



## ETHOSOMES- A NEW FRONTIER IN DRUG DESIGN

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### ABSTRACT

The present article is a descriptive study of the performances of ethosomes specialized delivery systems for transdermal drug delivery system. Vesicular systems, such as ethosomes are used in cosmetic and pharmaceutical products to encapsulate ingredients, to protect ingredients from degradation, to increase bioavailability, and to improve therapeutic efficacy. A review of literature is presented here and a sincere attempt has been made to highlight the properties and characteristics of ethosomes in transdermal drug delivery and cosmetic, veterinary and pharmaceutical applications. Interaction studies between ethosomes components and skin is also discussed along with the formulation aspects of ethosomes formulation. Our aim is to introduce the ethosomes as a carrier system for various applications of drugs and cosmetic, veterinary and pharmaceuticals.

**KEYWORDS:** Ethosomes, transdermal, liposomes, niosomes.

### INTRODUCTION

Transdermal drug delivery systems are the recently developed devices, which are non invasive to skin as compared to other routes for administration of drugs. Although the skin, particularly the stratum corneum presents a principle barrier to most drug absorption, it provides a large (1-2m) and accessible surface area for drug diffusion. Various types of transdermal drug delivery systems are utilized for long term continuous infusion of therapeutic agents, including antihypertensive, antifungal, analgesics, steroids and contraceptive drugs and also suitable for novel genetically engineered pharmaceuticals (peptides and proteins) to their site of action. Although transdermal delivery is currently limited to few drugs, it has achieved considerable commercial success.

Various types of transdermal drug delivery system include liposome, niosome ethosomes.

### LIPOSOMES

Liposome small vesicle of a bilayer of phospholipids encapsulating an aqueous space ranging from 0.03-10 $\mu$ m in diameter.

### NIOSOMES

Niosomes are non-ionic surfactants based multilamellar or unilamellar vesicles in which an aqueous solution of solute(s) is enclosed by a membrane resulted from the organization of surfactant macro-molecule as bilayer.

### ETHOSOMES

Ethosomes are vesicular carrier comprise of hydroalcoholic or hydro/alcoholic/glycolic phospholipids in which the concentration of alcohols or their combination is relatively high.

Ethosomes are recent development made in transdermal therapeutic systems. These are most advance devices which ignore demerits of liposome and niosome such as;

- Liposomes require special precautions and conditions for formulation and preparations.
- Complex method for routine and large scale production.
- Less chemical stability.
- High cost and while niosome possesses demerits like;
  - Fusion,
  - Aggregation,
  - Sedimentation,
  - Leakage on storage,
  - Physical instability.<sup>[2]</sup>
- They are not suitable for transdermal delivery because they cannot reach the deeper layer of the skin as they are trapped in the superior layers of stratum corneum.

### TRANSDERMALDRUG DELIVERY SYSTEMS

The skin acts as a major target as well as a principle barrier for transdermal drug delivery. They will improve the therapeutic efficacy and safety of drugs. This drug delivery system is also essential for the delivery of novel

genetically engineered pharmaceuticals (peptides and proteins) to their site of action.<sup>[3]</sup>

#### ADVANTAGES

- Avoidance of the 'first pass effect.
- Avoidance of exposure to the chemical, P<sup>H</sup>, Biological conditions of G.I.Tract.
- A stable and controlled blood level.
- Termination of further administration, if necessary. Long-term duration (ranging from a few hours to one week).
- Avoid unwanted adverse effects. Improved patient compliance and reduced inter and intra-patient variability.
- Self-administration is possible.

#### MECHANISM OF ACTION

There are two important layers in skin: the dermis and the epidermis. The outermost layer, the epidermis, is approximately 100 to 150 micrometers thick, has no blood flow and includes a layer within it known as the stratum corneum. The stratum corneum develops a thin, tough, relatively impermeable membrane which usually provides the rate limiting step in transdermal drug delivery system. This is the layer most important to transdermal delivery as its composition allows it to keep water within the body and foreign substances out. Beneath the epidermis, the dermis contains the system of capillaries that transport blood throughout the body. If the drug is able to penetrate the stratum corneum, it can enter the blood stream. The method was suitable for both

water-soluble and lipid soluble drugs. This is because "Lipid-soluble substances readily pass through the intercellular lipid bi-layers of the cell membrane whereas water-soluble drugs are able to pass through the skin because of hydrated intracellular proteins. Using drugs engineered in this manner, much more rapid and useful drug delivery is possible.<sup>[4]</sup>

#### LIMITATIONS

Transdermal drug delivery systems have some limitations

- The drug must have some desirable physico-chemical properties for penetration through stratum corneum.
- Skin irritation or contact dermatitis due to drug or excipients.
- The barrier function of skin changes from one site to another on the same person, from person to person and with age.

#### TRANSDERMAL MARKET

The market for transdermal products has been in a significant upward trend that is likely to continue for the foreseeable future. An increasing number of TDD products continue to deliver real therapeutic benefit to patients around the world. More than 35 TDD products have now been approved for sale in the US, and approximately 16 active ingredients are approved for use in TDD products globally.<sup>[5]</sup>

#### List of the Transdermal products

Table: 01 List of the Transdermal products.

Product name	Drug	Manufacturer	Indication
Catapres-TTS	Clonidine	Alza/Boehinger Ingelheim	Hypertension
CombiPatch	Estradiol/Norethindrone	Noven , Inc./Aventis	Hormone replacement therapy
Deponit	Nitroglycerin	Schwarz-Pharma	Angina pectoris

#### LIPOSOMES

liposomes are composed of small vesicles of a bilayer of phospholipids encapsulating an aqueous phase ranging from 0.03 to 10µm in diameter. They are composed of one or more lipid membranes enclosing discrete aqueous compartment. The enclosed vesicles can encapsulate water soluble drugs in aqueous space and lipid soluble drugs can be incorporated in to membrane. Liposomes are mainly composed of natural phospholipids and cholesterol. N "heads" up and "tails" down. The phospholipids composing liposomes are amphipathic possessing both a hydrophilic or polar head and hydrophobic or non-polar tail. Hydrophobic tail composed of 10-24 carbon atoms and polar end contains phosphoric acid bound to a water soluble portion. The liposome can be used as vehicle for administration of nutrients and pharmaceuticals.<sup>[6]</sup>

There are mainly two types of liposomes. Unilamellar (one bilayer) liposomes.

Unilamellar liposomes have a single phospholipid bilayer sphere enclosing the aqueous solutions.

#### MECHANISM OF ACTION

- Liposome encapsulates a region of aqueous solution inside a hydrophobic membrane.
- Dissolved the drug hydrophilic solutes.
- The hydrophilic solutes cannot readily pass through the lipids.
- Hydrophobic chemicals can be dissolved into the membrane, and in this way liposome can carry both hydrophobic molecules and hydrophilic molecules.<sup>[7]</sup>

#### METHODS OF PREPARATION

##### Supercritical Carbon dioxide Method

Bilayer vesicle preparation techniques have been developed to decrease the toxicity as well as the improvement of the stability of the vesicles. Supercritical fluid is a substance above its critical temperature (T<sub>c</sub>)

and critical pressure ( $P_c$ ). It has been used as a one step preparation for several bilayer vesicles at low temperature. At the critical point, supercritical fluids have the density as liquid and low viscosity with better flow property as gas. Carbon dioxide is a widely used gas to produce supercritical fluid because of its low critical temperature ( $T_c = 31.1^\circ\text{C}$ ) and pressure ( $P_c = 73.8$  bar). It has high solvating power at the conditions near the critical point and similar to non-polar solvent. It can be adjusted by changing pressures or temperatures. Bilayer vesicles can be prepared using supercritical carbon dioxide instead of organic solvents such as chloroform or ether. The procedure is consisted of two main parts which are the high-pressure part, in which the lipid components or cholesterol are dissolved under pressure in supercritical carbon dioxide and the low-pressure part, in which the homogeneous supercritical solution is expanded and simultaneously mixed with the aqueous phase to yield bilayer vesicles. Carbon dioxide is released when pressure is reduced. Supercritical carbon dioxide ( $sc\text{CO}_2$ ) can be used alternatively for organic solvent to form bilayer vesicles due to its advantages of being environmental friendly, non-toxic, non-inflammable and inexpensive. Ethanol is usually used as the co-solvent for the enhancement of the entrapment efficiency of various hydrophilic substances in bilayer vesicles using supercritical technique.<sup>[8]</sup>

#### Conventional method

The steps involved are the preparation of lipid for hydration, hydration of lipid film by agitation and sizing of the lipid lamellar dispersion to a homogeneous dispersion. The lipids are first dissolved and mixed in an organic solvent (usually chloroform or chloroform mixed with methanol) to assure a homogeneous mixture of lipids. Then, solvent is removed by evaporation to get a thin lipid film on the sides of a round bottom flask and left to dry overnight to remove residual organic solvent. Hydration of the dried lipid film is accomplished simply by adding an aqueous solution and agitating by swelling on the water bath at the temperature above the gel-liquid crystal transition temperature ( $T_c$  or  $T_m$ ) of the lipid. Hydration time may differ among lipid species and structures. However, a hydration time of 1 hour with vigorous shaking, mixing, or stirring is highly recommended. Suitable hydration media can be distilled water, buffer solutions, saline, and no electrolytes such as sugar solutions. Once the product of hydration which is a large, multilamellar vesicle (LMV) is obtained, the particles can be downsized by sonication or extrusion to obtain required sizes. This method appears to be a simple method. However, it has limitations because of low entrapment efficiency and difficult to scale to larger batch sizes.

#### Freeze-dried method

Bilayer vesicular formulations are specially needed to develop for protein and DNA delivery because of the easily chemical degradation of these entrapped materials. The advantage of the FDEL method is that it will not

expose the drug to high temperature of solvent evaporation and lyophilization, thereby reducing the risk of degradation. Various sizes of protein can be entrapped and stabilized in liposomes. Thus, this method will be very useful for the preparation of nanovesicles containing or complexes with protein.

#### VARIOUS METHODS FOR CHARACTERIZATION OF LIPOSOMES

- Visualization: Visualization of liposome can be done using transmission electron microscopy (TEM) and by dynamic light scattering (DLS).
- Vesicle size and size distribution: Optical microscopy, laser diffraction particle size analyzer, coulter submicron size analyzer.<sup>[9]</sup>
- Shape & surface morphological characterization: Optical microscopy, transmission electron microscope, scanning electron microscope.
- Zeta potential: liposome zeta potential was determined by DLVO theory.

#### NIOSOMES

Niosomes are non-ionic surfactant vesicles obtained from hydration of synthetic nonionic surfactants, with or without the incorporation of cholesterol or other lipids.<sup>[10]</sup> Niosomes have been investigated because of the instability of liposome. Niosomes can be prepared by the same procedure as liposome such as conventional chloroform film method reverse phase evaporation ether injection supercritical  $\text{CO}_2$  method Figure 03 shows the schematic representation of a niosome. Niosomes are vesicular systems similar to liposome that can be used as carriers of amphiphilic and lipophilic drugs. They are promising vehicles for drug delivery and being non-ionic. It is less toxic than liposome. It improves the therapeutic index of drug by restricting its action to target cells. Niosomes alleviate the disadvantages associated with liposomes such as chemical instability, variable purity of phospholipids, and high cost. Niosomes use alternative materials to phospholipids such as Span 60, Span 40 and Span 20, but the preparative methods of niosomes are very costly when compared to ethosomes.

#### METHOD OF NIOSOME PREPARATION

##### Ether Injection Method

This method provides a means of producing niosomes by slowly introducing a solution of surfactants dissolved in diethyl ether into warm water maintained at  $60^\circ\text{C}$ . The surfactant mixture in ether is injected through a 14-gauge needle into an aqueous solution. Vaporization of ether leads to the formation of the single layered vesicles. Depending upon the conditions used the diameters of the vesicle ranges from 50 to 1000 nm.

##### Microfluidization

Microfluidization is a recent technique used to prepare unilamellar vesicles of the defined size distribution. This method is based on submerged jet principle in which two fluidized streams interact at ultra high velocities, in

precisely defined micro channels within the interaction chamber. The impingement of the thin liquid sheet along a common front is arranged such that the energy supplied to the system remains within the area of niosome formation. Greater uniformity, smaller size and better reproducibility of the niosomes can be produced.<sup>[11]</sup>

### Hands Shaking Method

(Thin film hydration technique): The mixture of surfactants and cholesterol is dissolved in a volatile organic solvent (diethyl ether, chloroform or methanol) in a round bottom flask. The organic solvent is removed at room temperature (20°C) using a rotary evaporator leaving a thin layer of the solid mixture deposited on the wall of the flask. The dried surfactant film can be rehydrated with aqueous phase at 0-60°C with gentle agitation. This process forms typical multilamellar niosomes. Thermo sensitive niosome were prepared by evaporating the organic solvent at 60°C and leaving a thin film of lipid on the wall of the flask by a rotary evaporator. The aqueous phase containing the drugs was added slowly with intermittent shaking of the flask at room temperature followed by sonication.

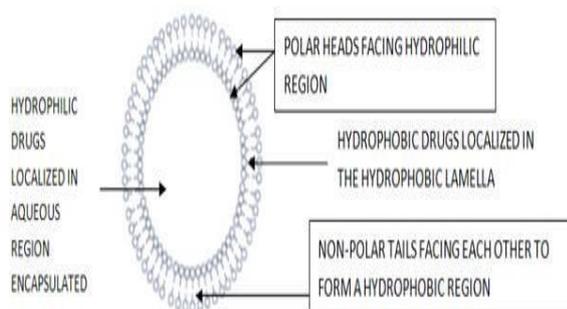


Figure: 02 Components present in niosomes.

### ETHOSOMES

Ethosomes are soft lipid vesicles containing phospholipids, alcohol (ethanol and isopropyl alcohol) in relatively high concentration and water. The size range of ethosomes may vary from nanometers to microns ( $\mu$ ). Ethosomes permeate through the skin layers more rapidly and possess significantly higher transdermal flux in comparison to conventional liposomes. Although, the exact mechanism for better permeation into deeper skin layers from ethosomes is still not clear. The 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 bilayers.

To improve the permeation of drugs through the skin various mechanisms have been investigated, including use of chemical or physical enhancers, such liposomes, niosomes, transferosomes but ethosomes also have been reported to enhance permeability of drug through the stratum corneum barrier. Permeation enhancers increase the permeability of the skin, so that the drugs can cross through the skin easily. Ethosomes can entrap drug

molecule with various physicochemical characteristics i.e. of hydrophilic, lipophilic, or amphiphilic. The ethosomes more advantages when compared to transdermal and dermal delivery. It delivers large molecules such as peptides, protein molecules. Simple method for drug delivery in comparison to Iontophoresis and Phonophoresis and other complicated methods. 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.

### INTERACTION BETWEEN SKIN AND ETHOSOMES

The enhanced delivery of actives using ethosomes over liposomes can be ascribed to an interaction between ethosomes and skin lipids. A possible mechanism for this interaction has been proposed. It is thought that the first part of the mechanism is due to the 'ethanol effect', whereby intercalation of the ethanol into intercellular lipids increasing lipid fluidity and decreases the density of the lipid multilayer. This is followed by the 'ethosomes effect', which includes inter lipid penetration and permeation by the opening of new pathways due to the malleability and fusion of ethosomes with skin lipids, resulting in the release of the drug in deep layers of the skin.<sup>[12]</sup>

### ETHANOL- AS PENETRATION ENHANCER

Substances that reversibly reduce the barrier resistance of the stratum corneum are known as chemical penetration enhancers. Ethanol is one of the most commonly used permeation enhancers. A number of mechanisms have been proposed for permeation enhancing action of ethanol. As a solvent, ethanol can be included in the formulation to enhance the solubility of the drug. This is particularly important for poorly soluble permeates, as they are prone to depletion in the donor vehicle. Ethanol is a relatively volatile solvent and will rapidly evaporate at skin temperature. Ethanol loss from a formulation may lead to the drug becoming supersaturated, which will influence drug flux across the membrane. In addition, ethanol is thought to alter the solubility properties of the stratum conium, facilitating improved drug partitioning.<sup>[16]</sup>

### METHODS OF PREPARATION

#### Cold Method

Ethosomes can be prepared from soybean phosphatidylcholine (Phospholipon 90), ethanol, drug and distilled water. Phospholipon 90 and drug should be dissolved in ethanol. Water has to be added in small quantities and the preparation mixed by mechanical stirring under controlled conditions.<sup>[1]</sup> Phospholipids and drug or fluorescent probe (Rhodamine-123) was dissolved in ethanol. This mixture was heated to  $30^{\circ}\pm 1^{\circ}\text{C}$  in a water bath. Double-distilled water heated to  $30^{\circ}\pm 1^{\circ}\text{C}$  was added slowly as a fine stream to lipid mixture with constant stirring at 700 rpm in a closed vessel. Mixing was continued for an additional 5

minutes, while maintaining the system at  $30^{\circ}\pm 1^{\circ}\text{C}$ . The resulting vesicle suspension was homogenized by passing through polycarbonate membrane of 400, 200, or 100 nm according to initial size of formulation using hand extruder for 3 cycles.<sup>[13]</sup>

### Hot Method

In this method, phospholipid is dispersed in water by heating in a water bath at  $40^{\circ}\text{C}$  until a colloidal solution is obtained. In a separate vessel ethanol and propylene glycol are mixed and heated to  $40^{\circ}\text{C}$ . Once both mixtures reach  $40^{\circ}\text{C}$ , the organic phase is added to the aqueous one. The drug is dissolved in water or ethanol depending on its hydrophilic/hydrophobic properties 69, 70. The vesicle size of ethosomal formulation can be decreased to the desired extent using probe sonication or extrusion method.<sup>[14]</sup>

### Ether injection method

In this method phospholipids are dispersed in ether and it was heated at  $40^{\circ}\text{C}$  until a colloidal solution is obtained. In a separate vessel ethanol and propylene glycol are mixed and heated to  $40^{\circ}\text{C}$ . Once both mixtures reach  $40^{\circ}\text{C}$ , the organic phase is added to the aqueous one. The drug is dissolved in water or ethanol depending on its hydrophilic/ hydrophobic properties.

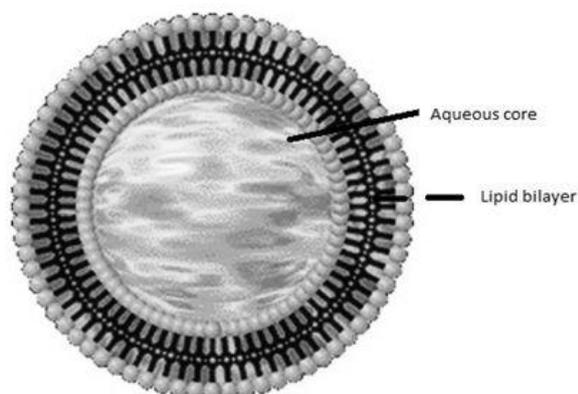


Figure: 03 components presents in ethosomes.

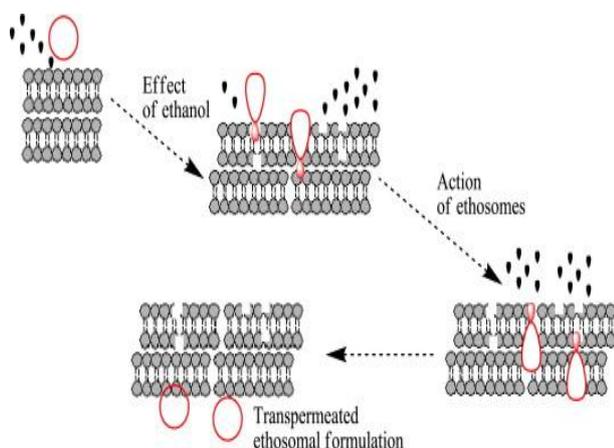


Figure: 06 picture of ethanol effect and ethosomal effect.

### MECHANISM OF PENETRATION

The enhanced delivery of actives using ethosomes over liposomes can be ascribed to an interaction between ethosomes and skin lipids. A possible mechanism for this interaction has been proposed. It is thought that the first part of the mechanism is due to the 'ethanol effect' whereby intercalation of the ethanol into intercellular lipids increasing lipid fluidity and decreases the density of the lipid multilayer. This is followed by the 'ethosome effect', which includes inter lipid penetration and permeation by the opening of new pathways due to the malleability and fusion of ethosomes with skin lipids, resulting in the release of the drug in deep layers of the skin as shown in Figure. The drug absorption probably occur.<sup>[17]</sup>

In following two phases.

#### 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.

#### Ethosomes Effect

Increased cell membrane lipid fluidity caused by the ethanol of ethosomes results increased skin permeability. So the ethosomes permeates very easily inside the deep skin layers, where it got fused with skin lipids and releases the drugs into deep layer of skin.<sup>[18]</sup>

### VARIOUS METHODS FOR CHARACTERIZATION OF ETHOSOMES

- Visualization: Visualization of ethosomes can be done using transmission electron microscopy (TEM) and by scanning electron microscopy (SEM).
- 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).
- Entrapment Efficiency: The entrapment efficiency of drug by ethosomes can be measured by the ultracentrifugation technique.
- Transition Temperature: The transition temperature of the vesicular lipid systems can be determined by using differential scanning calorimetry.
- 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.
- 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.
- Drug Content: Drug can be quantified by a modified high performance liquid chromatographic method.

### ADVANTAGES OF ETHOSOMES CAN BE ILLUSTRATED IN COMPARISON TO OTHER TRANSDERMAL DELIVERY SYSTEMS

- ✓ Ethosomes enhance the permeation of drug through skin for transdermal and dermal delivery.
- ✓ Ethosomes act as platform for the delivery of large and diverse group of drugs (peptides, protein molecules).
- ✓ Ethosomes composition is safe and the components are approved for pharmaceutical and cosmetic use.
- ✓ Low risk profile- The technology has no large-scale drug development risk since the toxicological profiles of the ethosomal.
- ✓ High patient compliance- The Ethosomal drug is administrated in semisolid form (gel or cream), producing high patient compliance by is high. In contrast, Iontophoresis and Phonophoresis are relatively complicated to use which will affect patient compliance.
- ✓ The Ethosomal system is passive, non-invasive and is available for immediate commercialization.
- ✓ Various application in Pharmaceutical, Veterinary, Cosmetic field.

### CONCLUSION

Ethosomes have been found to be much more efficient at delivering drug to the skin, than either liposomes or niosomes. Ethosomes have been tested to encapsulate hydrophilic drugs, cationic drugs, proteins and peptides. Ethosomal carrier opens new challenges and opportunities for the development of novel improved therapies. Ethosomes acts as a penetration enhancer and more permeation occurs in ethosomes than liposomes and niosomes. A wide variety of active agents of different therapeutic functions were formulated into ethosomes in transdermal and dermal drug delivery system. So on the basis of these studies we concluded that ethosomes are the present and future of vesicle system in transdermal and dermal delivery of various drugs.

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### REFERENCE

1. Touitou E, Compositions for applying active substances to or through skin. US Patent, 1996; 5540934.
2. Perrett S, Golding M, Williams WP. A Simple Method for the Preparation of Liposomes for Pharmaceutical Applications: Characterization of the Liposomes. *J. Pharm. Pharmacol*, 1991.
3. Cevc G. Lipid vesicles and other colloids as drug carriers on the skin. *Adv Drug Deliv Rev.*, 2004; 56: 675-711.
4. Roberts MS, Targeted drug delivery to the skin and deeper tissues: role of physiology, solute structure and disease. *Clin Exp Pharmacologic Physiology*, 1997 Nov; 24(11): 874-9.
5. Aulton.M.E, *Pharmaceutics; The science of dosage form design*, second edition, Churchill Livingstone, Harcourt publishers, 2002.
6. Ryan D. Gordon and Tim A. Peterson, *transdermal drug delivery, drug delivery technology*, www.drugdeliverytechnology.com
7. Kimball's Biology Pages, "Cell Membranes."
8. Wikipedia.2008.liposomesURL:http://en.wikipedia.org/wiki/liposomes.
9. Available: http://www.avantilipids.com/Preparation of Liposomes.html [2009, January 26].
10. Wiki pedía. 2008. neosomes URL:http://en.wikipedia.org/wiki/Ineosomes.
11. Niosome: An Unique Drug Delivery System Available: http://www.pharmainfo.net/reviews/niosome-unique-drug-delivery-system [2009, January 26].
12. Bouwsta JA, Honeywell-Nguye PL. Skin structure and mode of action of vesicles. *Adv Drug Del Rev.*, 2002; 54: S41-S55. Pub Med.
13. Sagar GH, Arunagirinathan MA., Bellare JR. Self-assembled surfactant nanostructures important in drug delivery: A Review. *Indian J Exp Biol*, 2007.
14. Godin B, Touitou E, Rubinstein E, Athamna A, and 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.
15. Jain S, Umamaheshwari RB, Bhadra D and Jain NK, Ethosomes-A novel vesicular carrier for enhanced transdermal delivery of an anti-HIV agent, *Indian J. Pharm. Sci.*, 2004.
16. Lodzki M et al, Godin B, Rakou L, Mechoulam R, Gallily R, Touitou E. Cannabidiol transdermal delivery and anti-inflammatory effect in a marine model. *J Control Rel.*, 2003; 93: 379-389.
17. Verma D D et al, Fahr A; Synergistic penetrations effect of ethanol and phospholipids on the topical delivery of Cyclosporin. *A J Control Release*, 2004; 97: 55-66. 22.
18. Available online http://www.ipharmsciencia.comISSN 2231-5896©2012 IPS.
19. Touitou E; Composition of applying active substance to or through the skin, US patent, 5,716,638, 1996. 27).