(Review Article)

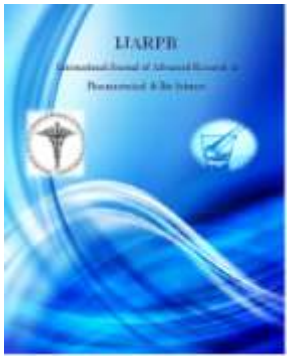
IJARPB
International Journal of Advanced Research in Pharmaceutical & Bio Sciences
(A peer reviewed International journal for Pharmaceutical and Bio Sciences)

Received on 17/07/2012; Revised on 25/07/2012; Accepted on 05/08/2012

Ethosome: A Novel Approach of Transdermal Drug Delivery System

Vivek Dave, Ashutosh Pareek, Sarvesh Paliwal

Department of Pharmacy, Banasthali University, Rajasthan 304022, India



Corresponding Author:

Vivek Dave
Department of Pharmacy, Banasthali University, Rajasthan 304022, India
E-mail: vivekdave1984@gmail.com

ABSTRACT

Transdermal drug delivery generated major interest among large pharmaceutical companies in the 1980s and 1990s due to potential advantages of avoidance of first-pass metabolism, avoidance of exposure to the chemical and biological conditions of the gastrointestinal tract, reduction or avoidance of adverse events, improved patient compliance. Ethosome delivery system was established to develop a patented, passive, non-invasive, transdermal drug delivery technology they are interesting and pioneering vesicular systems that have appeared in the field of pharmaceutical technology and drug delivery in recent years. This carrier presents interesting features correlated with its ability to permeate intact through the human skin due to its high deformability. Ethosomes are soft, malleable vesicles tailored for enhanced delivery of active agents. It has been shown that the physicochemical characteristics of ethosomes allow this vesicular carrier to transport active substances more efficaciously through the stratum corneum into the deeper layers of the skin than conventional liposomes . This aspect is of great importance for the design of carriers to be applied topically both for topical and systemic drug administration.

KEYWORDS: Ethosome, Transdermal drug delivery, Vesicle, Topical delivery

(Review Article)

INTRODUCTION

The skin acts as a major target as well as a principle barrier for topical/ transdermal (TT) drug delivery. The stratum corneum plays a crucial role in barrier function for TT drug delivery. Despite major research and development efforts in TT systems and the advantages of these routes, low stratum corneum permeability limits the usefulness of topical drug delivery. To overcome this, methods have been assessed to increase permeation. One controversial method is the use of vesicular systems, such as liposomes, ethosomes and niosomes, whose effectiveness depends on their physicochemical properties.

Touitou discovered lipid vesicular systems ethosomes embodying ethanol in relatively high concentration. Ethosomes contain phospholipids, alcohol (ethanol and isopropyl alcohol) in relatively high concentration and water. Unlike classical liposomes, ethosomes were shown to permeate through the stratum corneum barrier and were reported to possess significantly higher transdermal flux in comparison to liposomes. The exact mechanism for better permeation into deeper skin layers from ethosomes is still not clear. However, synergistic effects of combination of phospholipids and high concentration of ethanol in vesicular formulations have been suggested to be responsible for deeper distribution and penetration in the skin lipid bilayer. The use of lipid vesicles as drug delivery systems for skin treatment has attracted increasing attention in recent years. However, it is generally accepted that conventional ethosomes are of little value for this purpose. Ethosomes remain confined to the upper layer of stratum corneum (SC) and hence, are suitable for topical drug delivery. Only specially designed vesicles were shown to deliver drugs across the skin layers [1].

By far the most important reason for this is the low permeability of drugs in the stratum corneum, the outer- most layer of the skin acting as the main barrier in the skin [2, 3]. The structure of the stratum corneum is often com- pared with a brick wall, with the corneocytes as the bricks surrounded by the mortar of the intercellular lipid lamellae [4] (Fig. 1).

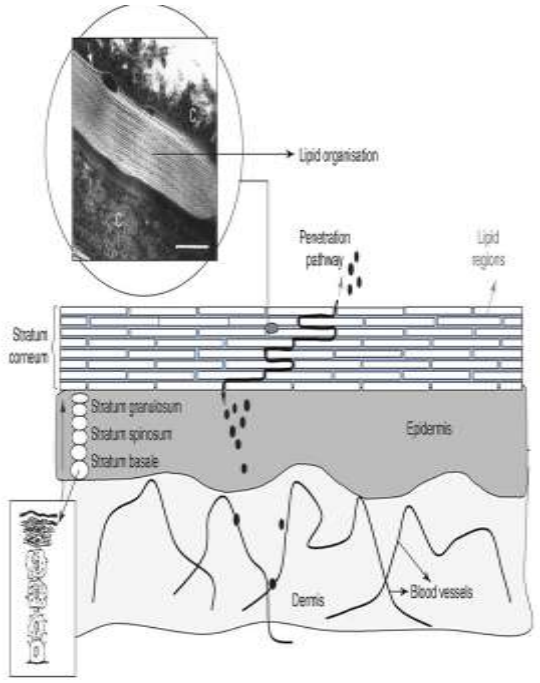


Figure 1: cross section of skin

It has been generally accepted that the highly organized crystalline lipid lamellae play an essential role in the barrier properties of the stratum corneum [5–10].Many techniques have been aimed to disrupt and weaken the highly organized intercellular lipids in an attempt to enhance drug transport across the intact skin [11-13] or to increase the driving force for permeation of drugs across this skin barrier. One of the most controversial methods is the use of vesicle

(Review Article)

formulations as skin delivery systems. If vesicles act as carrier systems, they might be able to transport large molecular weight drugs, such as proteins into the skin or even into the systemic circulation .If they act as penetration enhancers, however, the main mode of action is a perturbation of the lipid organization in the stratum corneum, thereby increasing the transport rate across the skin. The latter is only efficient for low molecular weight drugs. One of the most important characteristics of drug carrier systems is that drug and carrier should permeate along the same route across the skin (Fig. 2). In addition the vesicle material profile and the active compound profile in stratum corneum should be very similar.

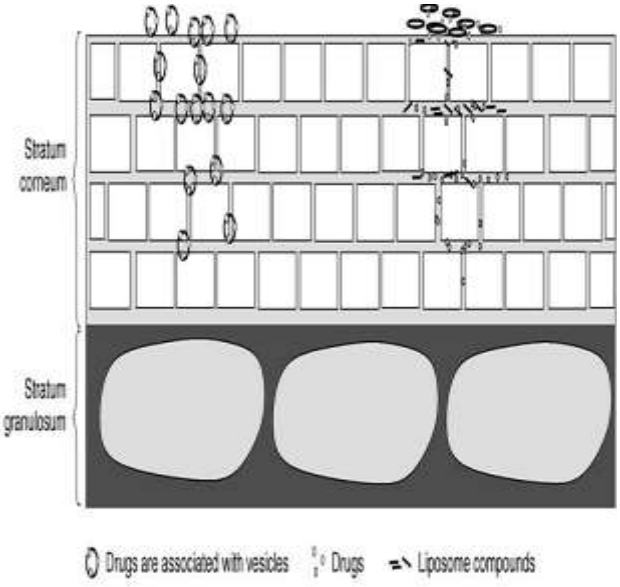


Figure 2: Pathway of Vesicle Carrier

Composition of ethosomes

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, ethosomes may contain phospholipids with various chemical structures like phosphatidylcholine (PC), hydrogenated PC, phosphatidic acid (PA), phosphatidylserine (PS), phosphatidylethanolamine(PE), phosphatidy-l glycerol (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 0.1-1percent 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 preparations. Cationic 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% [14]. Various polymer and combinations which is used for formulation of ethosomes are given in table 1.

Table 1: Different Additive Employed In Formulation of Ethosomes

Class	Example	Uses
Phospholipid	Soya phosphatidyl choline Egg phosphatidyl choline Dipalmityl phosphatidyl choline Distearyl phosphatidyl choline	Vesicles forming component
Polyglycol	Propylene glycol	As a skin penetration enhancer
Alcohol	Ethanol Isopropyl alcohol	For providing the softness for vesicle membrane As a penetration enhancer
Cholesterol	Cholesterol	For providing the stability to vesicle membrane
Dye	Rhodamine-123 Rhodamine red Fluorescene Isothiocynate(FITC) 6- Carboxy fluorescence	For characterization study
Vehicle	Carbopol Đ934, HPMC	As a gel former

Influence of high alcohol content

Ethanol is an established efficient permeation enhancer and is present in quite high concentration (20-50%) in ethosomes. However, due to the interdigitation effect of ethanol on lipid bilayers, it was commonly believed that vesicles could not coexist with high concentration of ethanol [15-17].

Lipid vesicular systems embodying ethanol in relatively high concentration and named them ethosomes. The basic difference between liposomes and ethosomes lies in their composition. The synergistic effect of combination of relatively high concentration of ethanol (20-50%) in vesicular form in ethosomes was suggested to be the main reason for their better skin permeation ability. The high concentration of ethanol (20-50%) in ethosomal formulation could disturb the skin lipid bilayer organization. Therefore, when integrated into a vesicle membrane, it could give an ability to the vesicles to penetrate the SC. Furthermore, due to high ethanol concentration the ethosomal lipid membrane was packed less tightly than conventional vesicles but possessed equivalent stability. This allowed a softer and malleable

(Review Article)

structure giving more freedom and stability to its membrane, which could squeeze through small openings created in the disturbed SC lipids. In addition, the vesicular nature of ethosomal formulations could be modified by varying the ratio of components and chemical structure of the phospholipids.

Advantages of Ethosomal Drug delivery

In comparison to other transdermal and dermal drug delivery systems,

1. The Ethosomal system is passive, non-invasive and is available for immediate commercialization
2. Ethosomes are platform for the delivery of large and diverse group of drugs (peptides, protein molecules)
3. Ethosome composition is safe and the components are approved for pharmaceutical and cosmetic use.
4. Low risk profile- The technology has no large-scale drug development risk since the toxicological profiles of the ethosomal components are well documented in the scientific literature.
5. The ethosomal drug is administrated in semisolid form (gel or cream), producing high patient compliance by is high. In contrast, Iontophoresis and phonopho-resis are relatively complicated to use which will affect patient compliance.
6. Ethosomes are enhanced permeation of drug through skin for transdermal and dermal delivery.
7. High market attractiveness for products with proprietary technology. Relatively simple to manufacture with no complicated technical investments required for production of ethosomes.
8. Various applications in pharmaceutical, veterinary, cosmetic field.

Method for Preparing Ethosomes

Ethosomal formulation may be prepared by hot or cold method as described below. Both the methods are convenient, do not require any sophisticated equipment and are easy to scale up at industrial level.

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 dissolve 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 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 [18] or extrusion [19] 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 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.

Injection Method

Ethosomes were prepared using different concentrations of lecithin, ethanol, isopropyl alcohol, propylene glycol. Phospholipids and drug was dissolved in ethanol and propylene glycol. The mixture was heated to 30º C in water bath. In this solution distilled water was added slowly in a fine stream with a constant mixing at 700 rpm in a closed vessel. The temperature was maintained at 30º C during the experiment. The mixing was continued for 5 minutes. The preparation was stored at 4º C. Ethosome prepared by the above procedure were subjected to sonication at 4ºc using probe sonicator in 3 cycles of 5 minutes with 5 minutes rest between the cycles.[29]

Physicochemical Characterizations and Properties of Ethosomal Formulation

Various methods of evaluations are as follows and summarized in Table 2.

Table 2: Methods for the Characterization of Ethosomal Formulation

Parameters	Methods
Vesicle shape (morphology)	Transmission electron microscopy Scanning electron microscopy
Entrapment efficiency	Mini column centrifugation method Fluorescence spectrophotometry
Vesicle size and size distribution	Dynamic light scattering method
Vesicle Skin interaction study	Confocal laser scanning microscopy Fluorescence microscopy Transmission electron microscopy Eosin-Hematoxylin staining
Phospholipid-ethanol interaction	31P NMR Differential scanning calorimeter
Degree of deformability	Extrusion method
Zeta potential	Zeta meter

Turbidity	Nephalometer
In vitro drug release study	Franz diffusion cell with artificial or biological membrane, Dialysis bag diffusion
Drug deposition study	Franz diffusion cell
Stability study	Dynamic light scattering method Transmission electron microscopy

Vesicle Morphology

Visualization by electron microscopy reveals an ethosomal formulation exhibited vesicular struct-ure 300-400 nm in diameter. The vesicles seem to be malleable as evident by their imperfect round shape.

Optical Microscope Observation

The ethosomal dispersion was spread on the glass slide using a glass rod. Formation of multilamellar vesicles was confirmed by examining the ethosomal suspension under an optical microscope with the magnification power of 100 X. Photographs of vesicles were taken using Olympus camera. As shown in (fig 3).

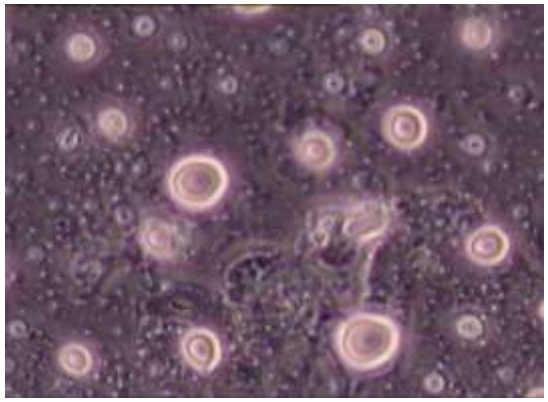


Figure 3: Microscopic images of Ethosome (Dave et.al, 2011)

Drug Entrapment Efficiency

The entrapment capacity of ethosomes was measured by the ultracentrifuge method. Vesicular preparations was kept overnight at 4˚C and centrifuged in a ultracentrifuge equipped with TLA-45 rotor at 4˚C, at 30 000 rpm for 2 h. suspension was assayed both in the sediment and in the supernatant. The entrapment capacity of suspension was calculated from the relationship [(T-C)/T] 100, where T is the total amount of drug that is detected both in the supernatant and sediment, and C is the amount of drug detected only in the supernatant. Differential scanning calorimetry thermograms and anisotropy measurement of AVPC (a fluorescent analog of phosphatidylcholine), revealed that ethosomes possessed lower Tm compared to classical liposomes and that the bilayers had a high degree of fluidity. This imparted a soft and malleable character to the vesicles[20]. Used confocal laser scanning microscopy (CLSM) to show that ethosomes can efficiently entrap both hydrophobic and hydrophilic fluorescent probes. Similar results were obtained using ultra-centrifugation method to measure entrapment of different drugs [21].

(Review Article)

pH Measurment

The pH measurement of the formulation was carried out using a pH meter by dipping the glass electrode completely into the semisolid formulation as to cover the electrode.

Vesicular shape and surface morphology

- **Scanning electronic microscopy**

Ethosome vesicles were analyzed by scanning electron microscopy (SEM) to characterize the surface morphology. Prior to analysis, the ethosome were mounted onto double-sided tape that has previously been secured on copper stubs and coated with platinum, then analyzed at different magnifications.

- **Vesicle Size and Size distribution**

The size of ethosomes ranges between tens of nanometers to microns and is influenced by the composition of the formulation. Dave et al prepared ethosomal with 30% ethanol and 2% phospholipids showed an average vesicle size of 161 ± 6.0 nm with a very low polydispersity index. In the ethanol concentration range of 10-50%, the size of the vesicles decreased with increasing ethanol concentration. The largest vesicles with 235 ± 8.0 nm sizes were present in the preparation containing 10% ethanol while the smallest vesicles of 91 ± 5.0 nm sizes were present in preparation containing 50% ethanol. Similarly, a decrease in the vesicle size (from 214 ± 8.0 nm to 82 ± 3.0 nm) was observed with increase in isopropyl alcohol concentration from 10 to 50%.[22]

Permeation Characteristics

These are compound, which promote skin permeability by altering the skin as a barrier to the flux of a desired penetrant. The flux, J, of drug across the skin can be written as:

$$J = D\, dc/dx$$

Where D is the diffusion coefficient and is a function of the size, shape and flexibility of the diffusing molecule as well as the membrane resistance, C is the concentration of the diffusing speacies, X is the spatial coordinate.

The in vitro skin permeation of acelecfenac from ethosomal formulation was studied using diffusion cell specially designed in our laboratory according to the literates. The effective permeation area of the diffusion cell is 2.011 cm^2 [30]. One of the most important features of ethosomal formulation is their sustained release characteristic. A significant prolongation of release across artificial membrane from ethosomal formulation as compared to drug solution was observed. In vitro and in vivo skin permeation studies have demonstrated the ability of ethosomal formulation to enhance permeation of both hydrophobic and hydrophilic molecules as compared to conventional liposomes. Different workers have reported 5-10 fold better skin permeation of drugs formulated in ethosomes as compared to conventional liposomal formulation [23-25].

Ethanol has long been known to have permeation enhancement property. However, the permeation enhancement from ethosomes was much greater than would be expected from ethanol alone, suggesting some kind of synergistic mechanism between ethanol, vesicles and skin lipids. Thus, ethanol that was earlier considered harmful to conventional liposomal formulations provided flexible characteristics to ethosomes, which allows them to easily penetrate into deeper layers of the skin. In

addition, the contribution of interaction between phospholipid vesicles with stratum corneum as proposed. [25] In enhancing the permeability of skin cannot be neglected.

Vesicle Skin Interaction Study

For evaluating the mechanism of better skin permeation of ethosomal formulation different visualization techniques e.g. transmission electron microscopy, eosin-hematoxylin staining, fluorescence microscopy and confocal scanning laser microscopy (CSLM) have been used. Often, when used in combination these visualization techniques gave better idea about structure modulation and penetration pathways of vesicles. To support the result of TEM study performed histological studies in order to visualize the changes in the ultrastructure of stratum corneum.

The results of eosin-haematoxyline staining study showed that ethosomal formulation affected the ultrastructure of stratum corneum. No change in the ultra-structure of viable tissue (epidermis or dermis) could be observed after treatment with conventional liposomal formulation. [80]

Touitou et al. reported the ability of ethosomes to deliver lipophilic molecules to deep layers of skin using a lipophilic fluorescent probe, Rhodamine red (RR) by CSLM. They found that intensity of fluorescence was much greater when ethosomal system was applied as compared to that when either a hydro alcoholic solution containing the same concentration of ethanol or an alcohol free liposomal system was applied. RR contained in ethosomes penetrated the mouse skin to a depth of approximately 140 mm. The probe fluorescence intensity was significantly greater from the ethosomal preparation whereas, deep penetration from conventional liposomal formulation was almost negligible. Similarly, Godin and Touitou [82] reported better skin permeation of fluoreceine isothiocyanate-bacitracin ethosomal formulation to deeper layer of skin as determined by CLSM.

In-vitro release studies through semi-permeable membrane

The in-vitro diffusion of the drug through semi-permeable membrane was performed. The semi permeable membrane soaked in a buffer for 6-8 hours. It was clamped carefully to one end of the hollow glass tube of 17 mm (area 2.011 cm^2) (dialysis cell). Which act as donor compartment. Phosphate buffer saline (PBS) 50 ml pH6.4 was taken in a beaker which was used as a receptor compartment.

A weight quantity was spread uniformly on the membrane. The donor compartment was kept in contact with the receptor compartment and the temperature was maintained at 37 ± 0.1 ºC. The solution of the receptor side was stirred by Teflon-coated magnetic bars. At predetermined time intervals, sample was withdrawn and replaced by 3 ml of PBS. The drug concentrations in the aliquot were determined at 275 nm against appropriate blank. This experiment was done in triplicate and average value was reported

Stability Study: (ICH Q1FC guidelines)

Products are normally required to have shelf – lives that are measured in year. Therefore, in addition to real time studies. (Storage at ambient condition) test must also be conducted under condition which accelerates any changes occur-ring at ambient temperature and humidity. Changes to stability testing requirements at an

international level have resulted in the following different stability long-term study conditions for hot and humid climates:

- 30°C/65%RH (e.g. WHO, ICH, SADC, GCC , Brazil)
- 30°C/70%RH (e.g. WHO previous, Cuba, Brazil previous)
- 30°C/75%RH (e.g. ASEAN)

Calculations based on meteorological data have demonstrated that the existing long-term stability conditions in WHO guidelines for Zone IV (30°C/65%RH) do not reflect climatic conditions in many countries which have hot and very humid areas, such as Brazil, Cuba, China, India and all of the ASEAN countries. Physical stability study was performed to investigate the leaking of the drug from ethosome during storage. The formulated ethosome dispersion were sealed in 10-ml USP type I amber colored glass vials and stored in at 4 ±2 °C and RT for a period of 45 days. Samples from each ethosome formulation were withdrawn at predetermined time intervals i.e. 45 days. Drug content and entrapment efficiency was determined.

Proposed Mechanism of Skin Permeation of Ethosomes

The stratum corneum lipid multilayers at physiological temperature are densely packed and highly conformational ordered. Ethosomal formulations contain ethanol in their composition that interacts with lipid molecules in the polar headgroup regions, resulting in an increased fluidity of the SC lipids. The high alcohol content is also expected to partial extract the SC lipids. These processes are responsible for increasing inter and intracellular permeability of ethosomes. In addition, ethanol imparts flexibility to the ethosomal membrane that shall facilitate their skin permeation. The interdigitated, malleable ethosome vesicles can forge paths in the disordered SC and finally release drug in the deep layers of skin. The transdermal absorption of drugs could then result from fusion of ethosomes with skin lipids. This is expected to result in drug release at various points along the penetration pathway [26-28].

Different Studies Related to the Application of Ethosomes as a Carrier System

Various studies employing ethosomal formulation have shown better skin permeability of drugs. The uses of ethosomes as carrier system for transdermal/ topical drug delivery are summarized below (Table 3).

Table 3: Application of Ethosomes as a Drug Carrier

Drug	Results
NSAIDS (Diclofenac)	Selective delivery of drug to desired side for prolong period of time
Acyclovir	Increase skin permeation Improved in biological activity two to three times Improved in Pharmacodynamic profile

Insulin	Significant decrease in blood glucose level Provide control release
Trihexyphenidyl hydrochloride	Improved transdermal flux Provide controlled release Improved patient compliance Biologically active at dose several times lower than the currently used formulation
DNA	Better expression of genes Selective targeting to dermal cells
Antibiotic Cannabidol Erythromycin	Improved skin deposition Improved biological activity Prolonging drug action
Bacitracin	Improved dermal deposition Improved intracellular delivery Increased bioavailability
Anti-HIV agents Zidovudine Lamivudine	Improved transdermal flux Improved in biological activity two to three times Prolonging drug action Affected the normal histology of skin
Azelaic acid	Prolong drug release

Ethosmes in the service of Cosmetic

Many cosmetic preparations contain active ingredients which can only act when they penetrate at least the outermost layer of the skin, the stratum corneum. However, due to the resistance of the stratum corneum to the transport into the skin, the efficacy of topically applied actives is often far from required. The efficiency of cosmetic products for skin whitening, alleviating broken capillaries, cellulite care, skin nutrition, protection from UV induced cellular damage and other targets depends on the delivery of the active agents to the deeper skin strata. For this purpose, carriers with adequate skin penetration enhancement properties are needed.

Ethosomes provides an excellent answer to these needs for a wide range of active ingredients.

(Review Article)

Revolutionary anti-cellulite creams

NTT has already on sale private label anti-cellulite products generating revenues to the company.

- US customer's product branded LipoductionTM being marketed at premium price in leading department stores.
- Japanese customer's product branded NoicellexTM being marketed in Japan via TV and print campaigns.

REFERENCES

1. Touitou, E., Merdan, V.M, (1998) Vesicular carriers for topical delivery. Acta Technologiae et legis Medicoment; 91: 1-6.
2. Blank, I.H. and Scheuplein, R.J. (1969) Transport into and within the skin.Br. J. Dermatol. 81, 4–10.
3. Scheuplein, R.J. and Blank, I.H. (1971) Permeability of the skin. Physiol. Rev. 51, 702–747.
4. Elias, P.M. (1983) Epidermal lipids, barrier function, and desquamation. J. Invest. Dermatol. 80 (Suppl.), 44s–49s
5. Williams, M.L. and Elias, P.M. (1987) The extracellular matrix of stratum corneum: role of lipids in normal and pathological function. Crit. Rev. Ther. Drug Carrier Syst. 3, 95–122.
6. Wertz, P.W. (2000) Lipids and barrier function of the skin. Acta Derm. Venereol. 208 (Suppl.), 7–11.
7. Bouwstra, J.A. et al. (2003) Structure of the skin barrier and its modulation by vesicular formulations. Prog. Lip. Res. 42, 1–36.
8. Bouwstra, J.A. et al. (2000) The lipid organisation of the skin barrier. Acta Derm. Venereol. 208 (Suppl.), 23–30.
9. Pilgram, G.S. et al. (1999) Electron diffraction providesne win formation of human stratum corneum lipid organization studied in relation to depth and temperature. J. Invest. Dermatol. 113, 403–409.
10. Bouwstra, J.A. et al. (1995) Lipid organization in pig stratum corneum. J. Lipid Res. 36, 685–695.
11. Barry, B.W. (2001) Novel mechanisms and devices to enable successful transdermal drug delivery. Eur. J. Pharm. Sci. 14, 101–114.
12. Schreier, H. and Bouwstra, J.A. (1994) Liposomes and niosomes as topical drug carriers: dermaland Trans dermal drug delivery. J.Control.Release30,1–15.
13. Prausnitz, M.R. et al. (2004) Current status and future potential of transdermal drug delivery. Nat. Rev. 3, 115–124.
14. Touitou, E. Composition of applying active substance to or through the skin. US patent, 5,540,934, 1998.
15. Touitou, E. Composition of applying active substance to or through the skin., US patent, 5,716,638, 1996.
16. Braun-Falco, O.; Kortung, H.C.; Maibach, H.I. Liposome Dermatitis, Springer-Verlag, Berlin Heideberg, 1992.
17. Berner, B.; Liu, P. (1995) Alcohol, In Percutaneous Enhancer, Smith, E.W.; Maibach, H.I., Ed.; CRC Press, Boca Raton, FI, pp 45-60.
18. Braun-Falco, O.; Kortung, H.C.; Maibach, H.I. Liposome Dermatitis, Springer-Verlag, Berlin Heideberg, 1992.
19. Berner, B.; Liu, P. (1995) Alcohol, In Percutaneous Enhancer, Smith, E.W.;

Maibach, H.I., Ed.; CRC Press, Boca Raton, FI, pp 45-60.
20. Touitou, E.; Dayan, N.; Bergelson L.; Levi-Schaffer F.; Piliponsky, (1998) J. Liposome Res. 8,112-114.
21. Godin, B. and Touitou, E. (2004) J. Control Release. 187, 1-15.
22. Lodzki, M.; Godin, B.; Rakou, L.; Mechoulam, R.; Gallily, R.; Touitou, E. (2003) J. Control. Release. 93, 377-387.
23. Touitou E. Godin B.; Bujanover S.; Becker Y. (2002) J. Control. Release. 80, 17.
24. Touitou, E.; Godin, B.; Dayan, N.; Weiss, C.; Piliponsky, A.; Levi-Schaffer, F. (2001) Biomaterials. 22, 3053-3059.
25. Esposito, E.; Menegatti, E.; Cortesi, R. (2004) J Cosmet. Sci. 55(3), 253-64.
26. Bouwstra, J.A. and Honeywell-Nguyen, P.L. Adv. Drug Deliv. Rev., 2002, 54(1), S41-S55.
27. Touitou, E.; Alkabes, M.; Dayan, N.; Eliaz, M. (1997) Pharm. Res. 14, S305-S306.
28. Touitou, E.; Dayan, N.; Bergelson L.; Levi-Scaffer. F.; Pilponsky, A. (1998) J. Lipid Res. 8, 113-114
29. Touitou E, Dayan N, Bergelson L, Godin B, Eliaz M, (2000) Ethosomes-novel vesicular carriers for enhanced delivery: characterization and skin penetration properties. J. Control. Release, 65:403
30. Dubey, V., Jain, N.K. (2007) Melatonin lodaded liposomes: physicochemical characterization and enhanced transdermal delivery. Eur. J. Pharm. 67, 398-405.

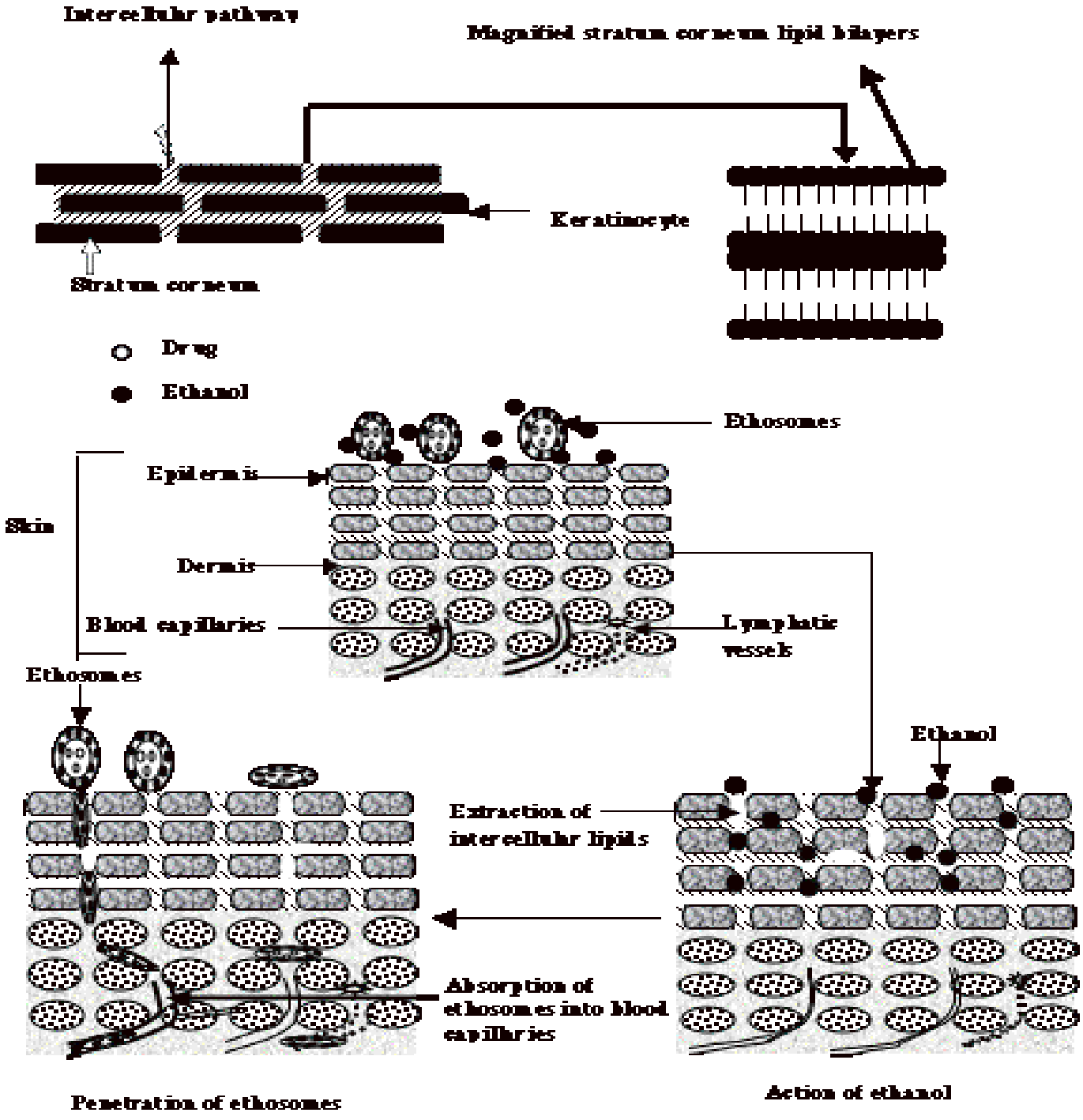


Figure 4: Proposed mechanism for penetration of molecule from ethosomal system across the lipid domain of stratum corneum