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Journal of Current Pharma Research 4 (2), 2014, 1172-1180.

Journal of Current Pharma Research
http://www.jcpronline.in

Review Article

Ethosome: A Versatile Tool for Novel Drug Delivery System

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Received 12 March 2014; received in revised form 22 March 2014; accepted 22 March 2014
Available online 23 March 2014

Abstract

Several approaches have been developed for increasing the skin penetration of drugs and many cosmetic chemicals is the use of vesicular systems, such as, liposomes and ethosomes. Ethosomal drug delivery system is one the approach having various application in pharmaceuticals. Ethosomes were developed by Touitou in 1997 as additional novel lipid carriers composed of ethanol, phospholipids and water. Ethanol is used as one of efficient permeation enhancer in ethosomes generally in concentration of 20-45%. Ethosomes were prepared by very simple methods as Hot and Cold methods characterized by vesicular size, Entrapment efficiency, transition temperature and veside stability. These are advantages like low toxicity better stability than liposomes, with better patient compliance. Ethosomes are having wide applications in drug delivery in treatment of AIDS and Parkinsonian syndrome and also in diabetic treatment. It a promising future in the development of novel improved therapies.

Keywords: Ethosomes, Transdermal, Skin permeation, enhanced drug delivery, Hydroethanolic solution.

1. Introduction

The skin forms a protective layer around the body. The rate of penetration of most drugs through the skin is controlled by the structure of stratum corneum. The ability of the skin to impede the permeation of molecules means that, to date; only a small number of pharmaceutically active compounds have been suitable for conventional transdermal delivery. Many drugs will be absorbed but in such small quantities per area of skin per hour that a very large application area would be needed to achieve levels of concentration in the blood high enough to have therapeutic effects. Other drugs, in particular large, charged molecules such as peptides and proteins barely pass the skin at all [1]. Ethosomes were designed to enhance the delivery of drugs into the deep layers of the skin.

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Ethosomes are considered as being safe for pharmaceutical and cosmetic use. Depending on the formulation, delivery can be targeted for local delivery or for systemic use. The structure of an ethosomes allows it to carry a wide variety of molecules with various physico-chemical properties. Ethosomal systems were found to be significantly superior at delivering drugs through the skin in terms of both quantity and depth, when compared to liposomes and many commercial transdermal and dermal delivery systems [1, 2]. Ethosomes are sophisticated vesicular delivery carriers that are capable of delivering various chemical applications. Visualization by dynamic light scattering showed that Ethosomes could be unilamellar or multilamellar through to the core. The size of Ethosomes can be modulated to range anywhere from 30 nm to a few microns. These novel delivery systems contain soft phospholipid vesicles in the presence of high concentrations of ethanol. Ethosomal systems are sophisticated conceptually, but characterized by simplicity in

their preparation, safety and efficiency, thus a rare combination that can expand their applications. It can interact with the polar head group region of the lipid molecules, resulting in the reduction of the melting point of the stratum corneum lipid, thereby increasing lipid fluidity and cell membrane permeability. Ethosomal systems were found to be significantly superior at delivering drugs through the skin in terms of both quantity and depth, when compared to liposomes and too many commercial transdermal and dermal delivery systems. Ethosomes has advantages over the liposomes because of low cost of manufacturing and easy to manufacture.

2. The Skin Barrier: Stratum Corneum

The stratum corneum is the outermost layer of the epidermis and is made of dead, flat skin call that shed about every two week. The cells of the stratum corneum are held together by an overlapping mechanism and with proteins that serve as a binding "glue". The stratum corneum serves an important barrier function by keeping molecules from passing into and out of the skin, thus protecting the lower layers of skin. The purpose of the stratum corneum is to form a barrier to protect underlying tissue from infection, dehydration, chemicals and mechanical stress. The stratum corneum, or horny layer, is the outermost layer of the skin and has been identified as the principal barrier for penetration of most drugs. The horny layer represents the final stage of epidermal cell differentiation. The thickness of this layer is typically 10 µm, but a number of factors, including the degree of hydration and skin location, influence this. The stratum corneum consists of 10-25 rows of dead corneocytes embedded in a lipid matrix. The cells are joined together by desmosomes, maintaining the cohesiveness of this layer.

The heterogeneous structure of the stratum corneum is composed of approximately 75-80% protein, 5-15% lipid and 5-10% unidentified on a dry weight basis. The main lipids located in the stratum corneum are ceramides, fatty acids, cholesterol, and cholesterol sulphate. These lipids are arranged in multiple bilayers called lamellae. Phospholipids are largely absent, a unique feature for a mammalian membrane. The ceramides are the largest group of lipids in the stratum corneum, accounting for approximately half of the total lipid mass, and are crucial to lipid organization of the stratum corneum (see in figure 1)[3].

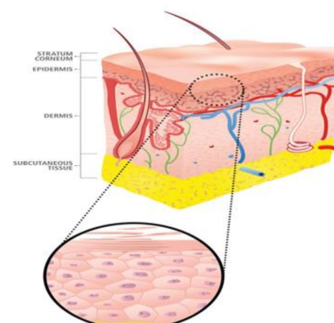


Fig.1: Skin barrier: stratum corneum

3. Ethosome

Ethosomes were developed by Touitou *et al.*, 1997, as additional novel lipid carriers composed of ethanol, phospholipids, and water. They are reported to improve the skin delivery of various drugs [2]. Ethosomes are the ethanolic phospholipide vehicles which are used mainly for transdermal delivery of drugs[3]. Ethosomes are soft, malleable vesicles composed mainly of phospholipids, ethanol (relatively high concentration) and water. These “soft vesicles” represents novel vesicular carrier for enhanced delivery to/through skin. The size of Ethosomes vesicles can be modulated from 10 nanometers to microns. They are composed mainly of phospholipids, (phosphatidylcholine, phosphatidylserine, phosphatitidic acid), high concentration of ethanol and water. The high concentration of ethanol makes the ethosomes unique. The ethanol in ethosomes causes disturbance of skin lipid bilayer organization, hence when incorporated into a vesicle membrane, it enhances the vesicle’s 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 [4].

3.1. Ethosomes composition

The 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 ethosomal system consists of phospholipids, ethanol and water[5]. The phospholipids with various chemical structure includes phosphatidylcoholine (PC), hydrogenated PC, phosphatidyl ethanolamine (PE), phosphatidyl glycerol (PPG), phosphatidy linositol (PI), hydrogenated PC etc. The nonaqueous phase range between 22% to 70% [6, 7]. 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 [8, 9]. Such composition delivers the high concentration of drug through the barrier of the skin. The delivery of drug modified by changing the concentration ratio of alcohol: water.

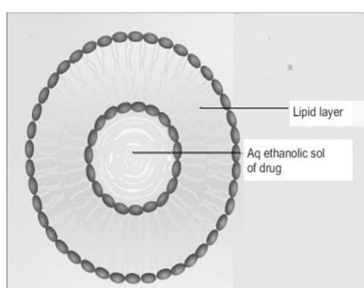


Fig. 2: Structure of ethosomes.

3.2. Advantages of Ethosomes

a) Enhanced permeation of drugs through the skin barrier
b) Better patient compliance
c) Low toxicity[10].
d) Better stability and solubility of drugs
e) Widely applied in Pharmaceutical, Veterinary, Cosmetic fields.
f) The Ethosomal system is passive, non-invasive and is available for immediate commercialization.
g) It Avoids first pass metabolism
h) Ethosomes are platforms for the delivery of large and diverse groups of drugs (peptides, protein molecules).
i) Simple method for drug delivery in comparison to Iontophoresis and Phonophoresis and other complicated methods.
j) Relatively simple to manufacture with no complicated technical investments required for production of Ethosomes [11].

3.3. Limitations of Ethosomes

a) Poor yield.
b) In case if shell locking is ineffective then the ethosomes may coalescence and fall apart on transfer into water [12].
c) Loss of product during transfer form organic to water media [13].

4. Mechanism of Drug Penetration

The main advantage of the ethosomal drug delivery system is penetration of the drug through the stratum corneum or skin barrier. Over the liposome, ethosomes increase the penetration of the drug. The enhanced delivery of actives using ethosomes over liposomes can be ascribed to an interaction between ethosomes and skin lipids. 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 3. [14]

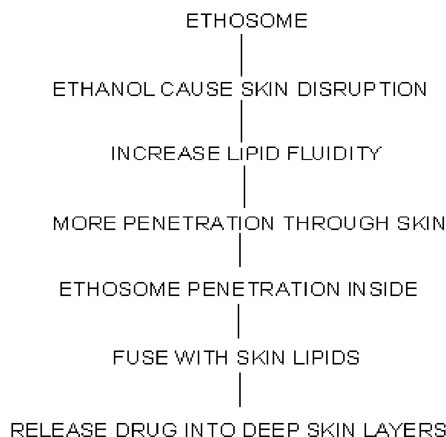


Fig.3. Mechanism of Drug Penetration from Ethosomes.

The drug absorption probably occurs in following two phases [15, 16]:

- Ethanol effect
- Ethosomes effect

Ethanol is an established efficient permeation enhancer [17] and is present in quite high concentration (20-50%) in Ethosomes. However, due to the interdigitation effect of

ethanol on lipid bilayer, it was commonly believed that vesicles could not coexist with high concentration of ethanol [18] (see in fig. 4).

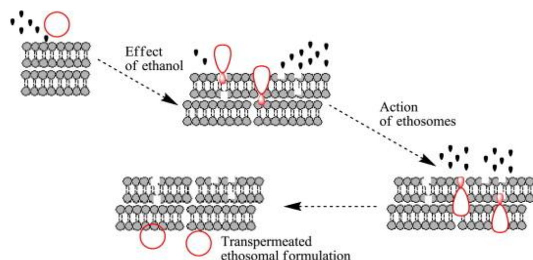


Fig.4: Effect of ethanol and Ethosomes.

4.1. Ethanol Effect

Ethanol acts as a penetration enhancer through the skin which is called as Ethanol Effect (see fig. 5). 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.

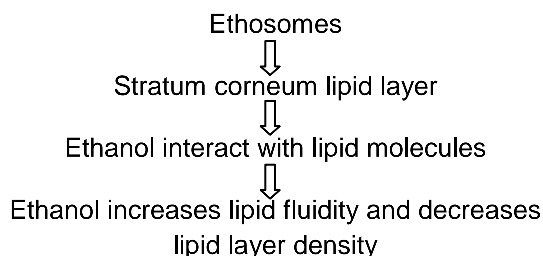


Fig.5: Action of Ethanol.

4.2. Ethosomal 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 (see in fig 6).

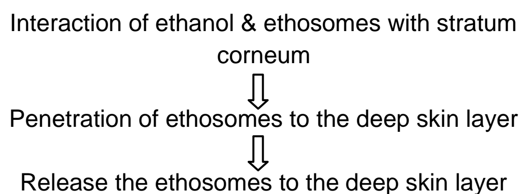


Fig.6: Action of Ethosomes.

4.3. Effect of High Alcohol Concentration

Ethanol is an established permeation enhancer and it is proposed that, it fluidizes the ethosomal lipids and stratum corneum bilayer thus allowing the soft, malleable vesicles to penetrate the disorganized lipid bilayer [19]. The relatively high concentration of ethanol (20 – 50 %) is the main reason for better skin permeation ability and is packed less tightly than conventional vesicles but has equivalent stability and better solubility of many drugs [5,20]. Moreover the vesicular nature of ethosomal formulation could be modified by varying the components ratio and phospholipids [21]. Ethanol confers a surface negative net charge to the ethosomes which causes the size of vesicles to decrease. The size of ethosomal vesicles increase with decreasing ethanol concentration [5]. 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.

5. Method of Preparation

There are two methods which can be used for the preparation of ethosomes namely hot method and cold method [5, 22]. Both the methods are very simple and convenient and do not involve any sophisticated instrument or complicated process (fig. 7).

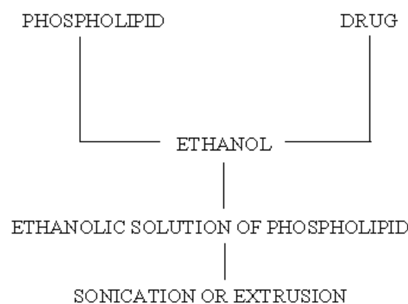


Fig.7: General method for Ethosomes preparation.

5.1. Hot Method

In this method phospholipid was dispersed in water by heating in a water bath at 40^0C until a colloidal solution is obtained. In a separate vessel ethanol and propylene glycol were mixed properly and heated upto 40^0C. Add the organic phase into the aqueous phase. Drug was dissolved in water or ethanol depending on its solubility [5, 22,23]. The vesicle size of ethosomal formulation can be decreased to the desire extent using probe sonication or extrusion method (see in fig. 8).

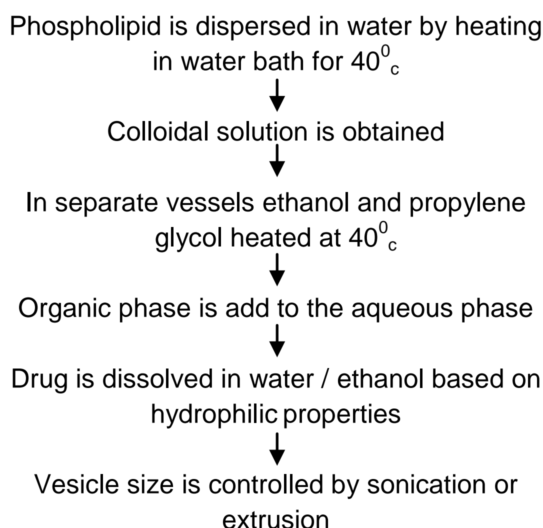
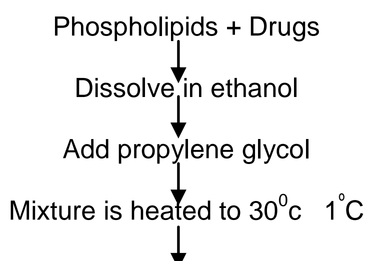


Fig.8: Flow chart hot method.

5.2. Cold Method

This is the most common and widely used method for the ethosomal preparation. Drug and phospholipid were dissolved in ethanol in which propylene glycol was added the mixture was heated upto 30^0 c for 1 hr. To prepared final dispersion double distilled water was added into constant stirring for 5 min. The vesicle size of ethosomal formulation can be decreased to desire extend using sonication [23,24] or extrusion [25] method. Finally, the formulation should be properly stored under refrigeration [5] (see in fig. 8).



Double distilled water is added with constant stirring for 5 mints.

↓

Vesicle size is controlled by using sonication and extrusion method

↓

Formation is stored in refrigerator

Fig.9: Flow chart cold method.

5.3. Classic Mechanical Dispersion Method

Soya phosphotidylcholine is dissolved in a mixture of chloroform: methanol (3:1) in round bottom flask. The organic solvents are removed using rotary vacuum evaporator above lipid transition temperature to form of a thin lipid film on wall of the flask. Finally, traces of solvent mixture are removed from the deposited lipid film by leaving the contents under vaccum overnight. Hydration is done with different concentration of hydroethanolic mixture containing drug by rotating the flask at suitable temperature [15,16].

5.4. Classic method:

The phospholipid and drug are dissolved in ethanol and heated to 30°C±1°C in a water bath. Double distilled water is added in a fine stream to the lipid mixture, with constant stirring at 700 rpm, in a closed vessel. The resulting vesicle suspension is homogenized by passing through a polycarbonate membrane using a hand extruder for three cycles [17].

6. Characterization of Ethosomes

1. Visualization

Visualization of Ethosomes can be done using transmission electron microscopy (TEM) and by scanning electron microscopy (SEM) [26,22.]

2. 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) [27, 28.]

3. Phospholipid -ethanol interaction

Phospholipid-ethanol interaction determined by ^{31}PNMR, Differential scanning calorimeter [29,26.]

4. Surface Tension Activity Measurement

Surface Tension Activity Measured by using Ring Method in a Du Nouy ring tensiometer [30,31].

5. Transition Temperature
Transition Temperature measured by Differential Scanning Calorimetry (DSC) [32,28].

6. Drug deposition study
Drug deposition study carried out by using Franz diffusion cell [33,34].

7. Entrapment Efficiency
The entrapment efficiency of drug by ethosomes can be measured by the ultracentrifugation technique [35,29].

8. Transition Temperature
The transition temperature of the vesicular lipid systems can be determined by using differential scanning calorimetry [36, 28].

9. 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 [37].

10. 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 [38].

11. Drug Content
Drug can be quantified by a modified high-performance liquid chromatographic method [39, 38].

12. Penetration and Permeation Studies
Depth of penetration from ethosomes can be visualized by confocal laser scanning microscopy (CLSM) [38].

13. Stability Study
Stability of the vesicles was determined by storing the vesicles at 4°C ± 0.5°C. Vesicle size, zeta potential, and entrapment efficiency of the vesicles was measured after 180 days using the method described earlier [40].

Table 1: All evaluation parameter with specific method are as follows.

Parameters	Methods	References
Visualization	Transmission electron microscopy (TEM) and Scanning Electron Microscopy (SEM)	22, 26
Vesicle size and Zeta potential	Dynamic Light Scattering (DLS), Photon Correlation Spectroscopy (PCS) and Zeta Meter	27
Entrapment Efficiency	Ultracentrifugation technique, uv spectroscopy, high performance liquid chromatography	29
Drug Content	High performance liquid chromatographic method	39, 38
Penetration and Permeation Studies	Confocal laser scanning microscopy (CLSM)	38
Phospholipid-ethanol interaction	^{31}PNMR,Differential scanning calorimeter	26, 29
Surface Tension Activity Measurement	Ring Method in a Du Nouy ring tensiometer	30, 31
Transition Temperature	Differential Scanning Calorimetry (DSC)	32, 28
In vitro **drug release study**	Franz diffusion cell with artificial or biological membrane, Dialysis bag diffusion	
Drug deposition study	Franz diffusion cell	33, 34
Stability study	Dynamic light scattering method, Transmission electron microscopy	26, 36
Turbidity	Nephalometer	11

7. Application
Ethosomes having wild variety of application in delivery of drug. Ethosomes are considered as being safe for pharmaceutical and cosmetic use. Maiden et al. [41]prepared and evaluated minoxidil ethosomal formulation. Minodixil is a lipid-soluble drug used topically on the scalp for the treatment of baldness. Conventional topical formulation has very poor skin permeation and retention properties. It was found that the quantity of minoxidil accumulated into nude mice skin after application of its ethosomal formulation was 2.0, 7.0 and 5.0 fold higher as compared to ethanolic phospholipids dispersion, hydroethanolic solution and ethanolic solution of drug each containing 0.5% of the drug. These results showed the possibility of using

ethosomes for pilosebaceous targeting of minoxidil to achieve its better clinical efficacy.
Touitou et al [5] compared the skin permeation potential of testosterone ethosomes (Testosome) across rabbit pinna skin with marketed transdermal patch of testosterone (Testoderm¨ patch, Alza). They observed nearly 30-times higher skin permeation of testosterone from ethosomal formulation as compared to that marketed formulation. The amount of drug deposited was significantly ($p < 0.05$) higher in case of ethosomal formulation. Dayan and Touitou [11] prepared ethosomal formulation of psychoactive drug trihexyphenidyl hydrochloride (THP) and compared its delivery with that from classical liposomal formulation. THP is a M1 muscarinic receptors antagonist and used in the treatment of Parkinson disease. Topical delivery of anti-arthritis drug is a better option for its site-specific delivery and overcomes the problem associated with conventional oral therapy. Cannabidol (CBD) is a recently developed drug candidate for treating rheumatoid arthritis. Its oral administration is associated with a number of problems like low bioavailability, first pass metabolism and GIT degradation. To overcome the above mention problem Lodzki et al. [42] prepared CBD-ethosomal formulation for transdermal delivery. Results of the skin deposition study showed significant accumulation of CBD in skin and underlying muscles after application of CBD-ethosomal formulation to the abdomen of ICR mice Plasma concentration study showed that steady state level was reached in 24 hr and maintained through 72 hr. Touitou E et al., [2] (1997) designed and evaluated novel vesicular carrier ethosomes of testosterone, molecular probes and minoxidil for characterization and skin penetration properties. Testosterone, molecular probes and minoxidil were formulated as ethosomes using soyabean phosphatidyl choline. The size distribution of ethosome vesicles was investigated using DLS. The size of the vesicles increased with decreasing ethanol concentration. No significant change in size was observed with change in phospholipid concentration. Drug entrapment by ethosomes containing 2% PL and 30% ethanol, and liposomes containing same concentration of PL with molecular probe, testosterone and monoxidil. Highest entrapment efficiency was observed by ethosomes. Stability study by DLS show highest stability with ethosomes containing 30% ethanol. Skin permeation study was carried out with Rhodamine red (RR) which show significant greater penetrability from ethosomal preparation while penetration from liposomes was negligible [43].

Table 2: Applications.

Drug	Applications	Ref.
Acyclovir	Treatment of Herpetic infection for Improved drug delivery	44
Anti-HIV agents Zidovudine Lamivudine	Treatment of AIDS for Improved transdermal flux	14
Trihexypenidyl HCl	Treatment of Parkinsonian syndrome for Increased drug entrapment efficiency, reduced side effect & constant systemic levels	11
Insulin	Significant decrease in blood glucose level	33

Conclusion

Ethosomal drug delivery is opens new challenges and opportunities for the development of novel improved therapies. It can be easily concluded that ethosomes can provide better skin permeation than liposomes. It is widely applied in pharmaceutical and cosmetic field. They improve the delivery of Pilosebaceous Targeting, Transdermal Delivery of Hormones, Delivery of anti-parkinsonism agent, Transcellular Delivery , Topical Delivery of DNA , Delivery of Anti-Arthritis Drug, Delivery of Antibiotics, Delivery of Anti-Viral Drugs, Delivery of Problematic drug molecules etc. Application of ethosomes provides the advantages such as improved permeation through skin and targeting to

deeper skin layers for various skin diseases. Thus ethosomes have a promising future in the Transdermal drug delivery system.

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Source of Support: Nil. Conflict of Interest: None declared

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