DOI: 10.21276/IJIPSR.2018.06.06.311

International Journal of Innovative Pharmaceutical Sciences and Research
www.ijipsr.com

REVIEW ON ETHOSOMES A RECENT VESICLE OF TRANSDERMAL DRUG DELIVERY

Chavan Sushmita*, Shetkar Suchita, Hulmajage Sonali, Khurde Sonali. Chandrawanshi Mayuri

*Department of Pharmaceutics, Channabasweshwar Pharmacy College, Latur, Maharashtra, **INDIA***

Abstract

Skin is the main target of topical and transdermal preparations. Major aim of transdermal drug delivery system is to cross the stratum corneum. Now-a-days we better know vesicles have importance in cellular communication. Ethosomal carriers are systems containing soft vesicles, composed of hydroalcoholic or hydro/glycolic phospholipids in which the concentration of alcohols is relatively high. The high concentration of ethanol brings increase in fluidity of lipids hence increase in permeability of the skin and improves the drug penetration. Ethosomal formulation may contain many drugs such as acyclovir, salbutamol, Insulin, cyclosporine, fluconazole, minodixil, etc. These are prepared by hot method and cold methods. The size of Ethosomal formulation can be decreased by sonication and extrusion method. The high concentration of ethanol makes the ethosomes unique and useful for transcellular delivery, delivery of hormones, anti-arthritis, anti-HIV etc. These are one of the best formulations developed.

Keywords: Ethosomes, Carriers, vesicles, Transdermal drug delivery.

Corresponding Author:

Chavan Sushmita

Department of Pharmaceutics,

Channabasweshwar Pharmacy College,

*Latur, Maharashtra, **INDIA***

***E-mail:** sushmitachavan4@gmail.com*

***Phone:** +91-9075365551*

DOI: 10.21276/IJIPSR.2018.06.06.311

INTRODUCTION

A number of techniques and efforts have been targeted to weaken and disrupt the highly organized intercellular lipids in an attempt to improve drug transport across the whole skin or to increase the permeation of drugs across this skin barrier. The vesicles have been well known for their important in cellular communication and particle transportation for many years. Researchers have known the properties of vesicular structures for better use in drug delivery, they incorporated drugs within their cavities that would allow for tagging the vesicle for cell specificity [1,2]. The soft, malleable vesicles adapt for superior delivery of active agents. Ethosomes are lipid vesicles containing phospholipids, alcohol (ethanol and isopropyl alcohol) in relatively high concentration and water [3]. The size of Ethosomes can be modulated to range anywhere from 30nm to a few microns. Ethosomes provides a number of important benefits including improving the drug efficacy, enhancing patient compliance and comfort and reducing the total cost of treatment [4]. Ethosomes are mainly used for the delivery of drugs through transdermal route. The transdermal delivery is one of the most significant routes of drug administration. The main factor which limits the application of transdermal route for drug delivery is the permeation of drugs through the skin. Human skin has selective permeability for drugs, only the lipophilic drugs having molecular weight < 500 dalton can pass through it. To overcome the stratum corneum barrier, various mechanisms have been investigated, including use of chemical or physical enhancers, such as sonophoresis, Iontophoresis, 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 [5,6]. Ethosomes were designed to enhance the delivery of drugs into the deep layers of the skin and through the skin. Depending on the formulation, delivery can be targeted for local delivery or for systemic use. Thus comparing to the other transdermal drug delivery systems, it is very important and with research point of view, an interesting system.

HUMAN SKIN

The human skin is the largest organ of the body, providing a protective coverage for the internal structure and organs. Skin comprises an area of between 16.1 ft2 and 21.6 ft2 (1.5 m2 and 2.0 m2), at an average thickness of 0.00394 in (0.1 mm), accounting for between 15% and 18% of the total body weight. The skin has three layers—the epidermis, dermis, and fat layer (also called the subcutaneous layer). Each layer performs specific tasks. Given its role as the protective shell for the internal organs and structure, the skin sustains constant contact and consequently, it is the most injured human organ.

DOI: 10.21276/IJIPSR.2018.06.06.311

Epidermis

The epidermis is the relatively thin, tough, outer layer of the skin. Most of the cells in the epidermis are keratinocytes. They originate from cells in the deepest layer of the epidermis called the basal layer. New keratinocytes slowly migrate up toward the surface of the epidermis. Once the keratinocytes reach the skin surface, they are gradually shed and are replaced by younger cells pushed up from below. The outermost portion of the epidermis, known as the stratum corneum, is relatively waterproof and, when undamaged, prevents most bacteria, viruses, and other foreign substances from entering the body.

The epidermis (along with other layers of the skin) also protects the internal organs, muscles, nerves, and blood vessels against trauma. In certain areas of the body that require greater protection (such as the palms of the hands and the soles of the feet), the outer keratin layer of the epidermis (stratum corneum) is much thicker. Scattered throughout the basal layer of the epidermis are cells called melanocytes, which produce the pigment melanin, one of the main contributors to skin color. Melanin's primary function, however, is to filter out ultraviolet radiation from sunlight which can damage DNA, resulting in numerous harmful effects, including skin cancer. The epidermis also contains Langerhans cells, which are part of the skin's immune system. Although these cells help detect foreign substances and defend the body against infection, they also play a role in the development of skin allergies.

Dermis

The dermis, the skin's next layer, is a thick layer of fibrous and elastic tissue (made mostly of collagen, elastin, and fibrillin) that gives the skin its flexibility and strength. The dermis contains nerve endings, sweat glands and oil glands, hair follicles, and blood vessels. The nerve endings sense pain, touch, pressure, and temperature. Some areas of the skin contain more nerve endings than others. For example, the fingertips and toes contain many nerves and are extremely sensitive to touch.

The sweat glands produce sweat in response to heat and stress. Sweat is composed of water, salt, and other chemicals. As sweat evaporates off the skin, it helps cool the body. Specialized sweat glands in the armpits and the genital region (apocrine sweat glands) secrete a thick, oily sweat that produces a characteristic body odor when the sweat is digested by the skin bacteria in those areas. The sebaceous glands secrete sebum into hair follicles. Sebum is an oil, that keeps the skin moist and soft and acts as a barrier against foreign substances.

The hair follicles produce the various types of hair found throughout the body. Hair not only contributes to a person's appearance but has a number of important physical roles including regulating body temperature, providing protection from injury, and enhancing sensation. A

DOI: 10.21276/IJIPSR.2018.06.06.311

portion of the follicle also contains stem cells capable of regrowing damaged epidermis. The blood vessels of the dermis provide nutrients to the skin and help regulate body temperature. Heat makes the blood vessels enlarge (dilate), allowing large amounts of blood to circulate near the skin surface, where the heat can be released. Cold makes the blood vessels narrow (constrict), retaining the body's heat. Over different parts of the body, the number of nerve endings, sweat glands and sebaceous glands, hair follicles, and blood vessels varies. The top of the head, for example, has many hair follicles, whereas the soles of the feet have none. Fat Layer: Below the dermis lies a layer of fat that helps insulate the body from heat and cold, provides protective padding, and serves as an energy storage area. The fat is contained in living cells, called fat cells, held together by fibrous tissue.

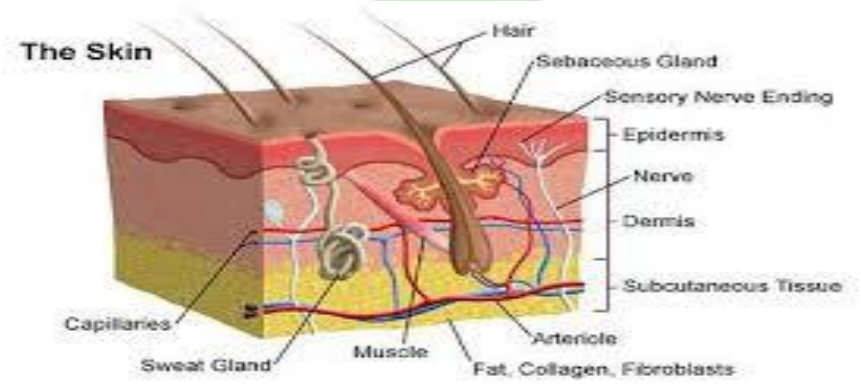

Fig.1: Structure of Skin

ROUTES OF PENETRATION

Human skin comes into contact with sebum, cellular debris, microorganisms and other materials, which somewhat affect the permeation of vesicles. The penetrant permeates by three potential pathways to the viable tissue:

- through hair follicles with associated sebaceous glands,
- via sweat ducts, or
- across continuous stratum corneum between these appendages. These pathways are important for ions and large polar molecules that struggle to cross intact stratum corneum.

ETHOSOMES

"Ethosomes are ethanolic liposomes" Ethosomes can be defined as noninvasive delivery carriers that enable drugs to reach deep into the skin layers and/or the systemic circulation. These are soft, malleable vesicles tailored for enhanced delivery of active agents. The vesicles have been well known for their importance in cellular communication and particle transportation for many years. Vesicles would also allow controlling the release rate of drug over an extended time, keeping the drug shielded from immune response or other removal systems and thus be able to release just the right amount of drug and keep that concentration

DOI: 10.21276/IJIPSR.2018.06.06.311

constant for longer periods of time. One of the major advances in vesicle research was the finding of a vesicle derivative, known as an ethosomes [7,8]. Ethosomal carriers are systems containing soft vesicles, ethanol at relatively high concentration and water. It was found that ethosomes penetrate the skin and allow enhanced delivery of various compounds to the deep strata of the skin or to the systemic circulation.

COMPOSITION

The ethosomes are composed of hydroalcoholic or hydro/glycolic phospholipids in which the concentration of alcohol is relatively high. Typically, ethosomes may contain phospholipids with various chemical structures like phosphatidylcholine (PC), hydrogenated PC, phosphatidic acid (PA), phosphatidylserine (PS), phosphatidyl ethanolamine (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% w/w. Cholesterol at concentrations ranging between 0.1-1% 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 preparationsCationic 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%. The concentration of the non-aqueous phase (alcohol and glycol combination) may range between 22 to 70% [9].

Table 1: Different additives employed in formulation of ethosomes:

Additives	Uses	Examples
Phospholipid	Vesicles forming component	Soya phosphatidyl choline, Egg phosphatidyl choline, Dipalmityphosphatidylcholine, Distearylphosphatidyl choline.
Polyglycol	Skin penetration enhancer	Propylene glycol, Transcutol
Cholesterol	Stabilizer	Cholesterol
Alcohol	For providing the softness for vesicle membrane as a penetration enhancer	Ethanol Isopropyl alcohol
Vehicle	As a gel former	Carbopol 934
Dye	For characterization study	6-Carboxy Fluorescence, Rhodamine-123, Rhodamine red, Fluorescene Isothiocynate

DOI: 10.21276/IJIPSR.2018.06.06.311

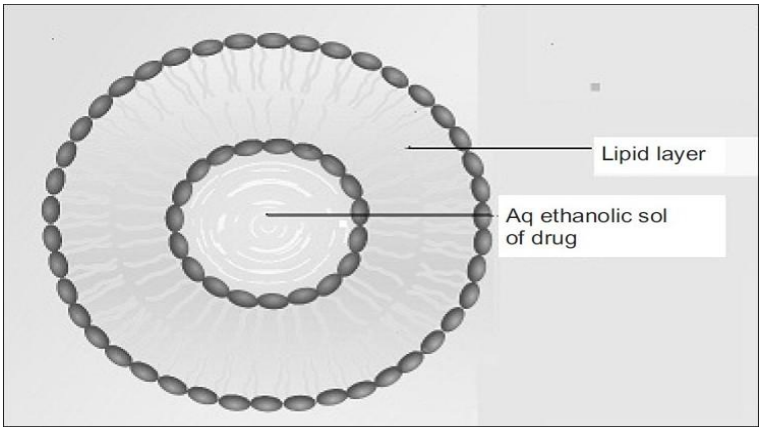

Fig. 2: Proposed diagram of ethosomes [10]

ADVANTAGES OF ETHOSOMAL DRUG DELIVERY [11]

- In comparison to other transdermal & dermal delivery systems-
- Enhanced permeation of drug through skin for transdermal drug delivery.
- Delivery of large molecules (peptides, protein molecules) is possible.
- It contains nontoxic raw material in formulation.
- High patient compliance- The ethosomal drug is administrated in semisolid form (gel or cream) hence producing high patient compliance.
- The Ethosomal system is passive, noninvasive and is available for immediate commercialization.
- Ethosomal drug delivery system can be applied widely in Pharmaceutical, Veterinary, Cosmetic fields.
- Simple method for drug delivery in comparison to Iontophoresis and Phonophoresis and other complicated methods.

DISADVANTAGES OF ETHOSOMAL DRUG DELIVERY SYSTEM [12-17]

- Drugs that require high blood levels cannot be administered – limited only to potent molecules, those requiring a daily dose of 10mg or less.
- Ethosomal administration is not a means to achieve rapid bolus type drug input, rather it is usually designed to offer slow, sustained drug delivery.
- Adequate solubility of the drug in both lipophilic and aqueous environments to reach dermal microcirculation and gain access to the systemic circulation.
- The molecular size of the drug should be reasonable that it should be absorbed percutaneously.
- Adhesive may not adhere well to all types of skin. Uncomfortable to wear.
- May not be economical.

DOI: 10.21276/IJIPSR.2018.06.06.311

- Poor yield.
- Skin irritation or dermatitis due to excipients and enhancers of drug delivery systems.
- In case if shell locking is ineffective then the ethosomes may coalescence and fall apart on transfer into water.
- Loss of product during transfer from organic to water media.
- The main advantage of ethosomes over liposomes is the increased permeation of the drug.

MECHANISM OF DRUG PENETRATION

It is thought that the first part of the mechanism is due to the 'ethanol effect' whereby intercalation of the ethanol into intercellular lipids increasing lipid fluidity and decreases the density of the lipid multilayer. This is followed by the 'ethosomal 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. Absorption of ethosomes is still not clear. The drug absorption probably occurs in following two phases: [18]

1. Ethanol effect
2. Ethosomes effect

ETHANOL EFFECT

Ethanol is major ingredient and acts as a penetration enhancer during the skin. The mechanism of its penetration enhancing effect is well known. Ethanol interacts with lipid molecules in the polar hard group region, resulting in a reducing the rigidity of the stratum corneum lipids, increasing their fluidity. The intercalation of ethanol into the polar head group environment can result in an increase in the membrane permeability. In addition to the effect of ethanol on stratum corneum structure, the ethosomes itself may interact with the stratum corneum barrier7.

ETHOSOMES EFFECT

Increased cell membrane lipid fluidity caused by the ethanol of ethosomes results increased skin permeability. In the case of ethosomes encapsulating drugs, the higher positive zeta potential imparted by the drug can improve skin attachment of the vesicles. While encapsulated drug in classic liposomes remained primarily at the surface of the skin the Ethosomal system was showed to be highly efficient carrier for enhanced drug delivery through the skin due to increased fluidity of the lipids.

DOI: 10.21276/IJIPSR.2018.06.06.311

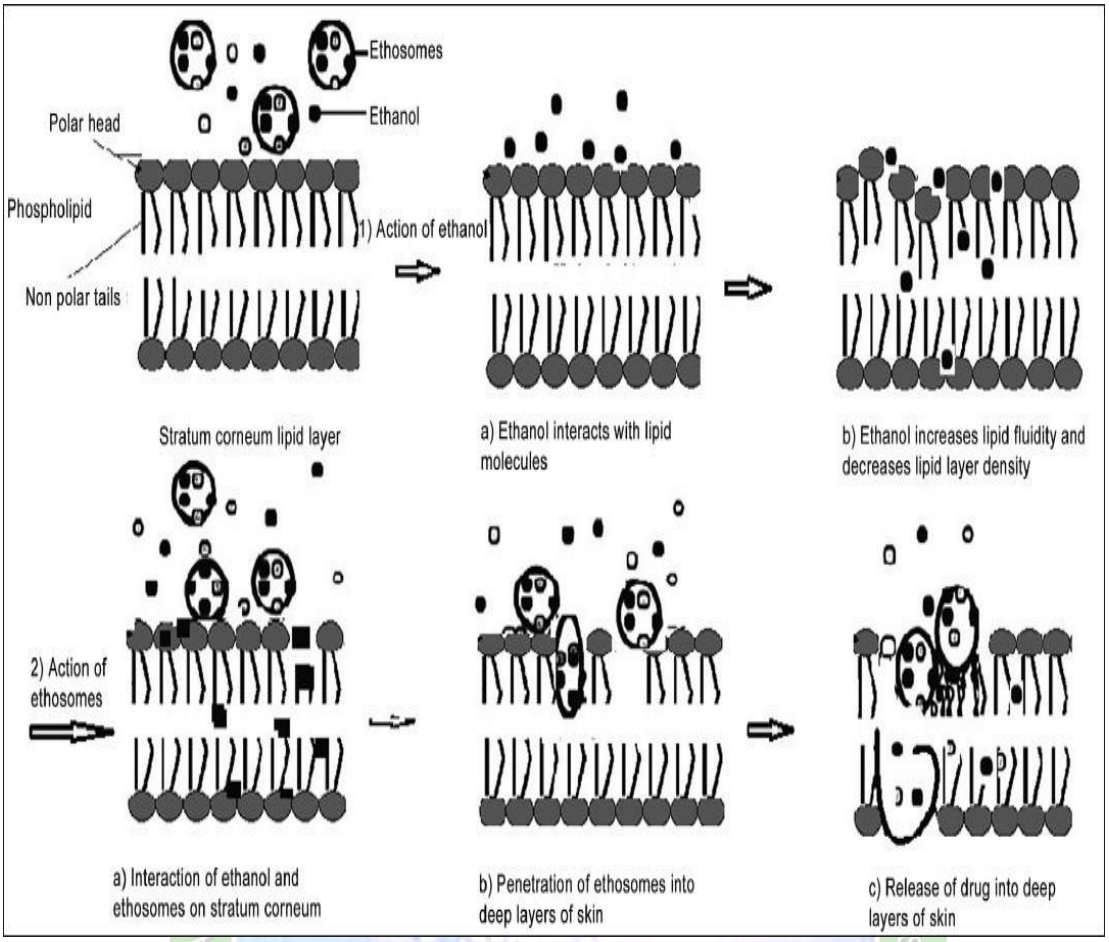

Fig. 3: Mechanism of Action of Ethosomes [10]

METHODS OF PREPARATION ETHOSOMES [19]

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 phospholipids, 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.

Hot method

In this method phospholipid is dispersed in water by heating in a water bath at 4000C 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

DOI: 10.21276/IJIPSR.2018.06.06.311

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.

VARIOUS METHODS OF CHARACTERIZATION OF ETHOSOMES [20-26]:

Vesicle shape

Ethosomes can be easily visualized by using transmission electron microscopy (TEM) and by scanning electron microscopy (SEM).

Vesicle size and zeta potential

Particle size of the ethosomes can be determined by dynamic light scattering (DLS) and photo n correlation spectroscopy (PCS). Zeta potential of the formulation can be measured by Zeta meter.

Transition temperature

The transition temperature of the vesicular lipid systems can be determined by using differenti al scanning calorimetry (DSC).

Drug entrapment

The entrapment efficiency of ethosomes can be measured by the ultracentrifugation technique.

Drug content

Drug content of the ethosomes can be determined using UV spectrophotometer. This can alsob e quantified by a modified high performance liquid chromatographic method.

Surface tension measurement

The surface tension activity of drug in aqueous solution can be measured by the ring method in a Du Nouy ring tensiometer.

Stability studies

The stability of vesicles can be determined by assessing the size and structure of the vesicles over time. Mean size is measured by DLS and structure changes are observed by TEM.

Skin permeation studies

The ability of the ethosomal preparation to penetrate into the skin layers can be determined by using confocal laser scanning

THERAPEUTIC APPLICATION OF ETHOSOMES

1. **In the treatment herpetic infection**

5% acyclovir ethosomal preparation compared to the 5 % acyclovir cream showed significant improvements in treatment of herpetic infections.

DOI: 10.21276/IJIPSR.2018.06.06.311

2. **Transcellular Delivery**

Ethosomes as compared to the marketed formulation suggested ethosomes to be an attractive clinical alternative for anti-HIV therapy [27].

3. **Ethosomes are used in pilosabeceous targeting**

Ethosomes, the high ethanol containing vesicles are able to penetrate the deeper layers of the skin and hence appear to be vesicles of choice for transdermal drug delivery of hydrophilic and impermeable drugs through the skin.

4. **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. The risk of failure of treatment is known to increase with each pill missed.

5. **Delivery of Anti-Arthritis Drug**

Topical delivery of anti-arthritis drug is a better option for its site-specific delivery and overcomes the problem associated with conventional oral therapy.

6. **Delivery of Anti-Viral Drugs**

Zidovudine is a potent antiviral agent acting on acquired immunodeficiency virus. Oral administration of zidovudine is associated with strong side effects. Therefore, an adequate zero order delivery of zidovudine is desired to maintain expected anti-AIDS effect. Jain et al [28] concluded that ethosomes could increase the transdermal flux, prolong the release and present an attractive route for sustained delivery of zidovudine. Acyclovir is another antiviral drug that widely used topically for treatment of Herpes labialis .The conventional marketed acyclovir external formulation is associated with poor skin penetration of hydrophilic acyclovir to dermal layer resulting in weak therapeutic efficiency. It is reported that the replication of virus takes place at the basal dermis. To overcome the problem associated with conventional topical preparation of acyclovir. Horwitz et al. formulated the acyclovir ethosomal formulation for dermal delivery. The results showed that shorter healing time and higher percentage of abortive lesions were observed when acyclovir was loaded into ethosomes.

7. **Delivery of anti-parkinsonism agent**

Dayan and Touitou 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. The results indicated better skin permeation potential of ethosomal-THP formulation and its use for better management of Parkinson disease.

DOI: 10.21276/IJIPSR.2018.06.06.311

CONCLUSION

As mentioned above, numerous studies have been published showing that ethosomes can substantially improve the permeation of drugs through the stratum corneum and thereby their efficacy. Ethosomes has initiated a new area in vesicular research for transdermal drug delivery. Ethosomes are soft, malleable vesicles and possible 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. The versatility of ethosomes for transdermal as well as topical drug delivery is evident from the research reports of enhanced delivery of quite a few drugs like minoxidil, testosterone, trihexyphenidyl hydrochloride, bacitracin, indinavir, salbutamol sulfate, azelaic acid and insulin. Delivery of Hepatitis B surface antigen (HBsAg) and DNA via ethosomes opens new opportunities to transcutaneous immunization (TCI) and gene therapy. Several excellent phytochemicals and herbal extracts have been successfully delivered via ethosomes and showed some distinct advantages over conventional drug delivery systems. As an alternative to conventional transdermal permeation enhancement techniques ethosomes are superior by offering safety, efficiacy, long term stability, simplified industrial manufacture as well as better patient compliance. Thus, it can be a logical conclusion that ethosomes can become a promising drug.

REFERENCES

1. Schreier H and Bouwstra JA. Liposomes and niosomes as topical drug carriers: dermal and transdermal drug delivery. J Control Rel. 1994;30:1– 15.
2. Anitha P, Ramkanth S, Uma Sankari K, Alagusundaram M, Gnanapraksah K and Devaki Devi P. Ethosomes - A noninvasive vesicular carrier for transdermal drug delivery. Int. J. Rev. Life. Sci 2011;1(1):17-24.
3. Akiladevi D and sachinandan basak. Ethosomes a noninvasive approach for transdermal drug delivery. IJCPR 2010; 2: 1-5.
4. Pavan K,Radhika PR and Sivakumar T. Ethosomes-A Priority in Transdermal Drug Delivery. International Journal of Advances in Pharmaceutical Sciences 2010;1: 2-3
5. Merdan VM, Alhaique F and Touitou E. Vesicular carriers for topical delivery. Acta Techno. Legis Medicament 1998; 12:1-6.
6. Upadhyay N, Mandal S, Bhatia L, Shailesh S and Chauhan P. A Review on Ethosomes: An Emerging Approach for Drug Delivery through the Skin. RRST-Pharmaceutics 2011; 3(7):1.

DOI: 10.21276/IJIPSR.2018.06.06.311

7. Manosroi A, Jantrawut P, Khositsuntiwong N, Manosroi W and Manosroi J. Novel Elastic Nanovesicles for Cosmeceutical and Pharmaceutical Applications. Chiang Mai. J. Sci. 2009; 36(2): 168-178.

8. Touitou E, Dayan N, Bergelson L, Godin B and Eliaz M. Ethosomesnovel vesicular carriers for enhanced delivery: characterization and skin penetration properties. J. Control. Release 2000; 65:403-418.

9. Verma DD and Fahr A. Synergistic penetration effect of ethanol and phospholipids on the topical delivery of Cyclosporine A. J. Control Release. 2004; 97: 55-66.

10. Verma P and Pathak k. Therapeutic and cosmoceutical potentials of ethosomes: An overview JAPTR 2010;(1):2-3

11. D Akiladevi, Sachinandan Basak, "Ethosomes Anoninvasive Approach For Transdermal Drug Delivery". Int J Curr Pharm Res, 2; 1-4.

12. Hitesh Jain, Jitendra Patel, Kruti Joshi, Parth Patel and Upadhyay U M. Ethosomes: A novel drug carrier, IJCP, 7(01), 2011, 1-4.

13. Upadhyay N, Mandal S, Bhatia L, Shailesh S, Chauhan P. A Review on "Ethosomes: An Emerging Approach for Drug Delivery through the Skin Rec, Res Sci Tech, 3(7), 2011, 19-24.

14. Sivakranth M, Anjuma Ara P, Krishnaveni C, Venkatesh E. Ethosomes: a novel vesicular drug delivery system, Int J Adv Pharm, 2(1), 2012, 16-27.

15. Kumar R, Aslam M D, Tripathi A, Prasad D, Chaudhary V, Jain V, Mishra S K and Singh R. Ethosomes: novel vesicular carriers in transdermal drug delivery, J Global Pharma Tech, 2(6), 2010, 1-7.

16. Rathore A R, Khambete H and Jain S. Preparation and Characterization of Repaglinide Loaded Ethosomal Gel for the Treatment of NIDDM, International Journal of Pharmaceutical and Biological Archives, 4(2), 2013, 385-390.

17. Shahwal V, Samnani A, Dubey B, Bhowmick M. Ethosomes: an overview, Int.J. Bio Adv Res, 2, 2011, 161-168.

18. Touitou E, Dayan N, Bergelson L, Godin B and Eliaz M. Ethosomesnovel vesicular carriers for enhanced delivery: characterization and skin penetration properties. J. Control. Release2000; 65:403-418.

19. Parashar Tarun, Soniya, Sachan Roopesh, Singh Vishal, Singh Gaurav, Tyagi Satyanand , Patel Chirag, Gupta Anil, "Ethosomes: A Recent Vesicle Of Transdermal Drug Delivery System". International Journal of Research and Development in Pharmacy and Life Sciences, 2013; 2: 285-292

DOI: 10.21276/IJIPSR.2018.06.06.311

20. Guo J, Ping Q, Sun G, Jiao C, Lecithin vesicular carriers for transdermal delivery of cy closporine A, Int. J. Pharm, 194(2), 2000, 201- 207.

21. Maghraby GMM, Williams AC, Barry BW, Oestradiol skin delivery from ultra deform able liposomes: refinement of surfactant concentration, Int. J. Pharm, 196(1), 2000, 63 - 74.

22. Fry DW, White JC, and Goldman ID, Rapid secretion of low molecular weight solutes from liposomes without dilution. Anal. Biochem, 90, 1978, 809- 815.

23. Cevc G, Schatzlein A, Blume G, Transdermal drug carriers: Basic properties, optimizat ion and transfer efficiency in case of epicutaneously applied peptides, J. Control. Relea se, 36, 1995,

24. Vanden Berge BAI, Swartzendruber VAB, Geest J, Development of an optimal protoc ol for the ultrastructural examination of skin by transmission electron microscopy, J. M icrosc, 187(2), 1997, 125- 133.

25. Dayan N, Touitou E, Carrier for skin delivery of trihexyphenidyl HCl: Ethosomes vs li posomes, Biomaterials, 21, 2002, 18791885.

26. Toll R, Jacobi U, Richter H, Lademann J, Schaefer H, Blume U,Penetration profile of microspheres in follicular targeting of terminal hair follicles, J. Invest.

27. Jain S. Vesicular approaches for transdermal delivery of bioactive agent. PhD thesis Dr. H.S. Gour University Sagar India 2005

28. Jain S, Umamaheshwari RB, Bhadra D, Jain NK, "Ethosomes: a novel vesicular carrier for enhanced transdermal delivery of an anti-HIV agent", Ind J Pharma Sci, 2004; 66: 72-81.