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RECENT ADVANCES AND TECHNOLOGICAL ASPECTS OF ETHOSOMES: A LACONIC REVIEW

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ABSTRACT

Over the years transdermal drug delivery has gained a lot concern due its advantages over oral route. To achieve the permeation through the skin, one simple and convenient approach that has been widely recognized is encapsulation of the drug in ethanol based liposomes i.e ethosomes. Ethosomes are the ethanolic phospholipid vesicles which are used mainly for transdermal delivery of drugs. Ethosomes have higher penetration rate through the skin as compared to liposomes. The increased permeation of ethosomes is probably due to its ethanolic content. Ethosomes provides a number of important benefits such as improved drug delivery, efficacy, patient compliance and comfort. This review focuses on the various aspects of ethosomes viz. skin permeation mechanism, preparation, advantages, characterization, composition, applications, marketed products based on ethosomes as delivery vesicle and recent patents on ethosomes.

KEYWORDS: Ethosomes, Transdermal, Topical, Skin, Phosphatidylcholine.

1. INTRODUCTION

Human skin is considered as first line of defense in human body. Skin has a multifunctional role including its key role as a barrier against endogenous substances such as water and of xenobiotics (chemicals and drugs).[1] The use of intact skin for the site of administration for topical preparations to obtain a pharmacological action in the skin or tissue has been documented from several years.[2] In the research area of drug delivery, most successful innovative route compared with oral treatment is the delivery of drug via skin i.e. transdermal drug delivery. In transdermal drug delivery system a specific amount of drug is delivered through the skin for systemic action.[3] Transdermal formulation maintain drug concentration within the therapeutic window for prolong period of time.[2] In comparison to oral drug

delivery promising result has been seen in transdermal drug delivery system (TDDS) system as it eliminates gastrointestinal interferences and first pass metabolism of the drug. The main negative aspect of drug delivery via skin is the barrier properties of the stratum corneum i.e. only the lipophilic drugs having molecular weight less than 500 daltons can pass through stratum corneum.[4] To overcome this problem various mechanisms have been investigated, including use of chemical or physical enhancers such as iontophoresis, sonophoresis etc.[5] Colloidal lipid-based vesicles such as liposome, niosomes and nanosomes were developed. As carrier systems in delivering drugs through transdermal route they have shown promising results.[6] Constant study with lipid based system has resulted in the introduction of two novel carriers, transfersomes and ethosomes. Transfersomes are deformable lipid vesicles consisting of phospholipids and an edge activator which is often a single chain surfactant molecule.[7] Ethosomes (Fig. 1) are modified form of liposome carriers containing phospholipid, and have a high concentration of ethanol and water. The important comparative parameters and descriptions of vesicular systems have been listed in Table **1**.

Table. 1: Comparison between liposomes, transferosomes and ethosomes.[14]

Characters	Liposome	Transferosomes	Ethosomes
Vesicles	Bilayer lipid vesicle	2nd generation elastic lipid vesicle carriers	3rdgeneration elastic lipid vesicle carriers
Composition	Phospholipids and cholesterol	Phospholipids and edge activator	Phospholipids and ethanol
Characteristics	Microscopic spheres vesicles	Ultra flexible liposome	Elastic liposome
Flexibility	Rigid in nature	High deformability	High deformability and elasticity
Permeation Mechanism	Diffusion/Fusion/Lipolysis	Deformation of vesicle	Lipid perturbation
Extent of Skin Penetration	Penetration rate is very less	Can easily penetrate through paracellular space	Can easily penetrate through paracellular space
Route of administration	Oral, parenteral, topical and transdermal	Topical and transdermal	Topical and transdermal

Ethosomes were first reported by Touitou *et al.*[8] Ethosomes are non-invasive delivery carriers that are capable to deliver the drugs to the deep skin layers and systemic circulation.[9] Ethosomes have become innovative liposome carriers with high deformability, high entrapment efficiency and good transdermal permeation rate in the drug-delivery systems which make them suitable for transdermal administration.[10] Ethosomes not only delivers the drug to the deep skin layer but also meet the essential criteria for efficient and safe administration of lipophilic or hydrophilic drugs.[11] Compared to conventional liposomes or hydro-ethanolic solutions, ethosomes due to their soft and flexible nature can

penetrate the skin and allow enhanced delivery of various active agents to deeper layers of skin or enhanced systemic circulation.[12] The size of ethosomes vesicles can be modulated from tens of nanometres to microns.[13-15]

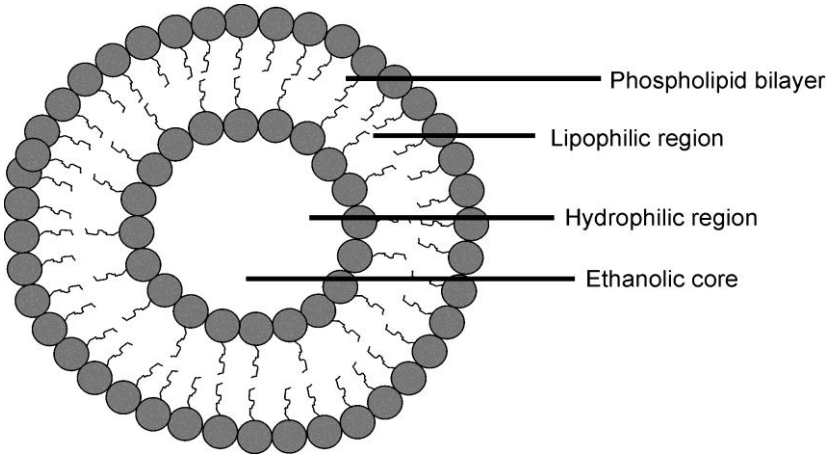

Fig. 1. Structure of Ethosome.

1.1Types of Ethosomal systems

There are three types of ethosomal systems based on their composition.

1.1.1 Classical ethosomes

Classical ethosomes are composed of phospholipids, water and high concentration of ethanol (40%). Classical ethosomes are better over classical liposomes due to small size, negative zeta potential and higher entrapment efficiency. Drugs having molecular weight ranging from 130.077 Da to 24 k Da can be entrapped in classical ethosomes. As compared to classical liposomes, ethosomes have better skin permeation and stability profiles.

1.1.2 Binary ethosomes

Binary ethosomes are prepared by adding another type of alcohol like propylene glycol (PG) and isopropyl alcohol (IPA) etc. to the classical ethosomes.[16]

1.1.3 Transethosomes

Transethosomes are the new form of ethosomal systems and were developed to combine the advantages of classical ethosomes and transfersomes in one formula. In their composition it contains basic components as that of classical ethosomes and a penetration enhancer or an edge activator (surfactant).[16,17]

1.2 Advantages of ethosomes

- Improved drug permeation as compared to liposomes for topical and transdermal delivery
- Macromolecules (proteins and peptides) can be easily delivered through skin.
- It is prepared using materials compatible and non-toxic to skin and body.
- The delivery of active ingredients using ethosomes is a passive and non-invasive technique. Its topical application in the form of gel or cream improves the patient compliance.
- Ethosomal drug delivery system can be applied widely in pharmaceutical, veterinary and cosmetic fields.
- Relatively simple to manufacture with no complicated technical investments required for production of ethosomes.[18]

1.3 Disadvantages of ethosomes

- Suitable for the drugs with low daily dose. Drugs that require high blood levels for therapeutic action cannot be administered.
- To reach dermal microcirculation and gain access to the systemic circulation adequate solubility of the drug in both lipophilic and aqueous environments is required.
- The molecular size of the drug should be rational for percutaneous absorption.
- May not be economical due to poor entrapment efficiency.
- Incompatibility of excipients of ethosomes with skin causing irritation or dermatitis.
- To manufacture a stable ethosomal system is an important limitation.
- Loss of product during transfer from organic to aqueous media.[19]

2. Ethosomes Composition

Ethosomes are composed of hydro-alcoholic and phospholipids, in which alcohol content is high. Ethosomes has been formulated using number of phospholipid such as phosphatidylcholine, hydrogenated phosphatidylcholine, phosphatidic acid, phosphotidylserine, phosphatidyl ethanolamine and phosphatidyl glycerol. Delivery of high concentration of active ingredients through skin is facilitated by this type of composition. Drug delivery can be adjusted by varying alcohol concentration. Non-ionic surfactants can be combined with phospholipids in the formulation of ethosomes. The concentration of the non-aqueous phase (alcohol and glycol combination) may range between 22 to 70%.[20]

2.1 Ethanol: Ethanol is one of the most commonly used permeation enhancers. There are number of mechanisms that have been proposed for permeation enhancing action of ethanol.

In ethosomal systems ethanol plays a significant role by giving the vesicles characteristics features in terms of size, zeta-potential, stability, entrapment efficacy and enhanced skin permeability. Ethanol in ethosomal systems have been reported to be used in concentration range 10%-50%.[21] Many researchers concluded that when the concentration of ethanol is increased, the size of the ethosomes would decrease. But, increasing ethanol concentration above the optimum level would cause the bilayer to be leaky which lead to a small increase in vesicular size and decrease in entrapment efficacy. Further, increase in concentration of ethanol will solubilize the vesicles.[22] Entrapment efficiency increased with increase in ethanol concentration. This effect applies to molecules of unreliable lipophilicities, because ethanol increases the solubility of the lipophilic and amphiphilic drugs, thus increases drug loading. This relationship was found to be linear with ethanol concentrations between 20% and 40%.[23] During the formulation process ethanol concentration should be optimized, as at low concentrations entrapment efficacy will be minimal, and at very high concentrations ethosomal membrane will be Leaky (Phospholipid can easily be dissolved in ethanol) leading to a significant reduction in entrapment efficacy.[24]

2.2 Phospholipids: In ethosomal system phospholipids from different sources have been used. During the development of ethosomal system the selection of phospholipid type and concentration of phospholipids are important factors as they will influence the size, entrapment efficacy, zeta potential, stability and penetration properties of the vesicles**.** In common, the concentration range of phospholipids in an ethosomal formulation is 0.5-5%.[25] Increasing the concentration of phospholipid will not only increase vesicular size slightly or moderately, but also increase entrapment efficiency considerably.[26] The relationship is true only until a certain concentration, whereby further addition in phospholipid concentration will have no effect on entrapment efficiency.[24]

2.3 Cholesterol: In ethosomal systems, cholesterol incorporation 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.[24,27]

2.4 Dicetyl phosphate: To prevent aggregation of the vesicles and enhance the stability of the formula dicetyl phosphate is commonly used. In the ethosomal formulation it is used at concentrations between 8% and 20% of the total phospholipid concentration. However, the effects of dicetyl phosphate on other ethosomal system properties are still unclear.[24,28]

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

2.5.1 Propylene glycol: Propylene glycol (PG) is a generally used penetration enhancer. It is used in the formulation of binary ethosomes at a concentration range of 5%-20% and found to affect the ethosomal properties of size, entrapment efficiency, permeation and stability.[24] In ethosomal systems, incorporation of PG will lead to further reduction in particle size in comparison to systems without PG. Significant decrease in particle size has been reported when PG concentration was increased from 0% to 20%v/v.[29] In order to get higher drug permeation, the ratio of ethanol:PG should be optimized in binary ethosomes. When stored at 4°C binary ethosomes were found to be stable than classical ethosomes.[30] Therefore, it is suggested that PG enhances ethosomal stability by increasing the viscosity and anti-hydrolysis property.[31]

2.5.2 Isopropyl alcohol

It has been found that IPA had a distinct effect on entrapment efficiency but less effect on drug release.

2.6 Drugs\Agents effects on ethosomal system properties

In ethosomal systems, most important factor to consider is the nature or physicochemical properties of the drugs/agents that is going to be incorporated. This is because the drugs/agents may affect the properties of the ethosomal systems, especially particle size and zeta potential.[24]

3. METHODS FOR PREPARATION

Methods which are used for the formulation and preparation of ethosomes are very simple and convenient and do not involve any sophisticated instrument or complicated process. Ethosomes can be formulated by following methods.

3.1 Hot method: In hot method, phospholipid is dispersed in water by heating on a water bath at 40°C until a colloidal solution is obtained (aqueous phase). In another vessel ethanol and propylene glycol are properly mixed and heated up to 40°C (organic phase). The organic phase is added to the aqueous phase under continuous stirring. Depending upon solubility of drug, it is dissolved in water or ethanol. By using probe sonication or extrusion method the vesicle size of ethosomal formulation can be decreased to the desire extent.[32]

3.2 Cold method

Cold method is one of the most extensively used techniques for preparation of Ethosomes. First phospholipid is dissolved in ethanol at room temperature by vigorous stirring and polyols such as propylene glycol etc are continuously added with constant stirring followed by heating at 30°C in water bath. Then water is heated at 30°C in a separate vessel, both mixtures are mix together following 5 min stirring in a covered vessel. The vesicle size of ethosomal formulation can be decreased to desire extend using sonication method.[33]

3.4 Classic mechanical dispersion method

In this method phospholipid is dissolved in an organic solvent or a mixture of organic solvents in a round bottom flask (RBF). The organic solvent is removed using a rotary vaccum evaporator to form a thin lipid film on the wall of the RBF. Traces of the solvent are removed from the deposited lipid film by leaving the contents under vaccum overnight. Lipid film is hydrated with hydro-ethanolic solution of drug by rotating the flask at suitable temperature. Finally, cool the resultant ethosomal suspension at room temperature.[34]

4. Mechanism of Drug Permeation

Ethanol influences the stratum corneum lipid multi-layer which are tightly packed and highly set at physiological temperature. Ethanol increases the fluidity of stratum corneum lipids by interacting with lipid molecules in polar head group region resulting in reduction in Tm of stratum corneum lipids, thus increasing their fluidity. Ethanol is penetration enhancer that acts by affecting the intercellular region of stratum corneum. A possible mechanism for the interaction between skin and ethosomes has been anticipated. It is thought that the first part of the mechanism is due to the **'ethanol effect'**, i.e ethanol increase the fluidity of cell membrane lipid and decrease the compactness of lipid multilayer of cell membrane. This is followed by the **'ethosomes effect'** i.e increase cell membrane lipid fluidity caused by ethanol of ethosomes results increase skin permeability so the ethosomes permeates very easily inside the deep skin layer.

Penetration enhancing effect of ethanol could be attributed to

(1) Increase in thermodynamic activity due to evaporation of ethanol known as "push effect"

(2) "Pull effect" in which penetration of drug molecules is increase due to reduction in barrier properly of stratum corneum by ethanol.[35,36]

5. Characterization of Ethosomes[37]

In general ethosomes can be characterized for the different parameters such as

- ***Vesicle shape:*** Ethosomes can be easily visualized by using transmission electron microscopy (TEM) and Scanning electron microscopy (SEM).
- ***Vesicle size and zeta potential:*** Particle size of the ethosomes can be determined by dynamic light scattering (DLS) and photo correlate on spectroscopy (PCS). Zeta potential of the formulation can be measured by Zeta sizer.
- ***Transition temperature:*** The transition temperature of the vesicular lipid systems can be determined by using differential Scanning calorimetry (DSC).
- ***Entrapment efficiency:*** The entrapment efficiency of ethosomes can be measured by the ultracentrifugation technique and membrane dialysis.
- ***Surface tension measurement:*** The surface tension activity of ethosomes 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).

6. Therapeutic Application of Ethosomes: Ethosomes can be used for many purposes in drug delivery. Various drugs have been used with ethosomal carrier. Some applications of ethosomes are following.

6.1 ***Pilosebaceous targeting:*** Hair follicles and sebaceous glands are increasingly recognized as important basics in the percutaneous drug delivery. Minoxidil is a lipid-soluble drug used topically on the scalp for the treatment of baldness is given by pilosebacious delivery. Use of pilosebaceous units as depots for localized therapy has gained interest, particularly for the treatment of follicle-related disorders such as acne and alopecia.[38]

6.2 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. In addition, along with these problems oral hormonal preparations highly relying on patient compliance. So, hormonal preparations can be given in ethosomal delivery.[39]

6.3 Delivery of Anti-Arthritis Drug

For site-specific delivery of anti-arthritis drugs, topical delivery is a better option and can overcome the problem associated with conventional oral therapy.[32]

6.4 Delivery of problematic drug molecules

Oral delivery of large biomolecules such as peptides or proteins such as insulin is difficult due to their complete degradation in GIT tract. Hence transdermal delivery is a better alternative. But, conventional transdermal formulation of such molecules has poor permeation. Formulating those into ethosomes can significantly increase permeation and therapeutic efficacy.[4,40]

6.5 Transcellular Delivery

As compared to the marketed formulation and ethosmal formulation of anti-HIV drugs zidovudine and lamivudine, has shown enhanced cellular application of anti-HIV in MT-2 cell line. That suggests ethosomes to be an attractive clinical alternative for anti-HIV therapy.[38]

6.6 Topical delivery of DNA

Ethosomes can be used as carrier for gene therapy application. It has been demonstrated that better intracellular uptake of DNA, better delivery and expression of genes in skin cells can be attained by ethosomal formulation. Recently, Scientists have reported immunization potential using transfersomal formulation. Therefore, better skin penetration ability of ethosomes enables the possibility of using these dosage forms for delivery of immunizing agents.[41]

6.7 Delivery of antibiotics

For increasing the therapeutic efficacy topical delivery of antibiotics is a better choice. Conventional oral therapy causes many side effects and conventional topical preparations possess low permeability to deep skin layers and tissues. By delivering sufficient quantity of antibiotic into deeper layers of skin ethosomes can overcome above problems. Ethosomes penetrate rapidly through the epidermis and delivers drug into the deeper layer of skin. The results of many studies and research showed that the ethosomal formulation of antibiotic could be highly efficient and would overcome the problems associated with conventional therapy.[42]

7. Ethosomes in Cosmeceuticals

Many researchers have investigated ethosomal delivery for cosmetic purpose. Esposito et al (2004) prepared ethosomal gel of azelaic acid for the treatment of acne and compared the *in vitro* release with conventional liposomes. The release rate was more rapid from ethosomal systems than from liposomal systems.[43] Koli et al (2008) have formulated antioxidant ethosomes for topical delivery using the synergistic properties of vitamin A palmitate, Vitamin E and Vitamin C. Usually antioxidants are not stable and are degraded by exposing to light. Research has revealed that the synergistic interaction of Vitamin C in the aqueous core and vitamin A and E in the lipid bilayer, provide complete protection from the oxidation in the ethosomes formulation.[44] The first commercial product based on ethosomes technology was marketed in 2000, and majority of products marketed so far are cosmeceutical products (Table **2**) viz. LipoductionTM, anticellulite formulation is marketed in USA. Many big pharmaceutical companies and cosmetic firms are now engaged in active research in product development using ethosome technology.[45]

Table. 2. Marketed cosmetic products based on ethosomal drug delivery system.[45,46]

Name of product	Uses	Manufacturer
Celltight EF	Topical cellulite cream	Hampden Health,USA
Skin genuity	Powerful cellulite buster	Physonics Nottingham, U.K
Nanominox	Contains 4% monoxidil, well-known hair growth promoter	Sinere, Germany
Noicellex	Topical anti-cellulite cream	Novel Therapeutic Technologies, Israel
Decorin cream	Anti-aging cream	Genome Cosmetics, Pennsylvania, USA
Supravir cream	For treatment in herpes viral infection	Trima, Israel

8. Patents on Ethosomal Drug Delivery System

Table. 3. list out the patents related to ethosomal drug delivery system.

Table. 3. Patents on ethosomes.[47]

S. No	Title	Patent no.	Year
1.	Chinese medicinal ethosome herpes gel patch for treating zoster	CN103536700 (A)	2014
2.	Leflunomide ethosome composition and its preparation method	CN103800277	2014
3.	Ethosome gel film coating agent with multiple wound repair effects.	CN103893394 (A)	2014
4.	Bullatacin ethosome gel	CN102552147 (A)	2012
5.	Daptomycin ethosome preparation	CN103006562 (A)	2013
6.	Ethosome preparation of male hormone medicaments.	CN102406605 (A)	2012
7.	Lidocaine ethosomes	CN102813624 (A)	2012
8.	Paclitaxel ethosome gel	CN102579323(A)	2012
9.	Progesterone ethosome,	CN102397255(A)	2012
10.	Acyclovir ethosomes	CN102133183 (A)	2011
11.	Podophyllotoxin ethosomes	CN102144972(A)	2011

9. CONCLUSION

Ethosomes are soft, malleable vesicles characterized with simple methods of preparation, safety and efficacy. It is a non-invasive carrier that enables drug to reach the deeper skin layers finally delivering to the systemic circulation. Ethosomes have better potential for topical and transdermal delivery of drugs than conventional liposomes. Further, it can easily delivers large molecules such as peptides, protein molecules either topical or transdermal. Ethosomal carrier opens new challenges and opportunities for the development of novel improved therapies. Further, research in this area will allow better control over drug release *in vivo* and long term safety data, allowing the therapy to be more effective. Ethosomes are characterized by simplicity in their preparation, safety and efficacy.

10. CONFILICT OF INTEREST

The author declares no conflict of interest.

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