soles having the most layers. Corneocytes are surrounded by a protein envelope, filled with waterretaining keratin proteins, attached through corneodesmosomes and surrounded in the extracellular space by stacked layer of lipid. The stratum corneum layer plays an important role in the barrier function of topical/ transdermal drug delivery. Human skin has selective permeability for drug, lipophilic drug can pass through the skin but the drug which are hydrophilic in nature cannot pass through skin.Water soluble drug show either very less or no permeation. To improve the permeation of drug through the skin various mechanisms have been investigated, including use of chemical or physical enhancer such as iontophoresis or sonophoresis. Liposomes, niosomes, transferosomes and ethosome are also enhancing the permeability of drug through stratum corneum. Permeability enhancer increase the permeability of the skin so that the drug can cross the through the skin easily. Ethosomes can enhance permeation through the stratum corneum barrier. (3, 23, 24) The thickness of stratum corneum layer is 10 micro grams and it consists of 10-25 rows of dead carnergie embedded in a lipid matrix. The heterogeneous structure of the stratum corneum is composed of approximately 75-80% protein, 5-15% lipid, 5-10% unidented on a drug weight basis.
Effects of Material use for Ethosomal system properties

**Ethanol**
Ethanol is an efficient penetration enhancer. It plays an important role in ethosomal systems by giving the vesicles unique characteristics in terms of size, potential, stability, entrapment efficacy, and enhanced skin permeability. Some studies have suggested that high concentrations of ethanol cause interpenetration of the ethanol hydrocarbon chain, which leads to a reduction in vesicular membrane thickness and hence reduces vesicular size. Vesicular charge is an important parameter that can influence vesicular properties, such as stability and vesicle-skin interaction. The high ethanol concentration in ethosomes has shifted the vesicular charge from positive to negative. Ethanol also has a significant effect on ethosomal system entrapment efficiency, and in general increasing ethanol concentration will increase entrapment efficiency. This effect applies to molecules of varying lipophilicities, whereby ethanol increases the solubility of the lipophilic and amphiphilic drugs and hence increases drug loading. This relationship was found to be linear, with ethanol concentrations between 20% and 40%. For this reason, ethanol concentration should be optimized during the formulation process, as at low concentrations entrapment efficacy will be minimal, and at very high concentrations ethosomal membrane will be more permeable because phospholipids can easily be dissolved in ethanol, leading to a significant reduction in entrapment efficacy.

**Phospholipids**
Phospholipids from different sources have been used in ethosomal system formulation. The selection of phospholipid type and concentration for the formulation are important factors during the development of ethosomal system because they will influence the size, entrapment efficacy, \( \zeta \)-potential, stability, and penetration properties of the vesicles. The different types of phospholipids used in the preparation of ethosomal systems are summarized in this fig.

In general, the concentration range of phospholipids in an ethosomal formulation is 0.5%–5%. Increasing phospholipid concentration will increase vesicular size slightly or moderately, but will increase entrapment efficiency significantly. However, the relationship is true only until a certain concentration, whereby further increment in phospholipid concentration will have no effect on entrapment efficiency.

**Cholesterol**
Cholesterol is a rigid steroid molecule, and its incorporation in ethosomal systems enhances the stability and entrapment efficiency of drugs. It prevents leakage and reduces vesicular permeability and vesicular fusion. Generally, it is used at a concentration of <3% but in some formulations it was used up to 70% of the total phospholipid concentration in the formulation. Several studies have reported that cholesterol increased the vesicular size of ethosomal systems. López-Pinto et al found that ethosomal size increased from 136±42 nm to 230±27 nm when 25.87 mM of cholesterol was used in the formulation. However, only one study has found that cholesterol had no stabilization effects on the ethosomes. The authors used phosphatidylethanolamine and 15% ethanol in the formulation. However, in vitro studies using Franz diffusion cells and confocal laser-scanning microscopy showed that these multilamellar vesicles (MLVs) were not able to pass across the stratum corneum due to the higher rigidity, and hence it was more difficult for the drug to permeate across the skin. The increased rigidity (ie, decreased elasticity) of the ethosomal vesicles upon the addition of cholesterol was also reported by other researchers.

**Other Alcohol**
Other alcohols, such as PG and IPA, are also used in the preparation of binary ethosomes along with ethanol.

**HPLC Essay**
The amount of drug permeated in the receptor compartment during in vitro skin permeation experiments and in MT-2 cell was determined by HPLC assay using methanol: distilled-water acetonitrile (70:20:10 vol/vol) mixture as mobile phase delivered at 1 mL/min by LC 10-AT pump (Shimadzu, Kyoto, Japan). A twenty-micro lite injection was eluted in C-18 column (4.6x150 mm, Luna, 54, Shimadzu) at room temperature. The column Eluent was monitored at...
271 nm using SPDM10A VP diode array UV detector. The coefficient of variance (CV) for standard curve ranged from 1.0% to 2.3%, and the squared correlation coefficient was 0.9968

**Preparation techniques and their effects on ethosomal properties**

**The Cold Method**
This is the simplest and most widely used method for the preparation of ethosomal systems, and if required it could be done under nitrogen protection. It was introduced by Touitou in 1996, and involves the preparation of the organic phase and aqueous phase separately. The organic phase is obtained by dissolving the phospholipids (in addition to surfactants or penetration enhancer for transethosomes) in ethanol or mixture of solvents (ethanol/PG) for the preparation of binary ethosomes, at room temperature, or at 30°C. The aqueous phase is either water, buffer solution, or normal saline solution. The aqueous phase is added to the organic phase in a fine stream, dropwise, or using a syringe pump at a constant rate of 1 or 200 μL/min. The mixture is stirred at a speed of 700–2,000 rpm, using an overhead or magnetic stirrer. The mixing is done for 5–30 minutes to obtain the required ethosomal suspension. The drug to be incorporated in the ethosomal system will be dissolved in either the aqueous or the organic phase, depending on its physicochemical properties. Figure 3 presents the preparation steps of an ethosomal system using the classical cold method.

**The Hot Method**
In this method phospholipid is dispersed in water by heating in a water bath at 400°C until a colloidal solution is obtained. In a separate vessel ethanol and propylene glycol are mixed and heated to 400°C. Once both mixtures reach 400°C, the organic phase is added to the aqueous one. The drug is dissolved in water or ethanol depending on its hydrophilic/hydrophobic properties. The vesicle size of ethosomal formulation can be decreased to the desire extent using probe sonication or extrusion method

**Characterization of Ethosomes**
- **Vesicle shape-transmission electron microscopy(TEM),Scanning electron microscopy(SEM)**
- **Entrapment efficiency-Mini column centrifugation method; Fluorescence spectrophotometry**
- **Vesicle skin interaction study-conofocal laser scanning microscopyFluorescence microscopy;TEM Eosin-hematoxylin staining**
- **Phospholipid-ethanol interaction-31P NMR; Differential scanning calorimeter**
- **Degree of deformability-Extrusion Method**
- **Zeta potential-Zeta meter**
- **Turbidity-Nephelometer**
- **In Vitro Drug release study-Franz diffusion cell with artificial or biological membrane, Dialysis bag diffusion**
- **Drug deposition study-Franz Diffusion cell**
- **Stability study-Dynamic light scattering method and TEM**

**Application of Ethosomes**
- Delivery of Antiviral drugs
- Topical delivery of DNA
- Transdermal delivery of Hormones
- Delivery of Anti-parkinsonism agent
- Transcellular Delivery
- Delivery of Anti-Arthritis Drugs
- Delivery of Problematic drugs molecules
- Delivery of Antibiotics
- Delivery of Antigen loaded Drugs
- Delivery of NSAIDS
- Widely used in Cosmeceuticals

**2. Conclusion**

The main disadvantage of transdermal drug delivery is the poor penetration of most compounds into the human skin. The main barrier of the skin is located within its uppermost layer, the stratum corneum. Ethosomes has initiated a new area in vesicular research for transdermal drug delivery which can provide better skin permeation than liposomes or hydro alcoholic solution. Ethosomes are soft, malleable vesicles and potential carrier for transportation of drugs. Ethosomes have been tested to encapsulate hydrophilic drugs, cationic drugs, proteins and peptides. Further, research in this area will allow better control over drug release in vivo and long-term safety data, allowing the therapy more effective.

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