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A REVIEW

Ethosomes in transdermal drug delivery

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ABSTRACT

Transdermal drug delivery system was first introduced more than 30 years ago. The technology generated tremendous excitement and interest amongst major pharmaceutical companies in the 1980s and 90s. By the mid to late 1990s, the trend of transdermal drug delivery system merged into larger organizations. Ethosomes are noninvasive delivery carriers that enable drugs to reach the deep skin layers and the systemic circulation. Although ethosomal systems are conceptually sophisticated, they are characterized by simplicity in their preparation, safety and efficacy a combination that can highly expand their application. Ethosomes are soft, malleable vesicles tailored for enhanced delivery of active agents. This article reviews various aspect of ethosomes including their preparation, characterization, potential advantages and their applications in drug delivery. Because of their unique structure, ethosomes are able to encapsulate and deliver through the skin highly lipophilic molecules such as cannabinoids, testosterone, and minoxidil, as well as cationic drugs such as propranolol, trihexyphenidil, Cyclosporine A, insulin, salbutamol etc. Ethosomes have become an area of research interest, because of its enhanced skin permeation, improved drug delivery, increased drug entrapment efficiency. Ethosomes including their mechanism of penetration, preparation, advantages, composition, characterization, application.

Key words : Ethosome, Ethanol, Transdermal delivery, Phospholipid, Vesicle

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INTRODUCTION

Transdermal drug delivery system (TDDS) showed promising result in comparison to oral drug delivery system as it eliminates gastrointestinal interferences and first passmetabolism of the drug but the main drawback of TDDS is it encounters the barrier properties of the Stratum Corneum *i.e.* only the lipophilic drugs having molecular weight < 500 Da can pass through it (Gangwar *et al.*, 2010 and Kumar *et al.*, 2010). 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. Liposomes, niosomes, transferosomes and ethosomes also have been reported to enhance permeability of drug through the stratum corneum barrier. Permeation

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enhancers increase the permeability of the skin, so that the drugs can cross through the skin easily. Unlike classic liposomes (Heeremans *et al.*,1995) that are known mainly to deliver drugs to the outer layers of skin, ethosomes can enhance permeation through the stratum corneum barrier (Asbill *et al.*, 2000 and Touitou *et al.*,1998). Ethosomes permeate through the skin layers more rapidly and possess significantly higher transdermal flux in comparison to conventional liposomes (Verma and Pathak, 2010, Jain *et al.*, 2004 and Touitou *et al.*, 2001).

The skin is one of the most extensive and readily accessible organs of the human body and the skin as a route of drug delivery can offer many advantages over traditional drug delivery systems including lower fluctuations in plasma drug levels, avoidance of gastrointestinal disturbances and first-pass metabolism of the drugs and high patient compliance. One of the greatest disadvantages to transdermal drug delivery is the skin's low permeability that limits the number of drugs that can be delivered in this manner. The skin offers an excellent barrier to molecular transport, as stratum corneum is the most formidable barrier to the passage of most of the drugs, except for lipophilic and low molecular weight drugs. For transdermal and topical drug delivery system to be effective, the drug must obviously be able to penetrate the skin barrier and reach the target site. During the past several decades, researchers have developed numerous techniques to weaken or disrupt the skin barrier and deliver drugs into the body through the intact skin. Chemical skin permeation enhancers, iontophoresis, sonophoresis, electroporation, microneedles, and many other methods have been investigated to increase the efficacy of transdermal transport. Owing to their limited efficacy, resulting skin irritation, complexity of usage and or high cost, none of these methods have been broadly applied to date. Lipid-based suspensions such as liposomes, niosomes and microemulsions, have also been proposed as low- risk drug carriers, but they do not offer much value in transdermal drug delivery because they do not deeply penetrate the skin, but rather remain on the upper layers of skin strata. Several researchers have developed novel elastic lipid vesicular systems in order to deeply and easily penetrate through the skin. Phospholipids,ethanol, bile salts and many surfactants have been used to prepare these elastic vesicles. The high flexibility of vesicular membranes allows these elastic vesicles to squeeze themselves through the pores in stratum corneum, which are much smaller than their vesicular sizes. In Cevc and Blume (1992) introduced the first generation elastic lipid vesicular carrier, Transfersomes, mainly consisting of phospholipids and an edge activator (non-ionicsurfactant). They were reported to penetrate intact skin and able to deliver the drug into and across the skin, when applied under non-occlusive conditions.

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. 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 nonelectrolytes are mostly determined within the stratum corneum (Fig. 1).

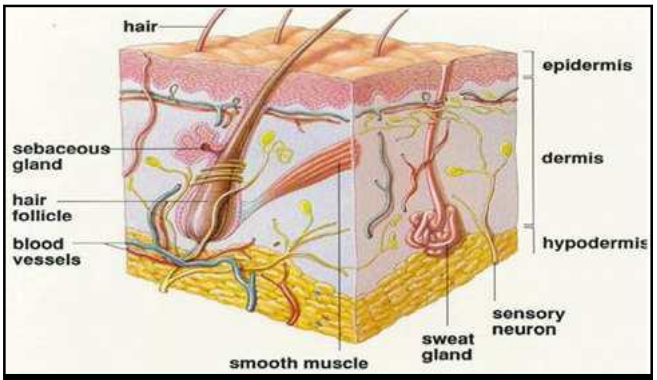

Fig. 1 : Structure of skin

Rational for transdermal drug delivery :

Given that the skin offers such an excellent barrier to molecular transport, the rationale for this delivery strategy needs to be carefully identified. There are several instances where the most convenient drug intake methods (oral route) were not feasible and alternative routes had to be sought. Although, intravenous introduction of the medicament avoids many of these shortfalls (such as gastrointestinal and hepatic metabolism), its invasive and

apprehensive nature (particularly for chronic administration) has encouraged the search for alternative strategies. Transdermal drug delivery (TDD) offers several distinct advantages including relatively large and readily accessible surface area for absorption, ease of application and termination of therapy. Further, evolution of better technologies for delivering drug molecules, safe penetration enhancers and the use of vesicular carriers have rejuvenated the interest for designing TDD system for drugs that were thought to be unfit for transdermal delivery.

Vesicular approaches for topical drug delivery :

Drug encapsulated in lipid vesicles prepared from phospholipids and nonionic surfactants is known to be transported into and across the skin. Lipids present in the skin contribute to the barrier properties of skin and prevent systemic absorption of drugs. Due to the amphiphilic nature, lipid vesicles may serve as non-toxic penetration enhancer for drugs. In addition,vesicles can be used for encapsulating hydrophilic and lipophilic as well as low and high molecular weight drugs. Therefore, these lipid rich vesicles are hypothesized to carry significant quantity of drugs across the skin thus, enhancing the systemic absorption of drugs. Drug delivery from liposomes in transdermal formulation has been studied for many purposes but unstable nature and poor skin permeation limits their use for topical delivery. In order to increase the stability of liposomes, the concept of proliposomes was proposed. This approach was extended to niosomes, which exhibited superior stability as compared to liposomes. However, due to poor skin permeability, liposomes and niosomes could not be successfully used for systemic drug delivery and their use was limited for topical use. To overcome problems of poor skin permeability Cevc *et al.* and Touitou (1996), recently introduced new vesicular carrier system ethosomes, for non-invasive delivery of drugs into or across the skin. Ethosomes incorporated penetration enhancers (alcohols and polyols), to influence the properties of vesicles and stratum corneum. The vesicles have been well known for their importance in cellular communication and particle transportation for many years. Researchers have understood the properties of vesicles structure for use in better drug delivery within their cavities, which would to tag the vesicle for cell specificity. One of the major advances in vesicle research was the development of vesicle derivatives, known as an ethosomes.

Ethosomes :

Ethosomes (Fig. 2) 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 (Gangwar *et al.,* 2010; Kumar *et al.,* 2010; Heeremans *et al.,* 1995; Asbill *et al.,* 2000; Touitou *et al.,* 1998 and Verma and Pathak, 2010). Ethosomes can entrap drug molecule with various physicochemical characteristics *i.e.* of hydrophilic, lipophilic, or amphiphilic. The size range of ethosomes may vary from tens of nanometers to microns (Bhalaria *et al.*, 2009 and Verma and Fahr, 2004). They are mainly used for the delivery of drugs through transdermal route. Drug can be entrapped in ethosomes which have various physicochemical characteristics *i.e.* hydrophilic, lipophilic, or amphiphilic. Ethosomes are soft, malleable vesicles used for delivery of drugs to reach the deep skin layers and/or the systemic circulation. The size range of ethosomes may vary from tens of nano meters to microns. Ethosomes are the modified forms of liposomes that are high in ethanol content. The ethosomal system is composed of phospholipid (Phosphatidylcholine, phosphatidylserine, phosphatitidic acid), high concentration of alcohol (ethanol and isopropyl alcohol) and water. The high concentration of ethanol makes ethosomes unique because ethanol causes disturbance of skin lipid bilayer organization, hence when incorporated into a vesicle membrane, it enhances the vesicles' ability to penetrate the stratum corneum.

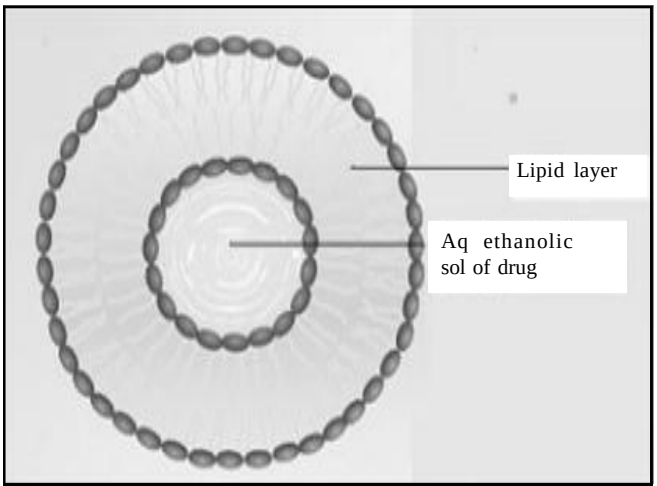

Fig. 2 : Diagram of Ethosomes

Advantages of Ethosomal drug delivery :

- Delivery of large molecules (peptides, protein molecules) is possible.
- It contains non-toxic raw material in formulation.
- Enhanced permeation of drug through skin for transdermal drug delivery.
- Ethosomal drug delivery system can be applied widely in pharmaceutical, veterinary, cosmetic fields.
- High patient compliance: The ethosomal drug is administrated in semisolid form (gel or cream) hence producing high patient compliance.
- Simple method for drug delivery in comparison to iontophoresis and phonophoresis and other complicated methods
- The Ethosomal system is passive, non-invasive and is available for immediate commercialization.

Ethosomes composition :

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). 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 per cent w/w. Cholesterol at concentrations ranging between 0.1 1 per cent can also be added to the preparation. 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 per cent. The concentration of the non-aqueous phase (alcohol and glycol combination) may range between 22 to 70 per cent (Table 1). The ethosomal system consists of phospholipids, ethanol and water. The phospholipids with various chemical structure includes phosphatidyl choline (PC), hydrogenated PC, phosphatidyl ethanolamine(PE), phosphatidyl glycerol (PPG), phosphatidyl inositol (PI), hydrogenated PC etc.The non-aqueous phase range between 22 per cent to 70 per cent. The alcohol may be ethanol or isopropyl alcohol. Dyes or amphiphilic fluorescent probe such as D– 289, Rhodamine- 123, fluorescence isothiocynate (FITC), 6 – carboxy fluorescence are often added to ethosomes for characterization study.

Table 1 : Different additive employed in formulation of Ethosomes (2)

Class	Example	Uses
Phospholipid	Soya phosphatidyl choline	Vesicles forming component
	Egg phosphatidyl choline	
	Dipalmityl phosphatidyl choline	
	Distearyl phosphatidyl choline	
Polyglycol	Propylene glycol	As a skin penetration enhancer
	Transcutol RTM	
Alcohol	Ethanol	For providing softness to the vesiclemembrane, as a
	Isopropylalcohol	penetration Enhancer
Cholesterol	Cholesterol	For providing stability to the vesiclemembrane
Dyes	Rhodamine -123	For characterization study
	Rhodamine red	
	Fluorescence isothiocyanate	
	6-carboxy fluorescence	
Vehicle	Carbopol 934	As a gel provider

Mechanism :

The depth of skin penetration from ethosomal systems can be assessed by confocal laser scanning microscopy (CLSM). For skin penetration studies various fluorescent probes with different physicochemical properties, like rhodamine red, rhodamine B, β-carotene (βC), rhodamine 6G, can be entrapped within the ethosomal vesicles (21,22). The transition temperature of the lipid in the vesicular systems can be determined as a measure of vesicle softness. Both the drug and concentration of ethanol influence the transition temperature of vesicular lipids. Storage stability of ethosomal systems can be determined by comparing the shape, average size and entrapment capacity of the vesicles over time at different storage conditions. Based on various stability studies performed, researchers suggest refrigerated condition (4-8°C) as the suitable storage condition for ethosomal formulations. Higher temperatures may cause degradation of vesicular lipids, lose of structural integrity of vesicles and an accelerated leakage of the entrapped contents.

Mechanism of drug penetration :

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 (Fig. 3).

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.

Ethosome effect :

Increased cell membrane lipid fluidity caused by the

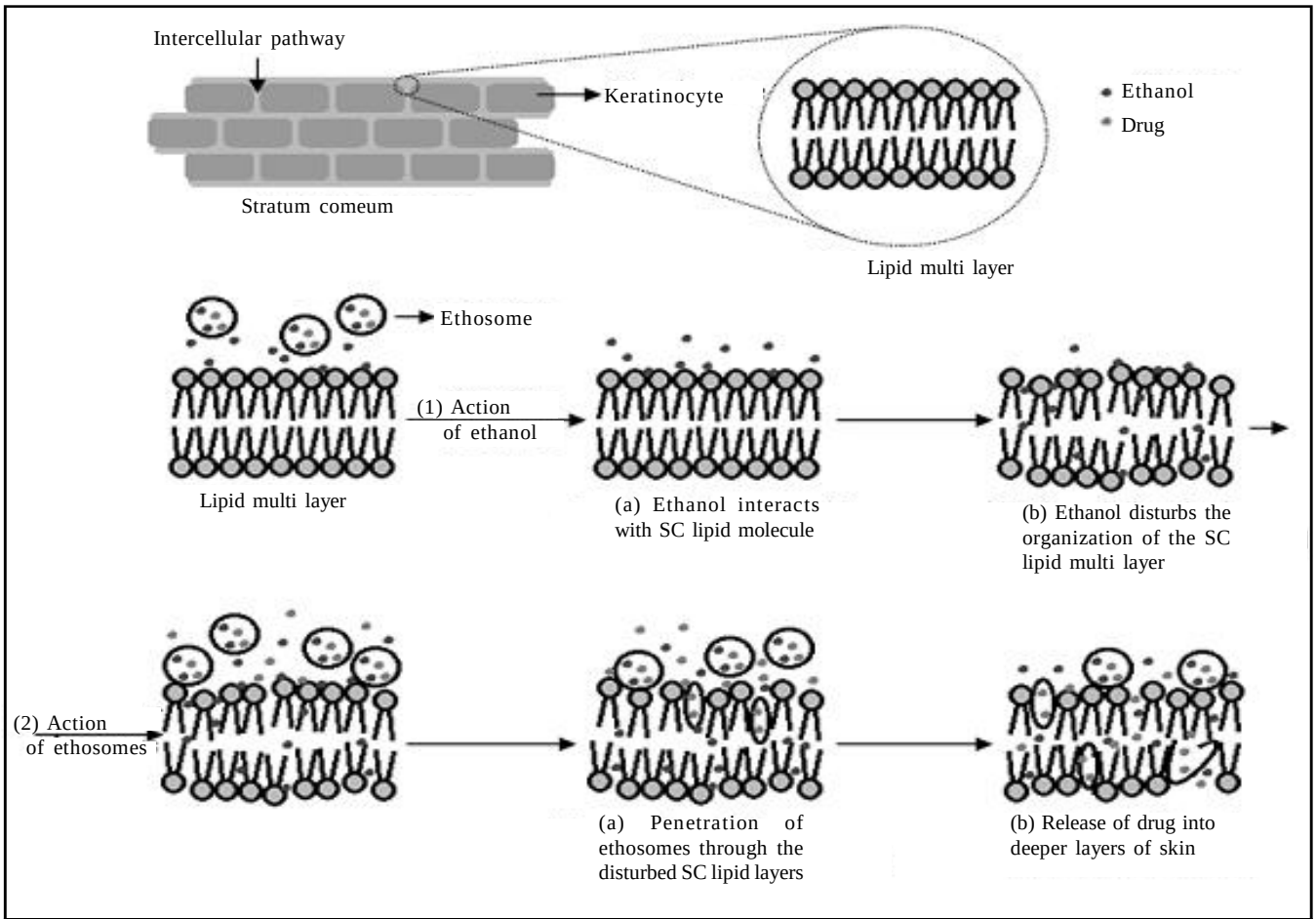

Fig. 3 : Mechanism of drug penetration

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 Ethosomes :

Ethosomes can be prepared by two very simple and convenient methods that are hot method and cold method.

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 300°C in a water bath. The water heated to 300°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 (Nikalje and Tiwari, 2012 and Dinesh *et al.,* 2009).

Hot method :

In this method phospholipid is dispersed in water by heating in a water bath at 400C 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 (Nikalje and Tiwari,2012 and Dinesh *et al.,* 2009).

Characterizations of Ethosomes :

Visualization :

Visualization of ethosomes can be done using transmission electron microscopy (TEM) and by scanning electron microscopy (SEM) (Nikalje and Tiwari, 2012).

Vesicle size and zeta potential :

Particle size and zeta potential can be determined by dynamic light scattering (DLS) using a computerized inspection system and photon correlation spectroscopy (PCS) (Maghraby *et al*., 2000).

Differential scanning calorimertry (*DSC*) :

Transition temperature (Tm) of the vesicular lipid systems was determined by using the Mettler DSC 60 computerized with Mettler Toledo star software system (Mettler, Switzerland).The transition temperature was measured by using the aluminium crucibles at a heating rate 10 degree/minute, within a temperature range from 20°C–300°C (Cevc *et al.*,1995 and Fry *et al.*,1978).

Surface tension activity measurement :

The surface tension activity of drug in aqueous solution can be measured by the ring method in a Du Nouy ring tensiometer (Cevc *et al.*,1995 and Fry *et al.*,1978).

Entrapment efficiency :

The entrapment efficiency of drug by ethosomes can be measured by the ultra centrifugation technique (Fry *et al.,* 1978).

Penetration and permeation studies :

Depth of penetration from ethosomes can be visualized by confocal laser scanning (Nikalje and Tiwari, 2012).

Vesicle stability :

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 (Cevc *et al*.,1996 and Fry *et al*.,1978).

Conclusion :

The main limiting factor of transdermal drug delivery system *i.e*. epidermal barrier can be overcome by ethosomes to significant extent. The ethosomes more advantages when compared to transdermal and dermal delivery. Ethosomes are the non-invasive drug delivery carriers that enable drugs to reach the deep skin layers finally delivering to the systemic circulation. It delivers large molecules such as peptides, protein molecules. Simple method for drug delivery in comparison to iontophoresis and phonophoresis and other complicated methods. High patient compliance as it is administrated in semisolid form (gel or cream) and various application in pharmaceutical, veterinary, cosmetic field.

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