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ETHOSOMES: A NOVEL TOOL FOR TRANSDERMAL DRUG DELIVERY

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ABSTRACT

Since skin offers an excellent barrier to molecular transport, as stratum corneum is the most formidable barrier to the passage of most of the drugs, except for highly lipophilic, low molecular weight drugs. Various attempts have been made for enhanced drug delivery into the body through the intact skin including using lipid vesicles like liposome, niosomes. Classic liposomes are of little or no value as carriers for transdermal drug delivery because they do not deeply penetrate the skin. One of the major advances in vesicle research was the finding that some specially designed vesicles possessed properties that allowed them to successfully deliver drugs in deeper layer of skin. Ethosomes are one of the specially designed lipid carriers recently developed by Touitou *et al*, showing enhanced skin delivery. Ethosomes are characterized by prolong physical stability with respect to liposomes.

Key Words: Stratum corneum (SC), Liposome, Niosomes, Classic Liposomes, Ethosomes.

INTRODUCTION

Transdermal delivery of drugs through the skin to the systemic circulation offers many advantages as compared to traditional drug delivery systems such as increased patient acceptability, avoidance of gastrointestinal disturbances and first pass metabolism of the drug

[1, 2]. Although the skin as a route for drug delivery can offers many advantages, the barrier nature of the skin makes it difficult for most drugs to penetrate into and permeate through it [3]. During the past decades there has been wide interest in exploring new techniques to overcome the stratum corneum barrier [4, 5]. Various physical and chemical methods has been investigated to overcome stratum corneum barriers such as iontophoresis, sonophoresis, radio frequency, electroporation, lipid vesicles like liposomes, niosomes, transferosomes etc [6,7,8].

Drug encapsulated in lipid vesicles prepared from phospholipids and nonionic surfactant serves as nontoxic penetration enhancer for drugs due to its amphiphilic nature. Lipid rich vesicles like liposome and niosomes can be used for encapsulation hydrophilic and lipophilic as well as low and high molecular weight drugs [9,10]. However these lipid rich vesicles has a problem of poor skin permeability. Introduction of ethosomes, another novel lipid carrier developed by Touitou *et al* has shown enhanced skin permeation as compared to conventional liposomes [2, 11].

Ethosomes

Ethosomes were developed by Touitou *et al* as noninvasive vesicular carriers very similar to liposomes produced in the presence of ethanol comprise of hydroalcoholic or hydro/alcoholic/glycolic phospholipids with high concentration of alcohol may be upto 50 % [2, 6, 12, 13, 14].

The ethosomal system consists of phospholipids, ethanol and water [15]. The phospholipids with various chemical structure includes phosphatidylcoholine (PC), hydrogenated PC, phosphatidylethanolamine (PE), phosphatidylglycerol (PPG), phosphatidylinositol (PI), hydrogenated PC etc (16). The nonaqueous phase ranges between 22 % to 70 %. 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 [3, 17,18].

Effect of high alcohol concentration

Ethanol is an established permeation enhancer and is proposed that it fluidizes the ethosomal lipids and stratum corneum bilayer thus allowing the soft, malleable vesicles to penetrate the

disorganized lipid bilayer [8]. 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 [3,19]. Moreover the vesicular nature of ethosomal formulation could be modified by varying the components ratio and phospholipids [20]. Ethanol confers a surface negative net charge to the liposome which causes the size of vesicles to decrease. The size of ethosomal vesicles increase with decreasing ethanol concentration[3].

Advantages of ethosomes

1. Enhanced permeation of drug molecules to and through the skin to the systemic circulation [3, 14].

2. Contrary to deformation liposomes, ethosomes improve skin delivery of drugs both under occlusive and non-occlusive conditions [21].

3. Ethosomes composition is safe and the components are approved for pharmaceuticals and cosmetic use.

4. Better patient compliance.

5. Better stability and solubility of many drugs as compared to conventional vesicles.

6. Various applications in pharmaceutical, veterinary and cosmetic field.

7. Relatively smaller size as compared to conventional vesicles.

Preparation of Ethosomes [16]

Methods of preparation of ethosomes

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 with the use of mixer. Propylene glycol is added during stirring. This mixture is heated to 30^0C in a water bath. The water heated to 30^0C 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 [22].

2. Hot method: In this method phospholipid is 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 are mixed and heated to 40^0C. Once both mixtures reach 40^0C, 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 [17, 18].

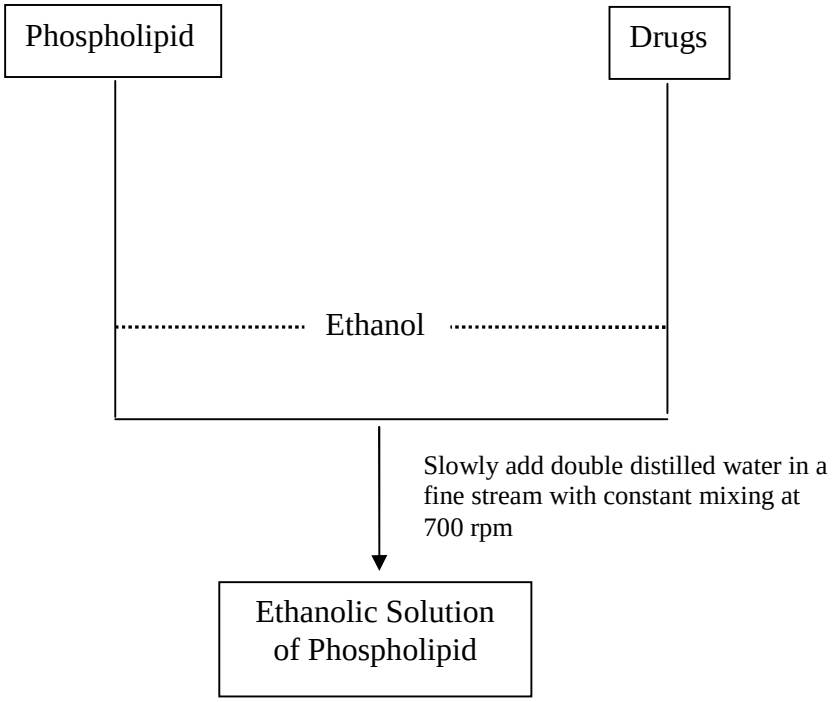

Fig 1: Method of preparation of ethosomes

Various methods of characterization of ethosomes

1. Vesicle Shape (Morphology): Morphology of ethosomes can be done using transmission electron microscope (TEM), Scanning electron microscope (SEM). TEM can be preformed using phosphotungstic acid as negative stain [23].

2. Vesicle size and size distribution: Ethosome size and size distribution can be done by dynamic light scattering method (DLS) using computerized inspection system [24, 25].

3. Entrapment efficiency: The ability of ethosomes to efficiently entrap lipophilic and hydrophilic drugs can be measured by ultracentrifugation technique, mini column centrifugation method and fluorescence spectrophotometry [20, 26, 27].

4. Transition temperature: The transition temperature of the vesicular lipid systems can be determined by using differential scanning calorimetry (DSC) [28].

5. Surface tension activity measurement: Surface tension activity of ethosomes can be measured in aqueous solution by Du Nouy ring tensiometer [29].

6. Turbidity: It can be measured by nephloturbidometer [14].

7. Vesicle skin interaction study: Vesicle skin interaction study can be done by examined under transmission electron microscopy or confocal laser scanning microscope (CSLM) or fluorescence microscope or eosin – hematoxylin staining. For fluorescence microscopy ethosomes should be loaded with fluorescence marker like Rhodamine123 [20, 24].

7. Degree of deformability or Elasticity Measurement: The elasticity of ethosomal vesicle membrane can be determined by extrusion method. The ethosomal formulation are extruded through filter membrane (pore diameter 50 nm) using stainless steel filter holder of diameter 25 nm, by applying a pressure of 2.5 bar [29].

8. Zeta potential: zeta potential can measure by zetometer or dynamic light scattering method (DLS) [20].

9. Phospholipid – ethanol interaction: Phospholipid – ethanol interaction can be assessed by ^{31}P NMR or by differential scanning calorimeter (DSC) [20].

10. Drug content: Drug content of ethosomal formulation can be quantified by a modified high performance liquid chromatographic technique (HPLC) [24].

11. Stability Study: The stability of the vesicle can be determined by assessing the size and structure of the vesicle over time by dynamic light scattering method or transmission electron microscope [30].

12. Penetration and permeation studies: Depth of skin penetration from ethosomes can be determined by confocal laser scanning microscope (CLSM) [31].

13. *In vitro* skin permeation/ deposition study: The in vitro permeation characteristic of ethosomal formulation can be done by using Franz diffusion cell with artificial or dialysis bag

diffusion or biological membranes such as heat separated human epidermis, human epidermis, male nude mouse abdominal skin, rat abdominal skin, rabbit pinna skin, dermatomed cadaver human skin, rat skin [14,26,32,33,34].

Mode of Action of Ethosomes

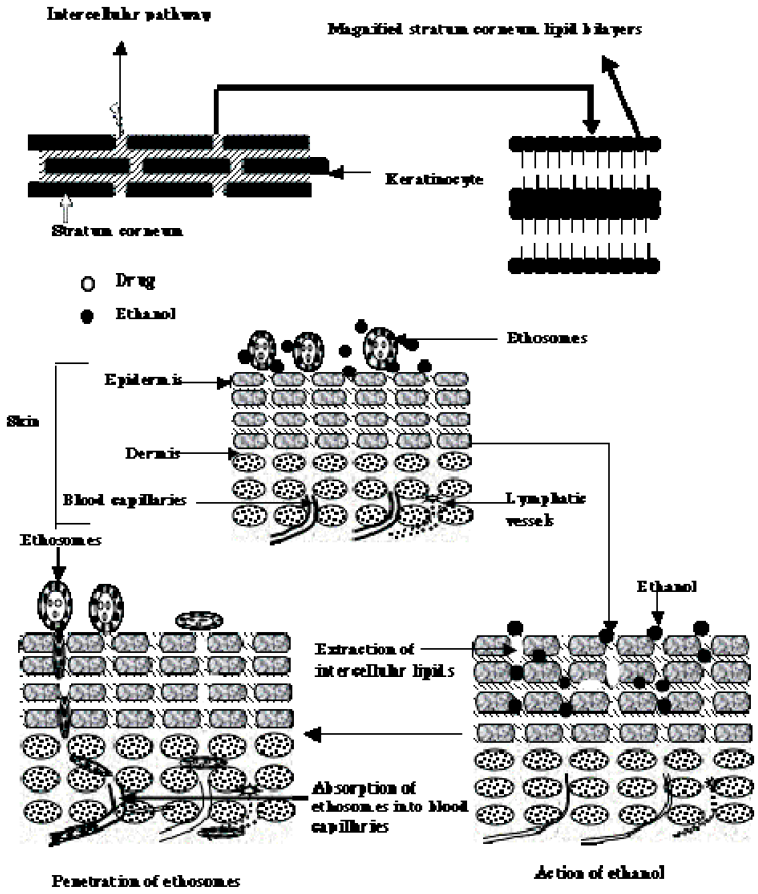

Fig. 2 Mode of action of ethosomes

The lipid multilayer, at physiological temperature is densely packed and highly conformational ordered and hence main barrier for permeation of drugs [35]. The enhanced delivery of drugs using ethosomes can be due to interaction between ethosomes and skin lipids. The possible mechanism of interaction is due to “ethanol effect” and “ethosomal effect” [36]. Ethanol increases the fluidity and decreases the density of lipid molecules by interacting with lipid molecules in the polar head group region which in turn result in increased permeability. Ethosomes effect include penetration of flexible ethosomal vesicles through disturbed stratum bilayers and 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 [3,16,20,21,37].

Application

Table.1 Applications of ethosomes

Drugs	Result
Anti- viral agents (Zidovudine) (38) (Lamivudine)(24) (Stavudine) (39)	Prolonged drug action, reduced drug toxicity. Control release for prolonged period of time. Improved biological anti-inflammatory activity, sustained effect.
NSAIDS (17,18) (Diclofenac)	Selective and prolong delivery of drug to desired side.
Acyclovir (4)	Increased skin permeation and biological activity two to three times.
Topical (40) Photodynamic Therapy (PDT) (5- aminolevulinic acid)	Greater penetration ability than that of liposomes, More entrapment efficiency
Insulin (14,41)	Significant decrease in blood glucose level.
Trihexyphenidyl Hydrochloride(14)	Higher entrapment capacity, improved tansdermal flux, improved patient compliance.
Antibiotic (42) (Erythromycin) (Cannabidol)	Complete inhibition of infection, prolonged drug action. Improved skin deposition and biological activity.

Pilosebaceous (2) Targeting (Minoxidil)	High penetration into deep layers of the skin.
Ammonium (26) glycrrhizinate	Improved biological anti-inflammatory activity, sustained effect.
Salbutamol sulfate (43)	Controlled release rate, enhanced skin permeation.
Propranolol (32)	Better skin permeation.
Testosterone (44)	Significantly higher permeation into the skin increased systemically delivery.
Bacitracin (45)	Reduced drug toxicity.
Methotrexate (46) (MTX)	Enhanced transdermal flux, lower lag time, higher entrapment efficiency and better stability profile

1. Pilosebaceous Targeting: Pilosebaceous units have been use for localized therapy, particularly for the treatment of follicle related disorders such as acne or alopecia. Ethosomal formulation of minoxidil a lipid soluble drug used for baldness accumulate into nude mice skin two to seven fold higher and thus can be use for pilosebaceous targeting for better clinical efficacy [2,20].

2. Transdermal Delivery: Since ethosomes enhance permeability of drug through stratum corneum barrier, it can be use for administration of drugs having poor skin permeation, low oral bioavailability, first pass metabolism and dose dependent side effect. Touitou *et al* reported that the skin permeation of testosterone from ethosomal formulation is nearly 30 times higher than the marketed transdermal patch of testosterone (Testosterone Patch, Alza). They also concluded that the ethosomal testosterone formulation area of application required

to produce the effective plasma concentration was 10 times less than required by commercially gel formulation [15, 20].

3. Topical Delivery of DNA: Another important application of ethosomes is their use for topical delivery of DNA molecules. Touitou *et al* demonstrated that better intracellular uptake of DNA, better delivery and expression of genes in skin cells can be achieved by ethosomal formulation [47]. Hence was concluded that ethosomes can be used carrier for gene therapy application that require transient expression of genes.

4. Delivery of Antiarthritis Drug: Arthritis treatment is associated with problems like low bioavailability; first pass metabolism, GIT degradation etc. To overcome above problems ethosomal formulation of antiarthritis drugs can be an alternative as it significantly increase skin permeation, accumulation and biological activity [20].

5. Delivery of Antibiotics: Conventional oral therapy of antibiotics is usually associated with several allergic reactions along with side effects and low therapeutic efficacy. Topical delivery of antibiotics is a better choice to increase therapeutic efficacy, but conventional topical preparation possess low permeability to deep skin layers and subdermal tissues. Ethosomes formulation of antibiotics could be highly efficient and over come the problems associated with conventional therapy since they penetrate rapidly into deeper layer of skin and suppress infection at their root [20, 42, 48].

6. Delivery of HIV Drugs: An effective antiretroviral therapy is required on a long term basis and is associated with strong side effects (35). Adequate zero order delivery of zidovudine, Lamivudine a potent antiviral agent is required to maintain expected anti – AIDS effect. Subheet Jain et al reported that ethosomal formulation of the above drugs prolong the release with increased Transdermal flux [25, 26]. Conventional topical preparation acyclovir an topically used antiviral drug for treatment of herpes labials show low therapeutic efficiency due to poor permeation through skin as replication of virus take places at the basal dermis. Ethosomal formulation of acyclovir show high therapeutic efficiency with shorter healing time and higher percentage of abortive lesions.

7. Delivery of Problematic Drug Molecules: Oral delivery of large biogenic molecules such as peptides or proteins and insulin is difficult because they are completely degraded in the GIT tract hence transdermal delivery is a better alternative. But conventional transdermal formulation of biogenic molecules such as peptides or protein and insulin has poor

permeation. Formulating these above molecules into ethosomes significantly increase permeation and therapeutic efficacy [49].

Future Perspective: For transdermal delivery of drugs, stratum corneum is the main barrier layer for penetration of drug. Various methods have been discovered to enhanced skin penetration of drugs lipid vehicle based enhancement approach has drawn considerable interest in recent past. Studies will continue further to improve skin delivery of drug using lipid vesicles.

Introduction of ethosomes has initiated a new area in vesicular research. Ethosomes has shown promising result and potential for delivery of various agents more effectively. Better control over drug release, non – invasive delivery of small, medium and large size drug molecules can be achieved by ethosomes. Ethosomes can be the promising tool for dermal/transdermal delivery of various agents and can be a alternate formulation for problematic drugs.

REFERENCE

1. Benson HAE. Transdermal drug delivery, penetration enhancement techniques. Current Drug Delivery, 2005; 2: 23 – 33.
2. Toutitou E, Dayan N, Bergelson L, Godin B, Eliaz, M. Ethosomes novel vesicular carriers for enhanced delivery, characterization and skin penetration properties. Journal of Control Release, 2000; 65: 403 – 418.
3. Mustafa MAE, Ossama YA, Viviane FN, Nawal MK. Lipid vesicles for skin delivery of drugs. Reviewing three decades of research. International Journal of Pharmaceutics, 2007; 332: 1 – 16.
4. Barry, BW. Novel mechanism and devices to enable successful Transdermal drug delivery. European Journal of Pharmaceutical Sciences, 2001; 14 (2): 101 – 114.
5. Honeywell–Nguyen PL, Bouwatra JA. Vesicles as a tool for transdermal and dermal delivery. Drug Discovery Today: Technologies, 2005; 2: 67 – 74.
6. Touitou E. Drug delivery across skin. Expert Opinion on Biological Therapy, 2002; 2 (7): 723 – 733.
7. Ranade V. Drug delivery system: Transdermal drug delivery. The Journal of Clinical Pharmacology, 1991; 31(6): 401 – 418.
8. Dinesh D, Amit AR, Maria S, Awaroop RL, Moh. Hassan GD. International Journal of Pharmacy and Pharmaceutical Sciences, 2009; 1 (1): 24 – 45.

9. Schreier H, Bovwstra J. Liposomes and niosomes as topical drug carriers: dermal and transdermal drug delivery. Journal of Control Release, 1994, 30(1): 1 – 15.
10. Touitou E, Junginger HE, Weiner ND, Nagai T, Mezei M. Journal of. Pharmaceutical Sciences, 1994; 83: 1189 -1203.
11. Touitou E, Godin B, Weiss C. Enhanced delivery of drugs into and across the skin by ethosomal carriers. Drug Development Research, 2000; 50: 406 – 415.
12. Touitou E, Godin G. Skin nonpenetrating sunscreens for cosmetic and pharmaceutical formulations. Clinics in Dermatology, 2008; 26: 375 -379.
13. Touitou E, Godin, B, Dyan, N, Weiss, C, Piliponsky A, Levi – Scha, F. Intracellular delivery mediated by an ethosomal carrier. Biomaterials, 2001; 22: 3053 – 3059.
14. Dayan N, Touitou E. Carriers for skin delivery of triexphenidyl HCl: Ethosome Vs Lipsomes. Biomaterials, 2000; 21: 1879 – 1885.
15. Touitou E. Drug delivery across skin. Expert Opinion on Biological Therapy, 2002; 2: 723 – 733.
16. Vyas, SP. Theory and practice in novel drug delivery system, (1st edition), CBS Publication and Distributors. New Delhi, 2009; 259 – 261.
17. Touitou E. Composition of applying active substance to or through the skin. US Patent: 1996; 5716638.
18. Touitou E. Composition of applying active substance to or through the skin. US Patent: 1998; 5540934.
19. Barry BW. Is Transdermal drug delivery research still important today? Drug Discovery Today, 2001; 6(19):967 – 971.
20. Biju SS, Sushama T, Mishra PR, Khar RK. Vesicular systems: An overview. Indian Journal of Pharmaceutical Sciences, 2006; 68(2): 141-153.
21. Elsayed MM, Abdallah OY, Naggar VF, Khalafallah NM. Deformable liposomes and ethosomes as carriers for skin delivery of ketotifen. Pharmazie, 2007; 62: 133 – 137.
22. Verma DD, Fahr A. Synergistic penetration effect of ethanol and phospholipids on the topical delivery of cyclosporine A. Journal of Control Release, 2004; 97(1): 55 – 66.
23. Guo J, Ping Q, Sun G, Jiao C. Lecithin vesicular carriers for Transdermal delivery of cyclosporine A. International Journal of Pharmaceutics, 2000 ; 194(2): 201 – 207.
24. Subjeet J, Ashok, KT, Bharti, S, Narendra KJ. Formulation and evaluation of ethosomes for transdermal delivery of lamivudine. APPS PharmSciTech, 2007; 8 (4): E1 – E9.

25. Maghraby GMM, Williams AC, Barry BW. Oestradiol skin delivery from ultradeformable liposomes: refinement of surfactant concentration. International Journal of Pharmaceutics, 2000; 196(1): 63 – 74.
26. Donatella P, Giuseppe L, Domenico M, Franco A, Massimo F. Ethosomes for skin delivery of ammonium glycyrrhizinate permeation through human skin and in vivo anti inflammatory activity on human volunteers. Journal of Controlled Release, 2005; 106: 99 – 110.
27. Fry DW, White JC, Goldman ID. Rapid seperation of low molecular weight solutes from liposomes without dilution. Analytical Biochemistry, 1978; 90: 809 – 815.
28. Torchilin VP, Weissig V. Liposomes a practical approach, (2nd edition), Oxford University Press, Oxford, 2003; 36 – 39.
29. Cevc G. Schatzlein A, Blume G. Transdermal drug carriers: Basic properties, optimization and transfer efficiency in case of epicutaneously applied peptides. Journal of Controlled Release, 1995; 36:3 – 16.
30. Vanden Berge BAI, Swartzendruber VAB, Geest, J. Development of an optimal protocol for the ultrastructural examination of skin by transmission electron microscope. Journal Microscopy, 1997; 187 (2): 125 – 133.
31. Toll R, Jacobi U, Richter H, Lademann J, Schaefer H, Blume, U. Penetration profile of microspheres in follicular targeting of terminal hair follicles. Journal of Investigative Dermatology, 2004; 123: 168 – 176.
32. Kirjavainen M, Urtti A, Valjakka–Koskela R, Kiesvaara J, Monkkoonen J. Liposome – skin interactions and their effects on the skin permeation of drugs. European Journal of Pharmaceutical Sciences, 1999; 7(4): 279 - 286.
33. Lopez–Pinto JM, Gonzalez–Rodriguez ML, Rabasco AM. Effect of cholesterol and ethanol on dermal delivery from DPPC liposomes. International Journal of Pharmaceutics, 2004; 298(1): 1 – 12.
34. Anibinder D, Touitou E. Testosterone ethosomes for enhanced transdermal delivery. Drug Delivery, 2005; 12(5): 297 – 303.
35. Jarivis B, Faulds D. Lamivudine: a review of its therapeutic potential in chronic hepatits B. Drugs, 1999; 58(1): 101 – 141.
36. Barry BW. Mode of action of penetration enhancers on the kinetics of percutaneous absorption. Journal of Control Release, 1987; 6: 85 – 97.
37. Sanjay P. Ethosomes: A promising tool for transdermal delivery of drug. Latest Reviews, 2005; 5 (2): 1 – 5.

38. Jain S, Umamaheshwari RB, Bhadra D, Jain NK. Ethosomes – A novel vesicular carrier for enhanced Transdermal delivery of an anti – HIV agent. Indian Journal Pharmaceutical Sciences, 2004; 66 (1): 72 – 81.
39. Sheo DM, Sunil KP, Anish KG, Gyanendra KS, Ram CD. Formulation development and evaluation of ethosome of stavudine. Indian Journal of Pharmaceutical Education and Research, 2010; 44(1): 102 – 108.
40. Yi-Ping F, Yi-Hung T, Pao-chu W, Yaw–Bin H. Comparison of 5- aminolevulinic acid – encapsulated liposome versus ethosome for skin delivery for photodynamic therapy. International Journal of Pharmaceutics, 2008; 356: 144 – 152.
41. Banga AK, Chein YW. Hydrogel – based iontotheraputic delivery devices for transdermal delivery of peptide/protein drugs. Pharmaceutical Research, 1993; 10: 697 – 702.
42. Godin B, Touitou E, Rubinstein F, Athamna A, Athamna M. A new approach for treatment of deep skin infections by an ethosomal antibiotic preparation: an in vivo study. Journal of Antimicrobial Chemotherapy, 2005; 55(6): 989 – 994.
43. Ehab RB, Mina IT. Enhanced transdermal delivery of salbutamol sulfate via ethosomes. AAPS PharmSciTech, 2007; 8 (4): E1 – E8.
44. Kaplun–Friscкhoff Y, Touitou E. Testosterone skin permeation enhancement by menthol through formation of eutectic with drug and interaction with skin lipids. Journal of Pharmaceutical Sciences, 1997; 86: 1394 – 1399.
45. Godin B, Touitou E. Mechanism of bacitracin permeation enhancement through the skin and cellular membrane from an ethosomal carrier. Journal of Controlled Release, 2004; 94: 365 – 379.
46. Vaibhav D, Dinesh M, Tathagata D, Manoj N, Saraf DK, Jain NK. Dermal and transdermal delivery of an anti – psoriatic agent via ethanolic liposomes. Journal of Controlled Release, 2007; 123: 148 – 154.
47. Touitou E. Composition and methods for intracellular delivery, 2002; PCT/IL02/00516.
48. Jia-You F, Chi-Tzong H, Wen-Ta C, Ying-Yue W. Effect of liposomes and niosomes on skin permeation of enoxacin. International Journal of Pharmaceutics, 2001; 219 (1): 61 – 72.
49. Jain S, Jain P, Jain NK. Transfersomes: a novel vesicular carrier for enhanced transdermal delivery: development, characterization and performance evaluation. Drug Development and Industrial Pharmacy, 2003, 29: 1013 – 1026.