Print ISSN: 2958-3675 | Online ISSN: 2958-3683
International Journal of Medical and Pharmaceutical Research
Available Online at https://ijmpr.in/
ORCiD ID: 0000-0002-3849-6959
Volume 3, Issue 2; Jul-Dec 2022; Page No. 09-14
Review Article Open Access

Ethosomal Drug Delivery And Its Applications - A Review

Rajesh Kumar[1]; Harendra Prasad[2]; Navneet Kumar Verma[3*]
[1]Associate Professor, Shri Babu Singh College of Pharmacy Sanyara Meethepur Kaushambi, UP, India
[2]Assistant Professor, Rajiv Gandhi College of Pharmacy, Nautanwa, Maharajganj, UP, India
[3]Associate Professor, Buddha Institute of Pharmacy, GIDA, Gorakhpur, UP, India-273209

ABSTRACT
Ethosomes are brand-new lipid vesicular carriers with a reasonably high ethanol content that are used as a transdermal drug delivery system to test the effectiveness of a targeted medication at the site of action. There are several applications in the creation of formulations for enhanced systemic circulation release. The amount of medicine in the formulation and the amount released to the site of action can both be observed, and this allows us to determine how these formulations will behave therapeutically. The concentration of alcohols or their combination is relatively high in vesicular carriers called ethosomes, which are made of hydroalcoholic or hydro/alcoholic/glycolic phospholipid. Drug molecules with different physicochemical properties, such as hydrophilic, lipophilic, or amphiphilic, can be captured by ethosomes. For individuals who cannot take medications orally, these formulations are a superior choice.

Keywords: *Ethosomes, Vesicular carrier, Transdermal drug delivery system.*

***Corresponding Author**
Navneet Kumar Verma
Associate Professor, Buddha Institute of Pharmacy, GIDA, Gorakhpur, UP, India-273209

INTRODUCTION

The main disadvantage of Transdermal drug delivery system (TDDS) is that it encounters the barrier properties of the Stratum Corneum, which means that only the lipophilic drugs with a molecular weight of less than 500 Dacan pass through it. Transdermal drug delivery system (TDDS) has shown promising results when compared to oral drug delivery system because it eliminates gastrointestinal interferences and first pass metabolism of the drug [1, 2]. Several strategies have been examined to boost the penetration of medications through the skin, including the use of chemical or physical enhancers, such as iontophoresis, sonophoresis, etc. Drug permeability across the stratum corneum has also been found to be improved by liposomes, niosomes, transferosomes, and ethosomes. Drugs can easily pass through the skin thanks to permeation enhancers, which make the skin more permeable. Unlike classic liposomes, [3] that are known mainly to deliverdrugs to the outer layers of skin, ethosomes can enhance permeation through the stratum corneum barrier [4, 5].Ethosomes permeate through the skin layers more rapidlyand possess significantly higher transdermal flux in comparison to conventional liposomes [6-8].Ethosomes (Fig. 1) are lipid vesicles containing phospholipids, alcohol (ethanol and isopropyl alcohol) in relatively highconcentration and water. Ethosomes are soft vesicles made of phospholipids and ethanol (in higher quantity) and water [1,6]. The size range of ethosomes may vary from tens of nanometers to microns (μ) [9, 10].

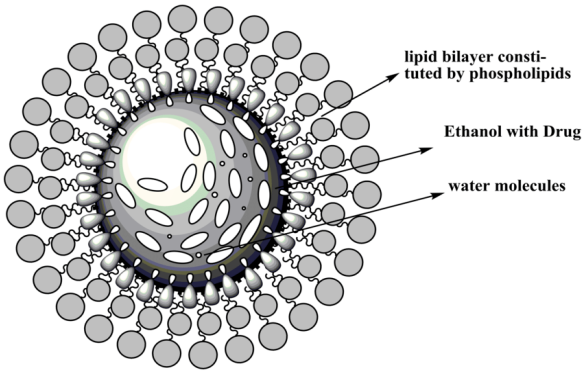

Figure1; Structure of Ethosome

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:

1. Ethanol effect
2. Ethosomes effect

1. 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.

2. Ethosome effect:

Increased cell membrane lipid fluidity caused bythe 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 [1].

METHODS OF PREPARATION ETHOSOMES

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

1. 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 withthe 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.[11, 12].

2. 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 [11, 12].

CHARACTERIZATIONS OF ETHOSOMES

1. Visualization

Visualization of ethosomes can be done using transmission electron microscopy (TEM) and by scanning electron microscopy (SEM) [11].

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) [13].

3. Differential Scanning Calorimetry (DSC)

Transition temperature (Tm) of the vesicular lipid systems was determined by using the Mettler DSC 60computerized 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 from20˚C–300˚C [14,15].

4. 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 [14,15].

5. Entrapment Efficiency

The entrapment efficiency of drug by ethosomes can be measured by the ultracentrifugation technique [15].

6. Penetration and Permeation Studies

Depth of penetration from ethosomes can be visualized by co focal laser scanning [11].

7. 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 [11,14,15].

EVALUATION TESTS

1. Filter Membrane-Vesicle Interaction Study by Scanning Electron Microscopy

Vesicle suspension (0.2 mL) was applied to filter membrane having a pore size of 50 nm and placed in diffusion cells. The upper side of the filter was exposed to the air, whereas the lower side was in contact with PBS(phosphate buffer saline solution), (pH 6.5). The filters were removed after 1 hour and prepared for SEM studies by fixation at 4°C in Karnovsky's fixative overnight followed by dehydration with graded ethanol solutions (30%, 50%, 70%, 90%, 95%, and 100%vol/vol in water). Finally, filters were coated with gold and examined in SEM (Leica, Bensheim, Germany) [2, 11,16].

2. Vesicle-Skin Interaction Study by Fluorescence Microscopy

Fluorescence microscopy was carried according to the protocol used for TEM and SEM study. Paraffin blocks are used, were made, 5-µm thick sections were cut using microtome (Erma optical works, Tokyo, Japan) and examined under a fluorescence micro Cytotoxicity AssayMT-2 cells (T-lymphoid cell lines) were propagated in Dulbecco's modified Eagle medium (HIMEDIA, Mumbai, India) containing 10% fetal calf serum, 100 U/M penicillin, 100 mg/mL streptomycin, and 2 mmol/Lglutamine at 37°C under a 5% CO2 atmosphere. Cytotoxicity was expressed as the cytotoxic dose 50(CD50) that induced a 50% reduction of absorbance at540 nm [1, 2, 11].

3. Vesicle-Skin Interaction Study by TEM and SEM

From animals ultrathin sections were cut (Ultracut, Vienna, Austria), collected on form var-coated grids and examined under transmission electron microscope. For SEM analysis, the sections of skin after dehydration were mounted on stubs using an adhesive tape and were coated with gold palladium alloy using a fine coat ion sputter coater. The sections were examined under scanning electron microscope [11, 16].

4. HPLC Assay

The amount of drug permeated in the receptor compartment during in vitro skin permeation experiments and in MT-2 cell was determined by HPLC assay using methanol: distilled-water: acetonitrile (70:20:10 vol/vol)mixture as mobile phase delivered at 1 mL/min by LC10-AT vp pump (Shimadzu, Kyoto, Japan). A twenty micro liter injection was eluted in C-18 column (4.6×150mm, Luna, 54, Shimadzu) at room temperature. The column eluent was monitored at 271 nm using SPDM10Avp diode array UV detector. The coefficient of variance(CV) for standard curve ranged from 1.0% to 2.3%, and the squared correlation coefficient was 0.9968 [2, 11,16].

5. Drug Uptake Studies

The uptake of drug into MT-2 cells (1×106 cells/mL) was performed in 24-well plates (Corning Inc) in which 100 µLRPMI medium was added. Cells were incubated with 100µL of the drug solution in PBS (pH 7.4), ethosomal formulation, or marketed formulation, and then drug uptake was determined by analyzing the drug content by HPLC assay [1, 2, 11, 16].

6. Skin Permeation Studies

The hair of test animals (rats) were carefully trimmed short (<2 mm) with a pair of scissors, and the abdominal skin was separated from the underlying connective tissue with a scalpel. The excised skin was placed on aluminiumfoil, and the dermal side of the skin was gently teased off for any adhering fat and/or subcutaneous tissue. The effective permeation area of the diffusion cell and receptor cell volume was 1.0 cm2 and 10 mL, respectively. The temperature was maintained at 32°C ±1°C. The receptor compartment contained PBS (10 mL of pH 6.5). Excised skin was mounted between the donor and the receptor compartment. Ethosomal formulation(1.0 mL) was applied to the epidermal surface of skin. Samples (0.5 mL) were withdrawn through the sampling port of the diffusion cell at 1-, 2-, 4-, 8-, 12-, 16-, 20-,and 24-hour time intervals and analyzed by high performance liquid chromatography (HPLC) assay [2,11].

7. 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 after180 days using the method described earlier [2].

PATENTED AND MARKETED FORMULATION OFETHOSOME

Ethosome was invented and patented by Prof. Elka Touitou along with her students of department of Pharmaceutics at the Hebrew University School of Pharmacy. Novel Therapeutic Technologies Inc (NTT) of Hebrew University has been succeeded in bringing a number of products to the market based on ethosome delivery system. Noicellex TM ananti – cellulite formulation of ethosome is currently marketed in Japan. Lipoduction TM another formulation is currently used in treatment of cellulite containing pure grape seed extracts (antioxidant) is marketed in USA. Similarly Physonicsis

marketing anti – cellulite gel Skin Genuity in London. Nanominox© containing monoxidil is used as hair tonic to promote hair growth is marketed by Sinere [17, 18].

APPLICATIONS OF ETHOSOMES

1. Delivery of Anti-Viral Drugs

Zidovudine is a potent antiviral agent acting on acquired immunodeficiency virus. Oral administration of zidovudineis associated with strong side effects. Therefore, an adequate zero order delivery of zidovudine is desired tomaintain expected anti-AIDS effect [20]. Jain *et al.* [7] concluded that ethosomes could increase the transdermalflux, prolong the release and present an attractive route for sustained delivery of zidovudine. Acyclovir is another anti-viral drug that widely used topically for treatment of Herpes labialis [21].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[22]. 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.

2. Topical Delivery of DNA

Many environmental pathogens attempt to enter the body through the skin. Skin therefore, has evolved into an excellent protective barrier, which is also immunologically active and able to express the gene [23]. On the basis of above facts another important application of ethosomes is to use them for topical delivery of DNA molecules to express genes in skin cells. Touitou et al. in their study encapsulated the GFP-CMV-driven transfecting construct into ethosomal formulation. They applied this formulation to the dorsal skin of 5-week male CD-1 nude mice for 48hr. After 48 hr, treated skin was removed and penetration of green fluorescent protein (GFP)formulation was observed by CLSM. It was observed that topically applied ethosomes-GFP-CMV-driventransfecting construct enabled efficient delivery and expression of genes in skin cells. It was suggested that ethosomes could be used as carriers for gene therapy applications that require transient expression of genes. These results also showed the possibility of using ethosomes for effective transdermal immunization. Gupta *et al*. recently reported immunization potential using transfersomal formulation. Hence, better skin permeationability of ethosomes opens the possibility of using these dosage forms for delivery of immunizing agents [2].

3. 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 [24]. Touitou *et al.* 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.

4. 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 [2].

5. Trans cellular Delivery

Touitou *et al.* in their study demonstrated better intracellular uptake of bacitracin, DNA and erythromycin using CLSM and FACS techniques in different cell lines. Better cellular uptake of anti-HIV drug zidovudine and lamivudine in MT-2 cell line from ethosomes as compared to the marketed formulation suggested ethosomes to bean attractive clinical alternative for anti-HIV therapy [6,8].

6. 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. Cannabidol(CBD) is a recently developed drug candidate for treating rheumatoid arthritis. Lodzki *et al.* prepared CBD ethosomal formulation for transdermal delivery. Results shows significantly increased in biological anti-inflammatory activity of CBD-ethosomal formulation was observed when tested by carrageen an induced rat pawedema model. It was concluded encapsulation of CBD in ethosomes significantly increased its skin permeation, accumulation and hence it's biological activity [2].

7. Delivery of Problematic drug molecules

Large biogenic molecules, such peptides or proteins, are difficult to give orally since the GI system totally degrades them. It is preferable to distribute proteins non-invasively in order to avoid the issues associated with oral delivery [25].

The impact of ethosomal insulin administration on decreasing blood glucose levels (BGL) in vivo in both normal and diabetic SDI rats was studied by Dkeidek and Touitou. In this investigation, an overnight fated rat's abdomen region was patched with a Hill Top patch containing insulin ethosomes. The findings demonstrated that both normal and diabetic rats' BGL significantly decreased (up to 60%) when insulin was given from this patch. On the other hand, administration of insulin from a control formulation failed to lower the BGL. For the treatment of inflammatory skin diseases including psoriasis, atopic dermatitis, and diseases of the hair follicle like alopecia areata, among others, Verma DD, Fahr A[26] described the use of cyclosporine A, ethosomal formulation. The possible use of ethosomes for the cutaneous administration of ammonium glycyrrhizin ate was examined by Paolino et al. [27]. Obtainable from Glycyrrhizinate Glabra, ammonium glycyrrhizinate is a naturally occurring triterpene that is useful for treating a variety of inflammatory skin conditions [28].

8. Delivery of Antibiotics

The therapeutic efficiency of antibiotics can be improved by administering them topically. Traditional oral medication has a number of adverse effects and allergic responses. Traditional external preparations have a low penetration to sub dermal tissues and deep skin layers [23]. By releasing appropriate amounts of antibiotic into the deeper layers of skin, ethosomes can get around this issue. Ethosomes quickly penetrate the epidermis, carry a sizable amount of medication into the deeper layer of skin, and inhibit infections from the inside out. Godin and Touitou created an ethosomal formulation filled with bacitracin and erythromycin for cutaneous and intracellular delivery with this goal in mind. The findings of this study demonstrated the potential for ethosomal antibiotic formulation to be highly effective and to circumvent the drawbacks of traditional therapy.

CONCLUSION

Ethosomes can significantly reduce the epidermal barrier, which is the principal obstacle to transdermal medication delivery systems. When compared to cutaneous and transdermal administration, the ethosomes have additional advantages. Drugs are delivered to the deep skin layers by ethosomes, a non-invasive drug delivery method, before reaching the systemic circulation. It transports big molecules like protein and peptide molecules. Compared to Iontophoresis, Phonophoresis, and other complex procedures, this drug delivery approach is simple. High patient compliance since it is applied in different pharmaceutical, veterinary, and cosmetic fields and is administered in semisolid form (gel or cream).

REFERENCES

1. Satyam, G., Shivani, S., & Garima, G. (2010). Ethosomes: A novel tool for drug delivery through the skin. J Pharm Res, 3(4), 688-91.
2. Kumar, K. P., Radhika, P. R., & Sivakumar, T. (2010). Ethosomes-a priority in transdermal drug delivery. International Journal of Advances in Pharmaceutical Sciences, 1(2).
3. Heeremans, J. L. M., Gerrttsen, H. R., Meusen, S. P., Mijnheer, F. W., Gangaram Panday, R. S., Prevost, R., ... & Crommelin, D. J. A. (1995). The preparation of tissue-type plasminogen activator (t-PA) containing liposomes: entrapment efficiency and ultracentrifugation damage. Journal of Drug targeting, 3(4), 301-310.
4. Asbill, C. S., El-Kattan, A. F., & Michniak, B. (2000). Enhancement of transdermal drug delivery: chemical and physical approaches. Critical Reviews™ in Therapeutic Drug Carrier Systems, 17(6).
5. Touitou, E., Dayan, N., Levi-Schaffer, F., & Piliponsky, A. (1998). Novel lipid vesicular system for enhanced delivery. J Lip Res, 8, 113.
6. Verma, P., & Pathak, K. (2010). Therapeutic and cosmeceutical potential of ethosomes: An overview. Journal of advanced pharmaceutical technology & research, 1(3), 274.
7. Jain, S., Umamaheshwari, R. B., Bhadra, D., & Jain, N. K. (2004). Ethosomes: a novel vesicular carrier for enhanced transdermal delivery of an antiHIV agent. Indian journal of pharmaceutical sciences, 66(1), 72.
8. Touitou, E., Godin, B., Dayan, N., Weiss, C., Piliponsky, A., & Levi-Schaffer, F. (2001). Intracellular delivery mediated by an ethosomal carrier. Biomaterials, 22(22), 3053-3059.
9. Bhalaria, M. K., Naik, S., & Misra, A. N. (2009). Ethosomes: A novel delivery system for antifungal drugs in the treatment of topical fungal diseases.
10. Verma, D. D., & Fahr, A. (2004). Synergistic penetration enhancement effect of ethanol and phospholipids on the topical delivery of cyclosporin A. Journal of controlled release, 97(1), 55-66.
11. Nikalje AP, Tiwari S(2012). "Ethosomes: A Novel Tool forTransdermal Drug Delivery", IJRPS, 2 (1), 1-20.
12. Dhamecha, D. L., Rathi, A. A., Saifee, M., Lahoti, S. R., & Dehghan, M. H. G. (2009). Drug vehicle based approaches of penetration enhancement. Int J Pharm Pharm Sci, 1(1), 24-46.
13. El Maghraby, G. M., Williams, A. C., & Barry, B. W. (2000). Oestradiol skin delivery from ultradeformable liposomes: refinement of surfactant concentration. International journal of pharmaceutics, 196(1), 63-74.
14. Cevc, G., Schätzlein, A., & Blume, G. (1995). Transdermal drug carriers: basic properties, optimization and transfer efficiency in the case of epicutaneously applied peptides. Journal of Controlled Release, 36(1-2), 3-16.

15. Fry, D. W., White, J. C., & Goldman, I. D. (1978). Rapid separation of low molecular weight solutes from liposomes without dilution. Analytical biochemistry, 90(2), 809-815.
16. Lopez-Pinto, J. M., Gonzalez-Rodriguez, M. L., & Rabasco, A. M. (2005). Effect of cholesterol and ethanol on dermal delivery from DPPC liposomes. International journal of pharmaceutics, 298(1), 1-12.
17. Touitou, E. (1996). U.S. Patent No. 5,540,934. Washington, DC: U.S. Patent and Trademark Office.
18. Touitou, E. (1998). U.S. Patent No. 5,716,638. Washington, DC: U.S. Patent and Trademark Office.
19. Anitha, P., Ramkanth, S., Sankari, K. U., Alagusundaram, M., Gnanapraksah, K., Devi, P. D., & Prasanna, R. I. (2011). Ethosomes-A noninvasive vesicular carrier for transdermal drug delivery. International journal of review in life sciences, 201(1), 1.
20. Kim, S., & Chien, Y. W. (1996). Toxicity of cationic lipids and cationic polymers in gene delivery. J. Control. Release, 40, 67-76.
21. Spruance, S. L. (1992, September). The natural history of recurrent oral-facial herpes simplex virus infection. In Seminars in dermatology (Vol. 11, No. 3, pp. 200-206).
22. Fiddan, A. P., Yeo, J. M., Strubbings, R., & Dean, D. (1983). Vesicular Approach for Drug Delivery into or Across the Skin. Br. Med. J, 286, 1699.
23. Fang, J. Y., Hong, C. T., Chiu, W. T., & Wang, Y. Y. (2001). Effect of liposomes and niosomes on skin permeation of enoxacin. International journal of pharmaceutics, 219(1-2), 61-72.
24. Johnsen, S., Bennett, E., & Jensen, V. G. (1974). Therapeutic effectiveness of oral testosterone. The Lancet, 304(7895), 1473-1475.
25. Chetty, D. J., & Chien, Y. W. (1998). Transdermal Delivery of CaCO3-Nanoparticles Containing Insulin. Crit Rev Ther Drug Carrier Syst, 15, 629-670.
26. Verma, D. D., & Fahr, A. (2004). Synergistic penetration enhancement effect of ethanol and phospholipids on the topical delivery of cyclosporin A. Journal of controlled release, 97(1), 55-66.
27. Paolino, D., Lucania, G., Mardente, D., Alhaique, F., & Fresta, M. (2005). Ethosomes for skin delivery of ammonium glycyrrhizinate: in vitro percutaneous permeation through human skin and in vivo anti-inflammatory activity on human volunteers. Journal of Controlled Release, 106(1-2), 99-110.
28. Fu, Y., Hsieh, T. C., Guo, J., Kunicki, J., Lee, M. Y., Darzynkiewicz, Z., & Wu, J. M. (2004). Licochalcone-A, a novel flavonoid isolated from licorice root (Glycyrrhiza glabra), causes G2 and late-G1 arrests in androgen-independent PC-3 prostate cancer cells. Biochemical and biophysical research communications, 322(1), 263-270.