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A Review on "Ethosomes: An Emerging Approach for Drug Delivery through the Skin"

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Abstract

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 hence these can be used widely in place of liposomes. The increased permeation of ethosomes is probably due to its ethanolic content. Ethanol increases the cell membrane lipid fluidity which results in increased skin penetrability of the ethosomes. 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. The interest in designing transdermal delivery systems was relaunched after the discovery of elastic vesicles: transferosomes and liposomes. 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, andminoxidil, as well as cationic drugs such as propranolol, trihexyphenidil, Cyclosporine A, insulin, salbutamol etc.. Ethosomes provides a number of important benefits including improving the drug's efficacy, enhancing patient compliance and comfort and reducing the total cost of treatment.

Key Words: Transdermal, Skin permeation, Vesicles, Enhanced drug delivery, Ethosomes

Introduction

The skin covers a total surface area of approximately 1.8m^{2}and provides the contact between the human body and the external environment. Dermal drug delivery is the topical application of drugs to the skin in the treatment of skin diseases and other inflammatory conditions. This has the advantage that high concentrations of drugs can be localized at the site of action, reducing the systemic side effects. Transdermal drug delivery uses the skin as an alternative route for the delivery of systemically acting drugs. 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 liposome Figure1. Ethosomes are lipid vesicles containing phospholipids, alcohol (ethanol and isopropyl alcohol) in relatively high concentration and water [1]. Transdermal drug delivery offers many advantages as compared to traditional drug delivery systems, including oral and parenteral drug delivery system. Advantages claimed are increased patient acceptability (noninvasiveness), avoidance of gastrointestinal disturbances and first pass metabolism of the drug[2].The traditional transdermal drug delivery systems involve a patch, in which the drug permeates through various layers of skin, via a passive diffusion pathway. However, this limits the basic potential of these systems, as stratum corneum is the most formidable barrier to the passage of most of the drugs, except for highly lipophilic, low molecular weight drugs [3]. 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. [4].

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 finding a vesicle derivatives, known as an Ethosomes [5]. 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 hence these can be used widely in place of liposomes. The increased permeation of ethosomes is probably due to its ethanolic content [6]. Ethanol increases the cell membrane lipid fluidity which results in increased skin penetrability of the ethosomes. These ethosomes permeates inside the skin and

fuse with cell membrane lipids and release the drug. Hot and cold methods are used for formulation of ethosomes [7]. Ciclopiroxolamine is an antifungal drug for treatment of cutaneous candidiasis infections. The goal of the current investigation is to evaluate the transdermal potential of novel vesicular carrier, ethosomes, bearing ciclopiroxolamine an antifungal having limited transdermal permeation. Ciclopirox olamine loaded ethosomes were prepared, optimized and characterized for vesicular shape and surface morphology, vesicular size, size distribution, entrapment efficiency, vesicles skin interaction and stability [8,9].

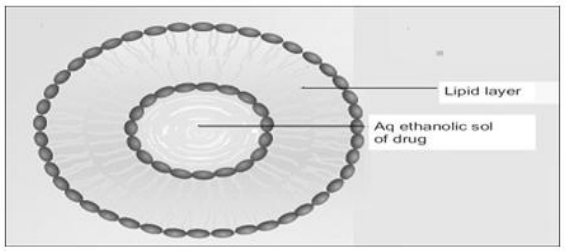

Fig 1:-proposed diagram of ethosomes.

Advantages of Ethosomal Drug Delivery

1. Ethosomes are enhanced permeation of drug through skin for transdermal and dermal delivery.
2. Ethosomes are platform for the delivery of large and diverse group of drugs (peptides, protein molecules)
3. Ethosome composition is safe and the components are approved for pharmaceutical and cosmetic use.
4. Simple method for drug delivery in comparison to Iontophoresis and Phonophoresis and other complicated methods.
5. It contains non-toxic raw material in formulation.
6. High patient compliance-The ethosomal drug is administrated in semisolid form (gel or cream) hence producing high patient compliance.
7. The Ethosomal system is passive, non-invasive and is available for immediate commercialization.
8. 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.

Mechanism of Drug 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 [10,11]. 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, shown in Figure 2.

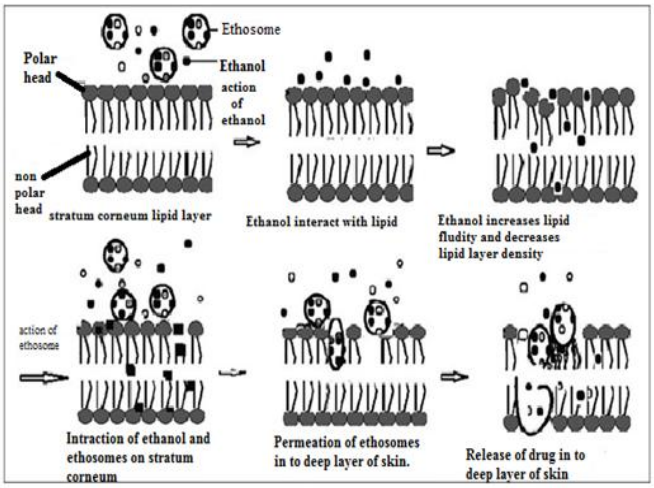

Fig 2:- Mechanism of action of ethosomes

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:

1. Ethanol effect
2. Ethosomes effect

- 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.

- 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.

Method of Preparation

There are two methods which can be used for the formulation and preparation of ethosomes. Both methods are very simple and convenient and not any sophisticated instrument use or any complicated process. Ethosomes can be made by following two methods

Hot method: - In this method phospholipid disperse in water by heating in a water bath at 400 ^{O}C until a colloidal solution is obtained. In a separate vessel properly mix ethanol and propylene glycol and heat up to 400c. Add the organic phase into the aqueous phase. Dissolve the drug in water or ethanol depending on its solubility [12,13]. The vesicle size of ethosomal can be decreased to the extent of our need using probe sonication or extrusion method.

Cold method: - This is the most common and widely used method for the ethosomal preparation. Dissolve phospholipid, drug and lipid materials in ethanol in a vessel at room temperature with vigorous stirring. Add propylene glycol during stirring. Heat the mixture upto 300 ^{O}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 6 or extrusion 13 method [14,15]. Finally, the formulation should be properly stored under refrigeration.

Various Methods for Characterization of Ethosomes

1. Vesicle shape: - Ethosomes can be easily visualized by using transmission electron microscopy (TEM) and by scanning electron microscopy (SEM) [16].
2. Vesicle 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 [17].
3. Transition temperature: - The transition temperature of the vesicular lipid systems can be determined by using differential scanning calorimetry (DSC) [18].
4. Drug entrapment: - The entrapment efficiency of ethosomes can be measured by the ultracentrifugation technique [19].
5. 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 [20].
6. 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.
7. 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.
8. 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).Table 1 shows Characterization of ethosomes. [21]

Table 1: Characterisation of ethosomes.

s.no	Parameter	Importance	Method
1	Size and shape	Determine skin penetration	SEM, TEM, DLS
2	Zeta potential	Stability of vesicles	Zeta Meter
3	Entrapment efficiency	Suitability of method	Ultracentrifugation
4	Drug content	Important in deciding the amount of vesicle preparation to be used.	UV, HPLC
5	Stability studies	To determine the shelf life of vesicle formulation	SEM, TEM, HPLC
6	Invitro dissolution	Determine the drug release rate from vesicle	Franz diffusion cell
7	Skin permeation	Determines rate of drug transport through skin	CLSM

Various vesicular structures as skin delivery systems

In the last years, the vesicular systems have been promoted as a mean of sustained or controlled release of drugs, because of their certain advantages, e.g. lack of toxicity, biodegradation, capacity of encapsulating both hydrophilic and lipophilic molecules, capacity of prolonging the existence of the drug in the systemic circulation by encapsulation in vesicular structures, capacity of targeting the organs and tissues,

capacity of reducing the drug toxicity and increasing its bioavailability. Vesicles are water-filled colloidal particles. The walls of these capsules consist of amphiphilic molecules (lipids and surfactants) in a bilayer conformation. In an excess of water these amphiphilic molecules can form one (unilamellar vesicles) or more (multilamellar vesicles) concentric bilayers. Hydrophilic drugs can be entrapped into the internal aqueous compartment, whereas amphiphilic, lipophilic and charged hydrophilic drugs can be associated with the vesicle bilayer by hydrophobic and/or electrostatic interactions [22, 23].

Most commonly, the vesicles are composed of phospholipids or nonionic surfactants. The reason for using vesicles in transdermal drug delivery is based on the fact that they act as drug carriers to deliver entrapped drug molecules across the skin, as well as penetration enhancers because of their composition. In addition, these vesicles serve as a depot for the sustained release of active compounds in the case of topical formulations, as well as rate-limiting membrane barrier for the modulation of systemic absorption in the case of transdermal formulations [24].

Liposomal formulations can be classified in two categories: rigid vesicles – liposomes and niosomes – and elastic or ultradeformable vesicles – transferosomes and ethosomes.

Niosomes: **-**Niosomes are vesicles composed of nonionic surfactants. The niosomes have been mainly studied because of their advantages compared with the liposomes: they are quite stable structures and require no special conditions for preparation and storage, they have no purity problems and the manufacturing costs are low. Unfortunately, the performed studies showed that, like liposomes, niosomes are not suitable for transdermal delivery, because they cannot reach the deeper layers of the skin, being trapped in the superior layers of stratum corneum. To overcomethis problem, the carried out researches introduced a novel generation of vesicular elastic systems: transferosomes (ultradeformable vesicles consisting of phospha- tidylcholine and an edge activator) and ethosomes (ultradeformable vesicles with high alcohol content) [25].

Transferosomes: -Transferosomes are a special type of liposomes, consisting of phosphatidylcholine and an edge activator. The concept of transferosomes was introduced in 1992 by Cevc and coworkers. These vesicular transferosomes are several orders of magnitude more elastic than the standard liposomes and thus well suited for the skin penetration [26].

Ethosomes

Ethosomes are deformable liposomes with high alcohol content (up to 45%). It is proposed that the alcohol fluidizes the ethosomal lipids and stratum corneum bilayer lipids thus allowing the soft, malleable ethosomes to penetrate. They have been introduced for the first time by Touitou in 1996. The ethanol from ethosomes composition plays the same role as the surfactant from the transferosomes, namely disorganizing the lipid bilayer, conferring a ten times higher deformability to the particles [27].

Evaluation of ethosomes

1. Visualization by scanning electron microscopy (SEM):- The size and shape of the vesicles were observed in the scanning electron microscopy. One drop of ethosomal suspension (F16) was mounted on a clear glass stub. It was then air dried and gold coated using sodium auro thiomalate to visualize under scanning electron microscope at 10,000 magnifications [28].

2. Determination of entrapment efficiency: - Entrapment efficiency of diclofenac potassium ethosomal vesicles was determined by centrifugation method. The vesicles were separated in a high speed cooling centrifuge at 20,000 rpm for 90 minutes in the temperature maintained at 4ºC. The sediment and supernatant liquids were separated; amount of drug in the sediment was determined by lysing the vesicles using methanol. From this, the entrapment efficiency was determined by the following equation [29],

Entrapment efficiency = DE/DT x 100 Where,

DE — Amount of drug in the ethosomal sediment

DT— Theoretical amount of drug used to prepare the formulation

(equal to amount of drug in supernatant liquid and in the sediment).

3.Vesicular size and size distribution: - Dynamic light scattering technique was used to determine the vesicular size and size distribution. One drop of ethosomal formulation was diluted to 10ml with hydroethanolic mixture used in the formulation and the measurements were taken. The size distribution of the liposome formulation was also determined after diluted with distilled water [30].

4. Comparison of in vitro skin permeation of drug forms various formulations: -Invitro skin permeation of diclofenac potassium in ethosomes, liposomes, hydroethanolic solution (1%w/v) and in phosphate buffer saline pH 7.4 (1%w/v) were studied using Franz diffusion cell with an effective permeation area of 2.54cm2. The ethosomal formulation was selected for the in vitro skin permeation on the basis of high entrapment efficiency and smaller vesicular size. Rats (male albino) 6 to 8 weeks old, weighing 120 to 150g were sacrificed for abdominal skin. After removing the hair, the abdominal skin was separated from the underlying connective tissue with scalpel. The excised skin was placed on aluminum foil and the dermal side of the skin was gently teared off for any adhering fat and/or subcutaneous tissue. The skin was checked carefully to ensure the skin samples are free from any surface irregularity such as fine holes or crevices in the portion that is used for transdermal permeation studies. The in vitro study was approved by the institutional ethical committee. The skin was mounted between donor and receptor compartment with the stratum corneum side facing upward into the donor compartment. Phosphate buffer saline pH 7.4 was taken in the receptor compartment. The formulation was applied on the skin in donor compartment which was then covered with aluminum foil to avoid any evaporation process. Samples were withdrawn at predetermined time intervals over 12 hours, and suitably diluted with phosphate buffer saline pH 7.4 to analyze the drug content in UV-Visible spectrophotometer at 276nm using phosphate buffer saline pH 7.4 as blank. The receptor medium was immediately replenished with equal volume of fresh medium to maintain the sink conditions throughout the experiment. The percentage of drug release was plotted against time to find the drug release pattern [31].

5. Skin retention studies: -The amount of diclofenac potassium retained in the skin was determined at the end of the 12 hours Invitro permeation studies. The formulation

remain in the invitro permeation experiment was removed by washing with distilled water. The receptor content was replaced by 50% v/v ethanol and kept for further 12 hours with stirring and the drug content was estimated spectrophotometrically at 276nm. This receiver solution diffused through the skin, disrupting any liposome and ethosome structure and extracting deposited drug from the skin.

6.FTIR studies: **-** Stability studies were carried out by storing the ethosomal formulations at two different temperatures 4ºC and 25±2ºC. The drug content was estimated for every 15 days to identify any change in the entrapment efficiency of ethosomal formulation.

7. Stability studies: - Stability studies were carried out by storing the ethosomal formulations at two different temperatures 4ºC and 25±2ºC. The drug content was estimated for every 15 days to identify any change in the entrapment efficiency of ethosomal formulation.

Therapeutic Application of Ethosomes

Touitou et al. experimentally tested the effect of an ethosomal insulin formulation that was applied to the skin on blood glucose level. The ethosomal formulation caused much as a 60% decrease in blood glucose levels in both normal and diabetic rats and kept the level constant for at least 8 hours [32]

Ethosomes as a carrier of various drug molecules has been listed below table 2.Horwitz et al. reported that a 5 % acyclovir ethosomal preparation compared to the 5 % acyclovir cream showed significant improvements in treatment of herpetic infections [33].

Esposito et al. investigated basic properties and the in vitrorelease rate kinetics of azelaic acid, alternatively vehiculated in different phospholipid-based vesicles such as ethosomes or liposomes. Diffusion of azelaic acid from ethosomal or liposomal dispersions and from ethosomes and liposomes incorporated in a viscous gel was investigated by a Franz cell assembled with synthetic membranes. The release rate was more rapid from ethosomal systems than from liposomal systems [34].

Table 2.Ethosomes as a carrier of various drug molecules has been listed below

Drug	Applications	Comments
Acyclovir	Treatment of Herpetic infection	Improved drug delivery
Zidovudine	Treatment of AIDS	Improved transdermal flux
TrihexyphenidylHCl	Treatment of Parkinsonian syndrome	Increased drug entrapment efficiency, reduced side effect & constant systemic levels
Erythromycin	Efficient healing of S. aureus -induced deep dermal infections	Improved drug penetration and systemic effect.
Insulin	Treatment of Diabetes	Improved therapeutic efficacy of drug
Testosterone	Treatment of male hypogonodism	Enhance skin permeation
Bacitracin	Treatment of dermal infections	Reduced drug toxicity
Minodixil	Hair growth promotion effect	Higher skin retention

Conclusion

Ethosomal carrier opens new challenges and opportunities for the development of novel improved therapies.Ethosomes are soft, malleable vesicles and potential 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 hydroalcoholic solution.It can be easily concluded that ethosomes can provide better skin permeation than liposomes. 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 diseases.

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