Review on ethosomes: a novel carrier for transdermal delivery system

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***Abstract*- Oral route is the normal route of drug delivery, which has many benefits such as easy delivery but has drawbacks such as low bioavailability and a propensity to produce rapid spikes in blood levels, thereby being a requirement for higher dose or repeated dosing, which is difficult for the patient and also high cost. With all these disadvantages in mind, there is a need for novel drug delivery technology with increased therapeutic efficacy and safety with controlled delivery to minimize the size and number of doses. This can be achieved by transdermal delivery which possesses several advantages such as avoids first-pass metabolism, eliminates gastrointestinal irritation reduces frequency of dosing, and rapid termination of drug action. Ethosomes have higher penetration rate through skin due to its ethanolic content. In this article reviews various aspect of ethosomes including their mechanism of penetration, preparation, advantages, characterization, composition, preparation. These carriers open new challenges and opportunities for the development of novel improved therapies.**

Key words: Ethosomes, Transdermal, Ethanol, Phospholipids

INTRODUCTION

Skin forms a protecting covering layer against the external environment and prevents water loss from the underlying tissue. It is flexible enough to resist permanent distortion from movement and thin enough to allow the perception of stimuli. It also performs many ancillary functions such as synthesis and metabolism and the production of sweat enables temperature control and excretion of waste products by means of sweating etc.1,2 It has been also reported that skin protects the body from antigenic stimuli by means of a part of the immune system known as skin associated lymphoid tissue[1] . 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[2,3]. 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. 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. The molecular structures and appearance of the molecules can be examined using molecular modeling computer programs.

ETHOSOMES

ETHs are easily formed vesicular systems composed of phospholipids, ethanol at high concentrations in water. These unique systems are suitable for transdermal applications of many active substances and vesicles are easily formed due to ethanol in the formulation (Touitou et al. 2000). Because of the synergistic effect of the presence of phospholipids and ethanol, the ETHs are very flexible and they permeate very easily inside the deep skin layers in comparison with LPs and their transdermal flux can be considerably high (Akiladevi and Basak 2010, Maheshwari et al. 2012). The mechanism of the drug delivery of ETHs can be explained in two ways: effect of ethanol and effect of ETHs. Ethanol is basically a penetration enhancer and acts by reducing the frequency of intercellular lipids. The fluidity of the lipids and the flexibility of the vesicles are high, therefore, the penetration of the parent drug enhances. The penetration of the interacting ETHs with lipids is facilitated and the active substance entrapped in the ETH can be transmitted to the deeper skin layers (Touitou et al. 2000, Chandel et al. 2012, Fatima Grace et al. 2014).

ETHOSOMAL TYPES

The following figure a gives a description about the types of Ethosomes.

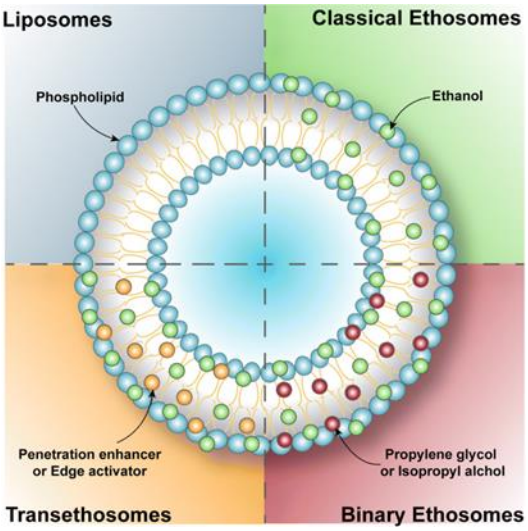

Figure 1 - Structure of different types of ethosomes

Classical ethosomes:

Classical ethosomes are a modification of classical liposomes and are composed of phospholipids, a high concentration of ethanol up to 45% w/w, and water.

Classical ethosomes

were reported to be superior over classical liposomes for transdermal drug delivery because they were smaller and had negative ζ-potential and higher entrapment efficiency. Moreover, classical ethosomes showed better skin permeation and stability profiles compared to classical liposomes[15,16] .The molecular weights of drugs entrapped in classical ethosomes have ranged from 130.077 Da to 24 kDa [17]

Binary ethosomes

Binary ethosomes were introduced by Zhou et al.Basically, they were developed by adding another type of alcohol to the classical ethosomes[18]. The most commonly used alcohols in binary ethosomes are propylene glycol (PG) and isopropyl alcohol (IPA). [19-21]

Transethosomes

Transethosomes are the new generation of ethosomal systems and were first reported by Song et al in 2012[22].This ethosomal system contains the basic components of classical ethosomes and an additional compound, such as a penetration enhancer or an edge activator (surfactant) in their formula. These novel vesicles were developed in an attempt to combine the advantages of classical ethosomes and deformable liposomes (transfersomes) in one formula to produce transethosomes. Many researchers have reported superior properties of transethosomes over classical ethosomes.[22-35] Different types of edge activators and penetration enhancers have been investigated to produce ethosomal systems with better characteristics. Transethosomes were reported to entrap drugs with molecular weights ranging from 130.077 Da to 200–325 kDa.[23,26]

ADVANTAGES OF ETHOSOMES:

1. Ethosome enhance permeation of drugs through skin for dermal, transdermal and intracellular delivery.
2. Deliver various molecules with different physicochemical properties, hydrophilic and lipophilic molecules, peptides, proteins and other macromolecules.
3. The components of the ethosomes are generally recognized as safe (GRAS), non-toxic and approved for pharmaceutical and cosmetic use.
4. Low risk profile- Ethosome structure has no largescale drug development risk as the ethosome feature toxicology profiles are well established in the scientific literature.
5. The ethosomal system is passive and non-invasive, and is suitable for immediate marketing[36,37]

DISADVANTAGES OF ETHOSOMES:

1. Allergic reaction can be identified if the patients are allergic to ethanol or any of the ethosomal components.
2. Unlike other carriers (solid lipid nanoparticles, polymeric nanoparticles, etc.) which can be used for multiple routes, ethosomal carriers are important only for transdermal use.
3. Due to the fact that ethanol is inflammable, sufficient care should be taken during planning, application, transport and storage.
4. Very poor yield so may not be economical.
5. Loss of product during transfer from organic to water media [36,37]

MECHANISM OF PENETRATION

The enhanced delivery of actives using ethosomes over liposomes can be ascribed to an interaction

between ethosomes and skin lipids. A possible mechanism for this interaction has been proposed. It is thought that the first part of the mechanism is due to the ethanol effect whereby intercalation of the ethanol into intercellular lipids increasing lipid fluidity and decreases the density of the lipid multilayer. This is followed by the ethosome effect, which includes inter lipid penetration and permeation by the opening of new pathways due to the malleability and fusion of ethosomes with skin lipids, resulting in the release of the drug in deep layers of the skin as shown in Figure b. The drug absorption probably occurs in following two phases:

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.Ethosomes 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. [38]

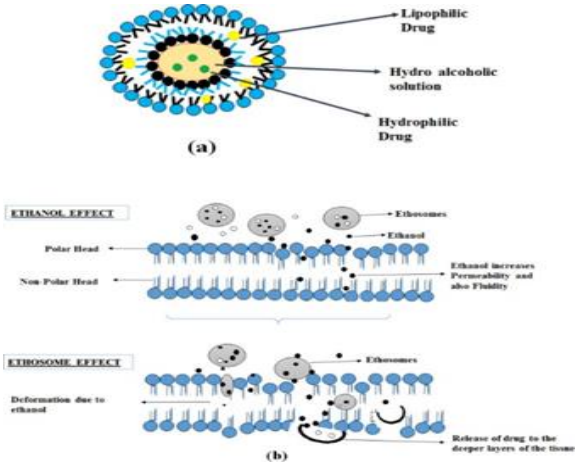

Figure 2 – Mechanism of penetration

COMPOSITION OF ETHOSOMES: [39]

Materials	Examples	Uses
Phospholipids	Soya phosphatidyl choline Egg phosphatidyl choline Dipalmityl phosphatidyl choline Distearyl phosphatidyl choline	Vesicles Forming
Alcohol	Ethanol Isopropyl alcohol	1. As a penetration enhancer 2. For providing the softness for vesicle membrane
Polyglycol	Propylene glycol Transcutol RTM	As a skin penetration enhancer
Cholesterol	Cholesterol	For providing stability
Dye	Rhodamine-123 Rhodamine red Fluorescen Isothiocynate (FITC) 6- Carboxy fluorescence	For characterization study
Vehicle	Carbopol 934	As a gel former

METHODS FOR PREPARATION

Cold Method:

This is the most common method utilized for the preparation of ethosomal formulation. In this method phospholipid, drug and other lipid materials are dissolved in ethanol in a covered vessel at room temperature by vigorous stirring with the use of mixer. Propylene glycol or other polyol is added during stirring. This mixture is heated to 30°C in a water bath. The water heated to 30°C in a separate vessel is added to the mixture, which is then stirred 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 is stored under refrigeration.[40-42]

Hot method:

In this method phospholipid is dispersed in water by heating in a water bath at 40°C until a colloidal solution is obtained. In a separate vessel ethanol and propylene glycol are mixed and heated to 40°C. Once both mixtures reach 40°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. [43]

Classic mechanical dispersion method:
Dissolve phospholipid in an organic solvent, or in a round bottom flask (RBF) mixture of organic solvents. Using a rotary vacuum evaporator above lipid transition temperature to remove the organic solvent to create a thin lipid film on the RBF wall; Traces of the solvent should be separated from the accumulated lipid film by leaving overnight in vacuum. Hydrate the lipid film with the drug's hydroethanol solution by spinning the flask with or without periodic sonication at the correct temperature and eventually cool the resulting ethosomal suspension at room temperature. The formulation should be stored under refrigeration.

The ethanol injection–sonication method:
In this process, the organic phase containing the dissolved phospholipid in ethanol is injected into the aqueous phase using a 200-flow syringe system38 μl / min, then homogenized for 5 minutes with an ultrasonic probe. [44]

CHARACTERIZATION OF ETHOSOMES

Vesicle shape: Ethosomes can be easily visualized by using transmission electron microscopy (TEM) and by scanning electron microscopy (SEM). [45]
Size and zeta potential: 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. [46]
Transition temperature: The transition temperature of the vesicular lipid systems can be determined by using differential scanning calorimetry (DSC). [47]
Drug entrapment: The entrapment efficiency of ethosomes can be measured by the ultracentrifugation technique. [48]
Drug content: Drug content of the ethosomes can be determined using UV spectrophotometer. This can also be quantified by a modified high performance liquid chromatographic method. [49]
Surface tension measurement: The surface tension activity of drug in aqueous solution can be measured by the ring method in a Du Nouy ring tensiometer.
Stability studies: 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.
Skin permeation studies: The ability of the ethosomal preparation to penetrate into the skin layers can be determined by using confocal laser scanning microscopy (CLSM). [50]

CONCLUSION

Ethosomes are found to a prominent novel drug carrier that has been used in transdermal drug delivery. The main limiting factor of transdermal drug delivery system i.e. epidermal barrier can be overcome by ethosomes to significant extent. Application of ethosomes provides the advantages such as improved permeation through skin and targeting to deeper skin layers for various skin disease. Hence ethosomes have been found to be much more efficient at delivering drug to the skin, than either liposomes or hydro alcoholic solutions. It can therefore be a fair inference that ethosomal formulations have promising future in the successful delivery of bioactive agents to the dermal / transdermal.

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