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Ethosomes as novel drug delivery system: A review

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Abstract
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. To provide continuous drug infusion through an intact skin, several transdermal therapeutic systems have been developed for topical application onto the intact skin surface to control the delivery of drug and its subsequent permeation through the skin tissue.
Transdermal route is promising alternative to drug delivery for systemic effect. An attempt was made to formulate the highly efficient ethosomal drug delivery system and enalapril meleate is used as model drug. The following conclusion are drown from the result and discussion described in the previous chapter. Liposomal formulation was also prepared by the thin film hydration method. The techniques used were simple and reproducible. The prepared ethosomes were spherical and discrete in shape. The size of vesicles were found to be in the range of 3.26-5.79 μm, 0.716-1.301 μm and 5.32 μm for unsonicated ethosomes, sonicated ethosomes and liposomes respectively. However ethosomes prepared by sonication method were more uniform and smaller in size, which is essential for skin permeation. While comparing the entrapment efficiency, ethosomes containing 30% w/w ethanol and prepared by sonication showed highest value with respect to all other formulation, so it is concluded ethosomes prepared by sonication and containing 30% w/w ethanol as the best formulation considering all other aspects. The highest value of transdermal flux for sonicated ethosomes containing 30% w/w ethanol is the indication of complete and rapid penetration through the skin may be because of tiny vesicular size.

Keywords: Composition of ethosomes, Method of preparation, Mechanism of penetration, Therapeutic applications etc.

Introduction
Continuous intravenous infusion is recognized as a superior mode of drug administration not only to bypass hepatic "first-pass" metabolism, but also to maintain a constant and prolonged drug level in the body. A closely monitored intravenous infusion can provide the advantages of both direct entry of drug into the systemic circulation and control of circulating drug levels. However, such mode of drug administration entails certain risks and, therefore, necessitates hospitalization of the patients and close medical supervision of administration. [1-5]
Recently, it is becoming evident that the benefits of intravenous drug infusion can be closely duplicated, without its hazards, by using the skin as the port of drug administration to provide continuous transdermal drug infusion into the systemic circulation. [6]
To provide continuous drug infusion through an intact skin, several transdermal therapeutic systems have been developed for topical application onto the intact skin surface to control the delivery of drug and its subsequent permeation through the skin tissue. It is exemplified by the development and marketing of scopolamine-releasing transdermal therapeutic system for 72-hr prophylaxis or treatment of motion-induced nausea, of nitroglycerin and isosorbide dinitrate-releasing trans-dermal therapeutic systems for once-a-day medication of angina pectoris, and of clonidine-releasing transdermal therapeutic system for weekly treatment of hypertension. The intensity of interests in the potential biomedical applications of transdermal controlled drug administration is demonstrated in the increasing research activities in a number of health care institutions in the development of various types of transdermal therapeutic systems for long term continuous infusion of therapeutic agents, including antihypertensive, anti-anginal, anti-histamine, anti-inflammatory, analgesic, anti-arthritic steroidal, and contraceptive drugs

Skin and drug permeation [7-10]
For understanding the concept of transdermal drug delivery systems, it is important to review the structural and biochemical features of human skin and those characteristics which contribute to the barrier function and the rate of drug access into the body via skin.

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The skin is one the most extensive organs of the human body covering an area of about 2m2 in an average human adult. The skin separates the underlying blood circulation network from the outside environment, serves as a barrier against physical, chemical and microbial attacks, acts as a thermostat in maintaining body temperature, protects against harmful ultraviolet rays of the sun and plays a role in the regulation of blood pressure. Anatomically, the skin has many histologic layers but in general, it is described in terms of three major tissue layers: the epidermis, the dermis and the hypodermis.

The epidermis results from an active epithelial basal cell population and is approximately 150 micrometers thick. It is the outermost layer of the skin and the process of differentiation results in migration of cells from the basal layer towards the skin surface. The end result of this process is the formation of a thin, stratified and extremely resilient layer at the skin surface. Below this layer are the other layers of the epidermis - the stratum lucidum, stratum granulosum, stratum spinosum and stratum germinativum. Together, these other layers constitute the viable epidermis.

The stratum corneum or the horny layer is the rate-limiting barrier that restricts the inward and outward movement of chemical substances. The interior of the cells is crisscrossed with densely packed bundles of keratin fibres. Due to this, the dry composition of the horny layer is 75-85% protein, most of which is the intracellular keratin and a part being associated with a network of cell membranes. The bulk of the remainder of the substance of the stratum corneum is a complicated mixture of lipids which lies between regions, the mass of intracellular protein and the intercellular lipoidal medium. The epidermis rests on the much thicker dermis. The dermis essentially consists of about 80% of protein in a matrix of muco-polysaccharide "ground substance". A rich bed of capillaries is encountered 20 m or so into the dermal field. Also contained within the dermis are lymphatics nerves and the epidermal appendages such as hair follicles, sebaceous glands and sweat glands. Excepting the soles of the feet, the palms of the hand, the red portion of the lips and associated with one or more sebaceous glands which are outgrowths of epithelial cells. The sweat gland are divided into the eccrine and apocrine types and are widely distributed over the surfaces of the body. The sweat glands serve to control body heat by secretion of a dilute salt solution.

Percutaneous Absorption [8-43]
Percutaneous absorption involves passive diffusion of substances through the skin. The mechanism of permeation can involve passage through the epidermis itself or diffusion through shunts, particularly those offered by the relatively widely distributed hair follicles and eccrine glands.

Transepidermal absorption [9-43]
The trans-epidermal pathway is principally responsible for diffusion across the skin. The main resistance encountered along this pathway arises in the stratum corneum. Permeation by the trans-epidermal route first involves partitioning into the stratum corneum. Diffusion then takes place across this tissue. The current popular belief is that most substances diffuse across the stratum corneum via the intercellular lipoidal route. However, there appears to be another microscopic path through the stratum corneum for extremely polar compounds and ions. When a permeating drug exits at the stratum corneum, it enters the wet cell mass of the epidermis and since the epidermis has no direct blood supply, the drug is forced to diffuse across it to reach the vasculature immediately beneath.

It is a permeable field that functions as a viscid watery regime to most penetrants. It appears that only ions and polar non-electrolytes found at the hydrophilic extreme and lipophilic non-electrolytes at the hydrophobic extreme have any real difficulty in passing through the viable field. The epidermal cell membranes are tightly joined and there is little to no intercellular space for ions and polar non-electrolyte molecules to diffusionally squeeze through.

Passage through the dermal region represents a final hurdle to systemic entry. Permeation through the dermis is through the interlocking channels of the ground substance. Since the viable epidermis and dermis lack major physicochemical distinction, they are generally considered as a single field of diffusion, except when penetrants of extreme polarity are involved, as the epidermis offers measurable resistance to such species.

Transfollicular (shunt pathway) absorption
The skin's appendages offer only secondary avenues for permeations. Sebaceous and eccrine glands are the only appendages which are seriously considered as shunts bypassing the stratum corneum since these are distributed over the entire body. Though eccrine glands are numerous, their orifices are tiny and add up to a miniscule fraction of the body's surface. Moreover, they are either evacuated or so profusely active that molecules cannot diffuse inwardly against the gland's output. For these reasons, they are not considered as a serious route for percutaneous absorption. However, the follicular route remains an important avenue for percutaneous absorption since the opening of the follicular pore, where the hair shaft exits the skin, is relatively large and sebum aids in diffusion of penetrants. Partitioning into sebum, followed by diffusion through the sebum to the depths of the epidermis, is the envisioned mechanism of permeation by this route. Vasculature subserving the hair follicle located in the dermis is the likely point of systemic entry. [11-44]

Clearance by local circulation
The earliest possible point of entry of drugs and chemicals into the systemic circulation is within the papillary plexus in the upper dermis. The process of percutaneous absorption is general, regarded as ending at this point. However, some molecules bypass the circulation and diffuse deeper in the dermis. [10]

Basic components of transdermal drug delivery systems [11-15]
The components of transdermal devices include:
1. Polymer matrix or matrices
2. Drug
3. The drug permeation enhancers
4. Other excipients

1. Polymer matrix
The polymer controls the release of the drug from the device. The following criteria should be satisfied for a polymer to be used in a transdermal system (Kydoineus & Berner, 1987):
1. Molecular weight, glass transition temperature and chemical functionality of the polymer should be such that the specific drug diffuses properly and gets released through it.
2. The polymer should be stable, non-reactive with the drug, easily manufactured and fabricated into the desired product; and inexpensive.
3. The polymer and its degradation products must be non-

toxic or non-antagonistic to the host.
4. The mechanical properties of the polymer should not deteriorate excessively when large amounts of active agent are incorporated into it.

Possible useful polymer for trans-dermal devices are:

Natural polymers
Cellulose derivatives, Zein, Gelatin, Shellac, Waxes, Proteins, Gums and their derivatives, Natural rubber, starch etc.

Synthetic elastomers
Polybutadiene, Hydrin rubber, Polysiloxane, Silicone rubber, Nitrile, Acrylonitrile, Butyl rubber, Styrenebutadiene rubber, Neoprene etc.

Synthetic polymers
Polyvinyl alcohol, Poly vinyl chloride, Polyethylene, Polypropylene, Polyacrylate, Polyamide, Polyurea, Polyvinylpyrrolidone, Polymethylmethacrylate, etc.

2. Drug
For successfully developing a trans-dermal drug delivery system, the drug should be chosen with great care. The following are some of the desirable properties of a drug for trans-dermal delivery

Physicochemical properties
1. The drug should have a molecular weight less than approximately 1000 daltons.
2. The drug should have affinity for both lipophilic and hydrophilic phase. Extreme portioning characteristics are not conducive to successful drug delivery via the skin.
3. The drug should have a low melting point.

Biological properties
1. The drug should be potent with a daily dose of the order of a few mg/day.
2. The half-life (t1/2) of the drug should be short.
3. The drug must not induce a cutaneous irritant or allergic response.
4. Drugs which degrade in the GI tract or are inactivated by hepatic first pass effect are suitable candidates for trans-dermal delivery.
5. Tolerance to the drug must not develop under the near zero-order release profile of trans-dermal delivery.
6. Drugs which have to be administered for a long period of time or which cause adverse effects to non-target tissues can also be formulated for trans-dermal delivery.

3. Permeation enhancers
These are compounds which promote skin permeability by altering the skin as a Barrier to the flux of a desired penetrant
The flux, J, of drugs across the skin can be written as:
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 species; x is the spatial coordinate.
Enhancement of flux across membranes reduces to considerations of:
• Thermodynamics (lattice energies, distribution coefficients)
• Molecular size and shape
• Reducing the energy required to make a molecular hole in the membrane

These may conveniently be classified under the following main headings:

Solvents
These compounds increase penetration possibly by swelling the polar pathway. Examples include water alcohols – methanol and ethanol; alkyl methyl sulfoxides – dimethyl sulfoxide, alkyl homologs of methyl sulfoxide, dimethyl acetamide and dimethyl formamide; pyrrolidones – 2 pyrrolidone, N-methyil, 2-pyrrolidone; laurocapram (Azone) miscellaneous solvents – propylene glycol, glycerol, silicone fluids, isopropyl palmitate.

Surfactants [16-22]
These compounds are proposed to enhance polar pathway transport, especially of hydrophilic drugs. The ability of a surfactant to alter penetration is a function of the polar head group and the hydrocarbon chain length. These compounds are, however, skin irritants, therefore, a balance between penetration enhancement and irritation has to be considered. Anionic surfactants can penetrate and interact strongly with the skin. Once these surfactants have penetrated the skin, they can induce large alterations. Cationic surfactants are reportedly more irritant than the anionic surfactants, the nonionics have long been recognized as those with the least potential for irritation and have been widely studied. Examples of commonly used surfactants are:

Anionic surfactants
Dioctyl sulphosuccinate, Sodium lauryl sulphate, Decodecylmethyl sulphoxide etc.

Nonionic surfactants
Pluronic F127, Pluronic F68, etc

Bile salts
These systems apparently open up the heterogeneous multi-laminate pathway as well as the continuous pathways. Examples include: propylene glycol-oleic acid and 1, 4-butane diol-linoleic acid

Miscellaneous chemicals
These include urea, a hydrating and keratolytic agent; N, N-dimethyl-m-tolumide; calcium thioglycolate; anti-cholinergic agents.

4. Other excipients
Adhesives
The fastening of all trans-dermal devices to the skin has so far been done by using a pressure sensitive adhesive. The pressure sensitive adhesive can be positioned on the face of the device or in the back of the device and extending peripherally. Both adhesive systems should fulfill the following criteria
1. Should not irritate or sensitize the skin or cause an imbalance in the normal skin flora during its contact time with the skin.
2. Should adhere to the skin aggressively during the dosing interval without its position being disturbed by activities such as bathing, exercise etc.
3. Should be easily removed.
4. Should not leave an unwashable residue on the skin
5. Should have excellent (intimate) contact with the skin at macroscopic and microscopic level.

The face adhesive system should also fulfill the following criteria.
1. Physical and chemical compatibility with the drug, excipients and enhancers of the device of which it is a part.
2. Permeation of drug should not be affected.
3. The delivery of simple or blended permeation enhancers should not be affected.

Backing membrane:- Backing membranes are flexible and they provide a good bond to the drug reservoir, prevent drug from leaving the dosage form through the top and accept printing. It is impermeable. Substance that protects the product during use on the skin e.g. metallic plastic laminate, plastic backing with absorbent pad and occlusive base plate, adhesive foam pad with occlusive base plate etc.

Approaches used in development of trans-dermal drug delivery systems [16-25]
Four different approaches have been utilized to obtain trans-dermal drug delivery systems:

Membrane permeation – controlled systems
In this type of system, the drug reservoir is totally encapsulated in a shallow compartment moulded from a drug-impermeable metallic plastic laminate and a rate controlling polymeric membrane which may be micro-porous or non-porous e.g., ethylene vinyl acetate (EVA) copolymer, with a defined drug permeability property. A cross-sectional view of this system is shown in figure. The drug molecules are permitted to release only through the rate-controlling membrane. In the drug reservoir compartment, the drug solids are either dispersed in a solid polymer matrix or suspended in an unleachable, viscous liquid medium such as silicone fluid to form a paste like suspension. A thin layer of drug compatible, hypoallergenic adhesive polymer e.g. silicone or polyacrylate adhesive may be applied to the external surface of the rate controlling membrane to achieve an intimate contact of the trans-dermal system and the skin surface the rate of drug release from this type of trans-dermal drug delivery system can be tailored by varying the polymer composition, permeability coefficient and thickness of the rate limiting membrane and adhesive

Adhesive dispersion type systems
This is a simplified form of the membrane permeation controlled system. As represented in figure the drug reservoir is formulated by directly dispersing the drug in an adhesive polymer eg. Poly (isobutylene) or poly (acrylate) adhesive and then spreading the medicated adhesive, by solvent casting or hot melt, on to the flat sheet of drug impermeable metallic plastic backing to form a thin drug reservoir layer.
On top of the drug reservoir layer, thin layers of non-medicated, rate controlling adhesive polymer of a specific permeability and constant thickness are applied to produce an adhesive diffusion-controlled delivery system. An example of this type of system is isosorbide dinitrate releasing trans-dermal therapeutic system for once a day medication of angina pectoris. This adhesive diffusion controlled drug delivery system is also applicable to the trans-dermal controlled administration of verapamil.

Matrix diffusion controlled systems
In this approach, the drug reservoir is prepared by homogeneously dispersing drug particles in a hydrophilic or lipophilic polymer matrix. The resultant medicated polymer is then moulded into a medicated disc with a defined surface area and controlled thickness. The dispersion of drug particles in the polymer matrix can be accomplished by either homogeneously mixing the finely ground drug particles with a liquid polymer or a highly viscous base polymer followed by cross linking of the polymer chains or homogeneously blending drug solids with a rubbery polymer at an elevated temperature. The drug reservoir can also be formed by dissolving the drug and polymer in a common solvent followed by solvent evaporation in a mould at an elevated temperature and/or under vacuum. This drug reservoir containing polymer disc is then pasted on to an occlusive polymer is then spread along the circumference to form a strip of adhesive rim around the medicated disc.
This type of trans-dermal system is exemplified by the nitroglycerin releasing trans-dermal therapeutic systems. These are designed to be applied to the intact skin to provide a continuous trans-dermal infusion of nitroglycerin at a daily dose of 0.5 mg/cm2 for therapy of angina pectoris. It is a modified version of NitroDur in which the drug is dispersed in an acrylic based polymer adhesive with a resinous cross linking agent which results in a much thinner and more elegant patch. Patent disclosures have also been filed for applying this drug delivery system for trans-dermal controlled administration of estradiol discetyate and verapamil.

Micro-reservoir type or micro-sealed dissolution controlled system [26-30]
This can be considered a combination of the reservoir and matrix diffusion type drug delivery systems. Here the drug reservoir is formed by first suspending the drug solids in an aqueous solution of a water soluble liquid polymer and then dispersing the drug suspension homogeneously in a lipophilic polymer viz. silicone elastomers by high energy dispersion technique to form several discrete, unleachable microscopic spheres of drug reservoirs. The quick stabilization of this thermodynamically unstable dispersion is accomplished by immediately cross linking the polymer chains in situ which produces a medicated polymer disc with a constant surface area and a fixed thickness. Depending upon the physiochemical property of the drug and the desired rate of drug release, the device can be further coated with a layer of biocompatible polymer to modify the mechanism and rate of drug release. A trans-dermal therapeutic system is produced by positioning the medicated disc at the centre and surrounding it with an adhesive rim.
In the past decade, topical delivery of drugs by liposomal formulations has evoked considerable interest. Recently, it has value as carriers for transdermal drug delivery, because they do not deeply penetrate skin but remain confined to upper layers of the stratum corneum. Confocal microscopy studies have shown that intact liposomes are not able to penetrate into the granular layers of the epidermis. Recent approaches to modulating drug delivery through skin have resulted in the design of a novel vesicular carrier, ethosomes. Ethosomes were developed by Touitou et al as additional novel lipid carriers composed of ethanol, phospholipid, and water. Ethosomes were reported to improve the skin delivery of various drugs]. Ethanol is known as an efficient permeation enhancer that is believed to act by affecting the intercellular region of the stratum corneum. Its inclusion in liposomes to form ethosomes has already been investigated. Transdermal drug delivery systems offer many advantages over their

corresponding classical oral, injectable, and inhaler systems, including (1) improving the systemic bioavailability of drugs because the first-pass metabolism by the liver and digestive system is avoided, and achieving a controlled constant drug delivery profile, which is especially important for those suffering from nocturnal attacks and need a longer duration of therapeutic action from a single application. Despite the many advantages of the skin as a site of drug delivery, only few drugs are currently in the market for transdermal delivery system. The primary reason for this is the low permeability of drugs in the stratum corneum, as stratum corneum (outermost layer) acts as the main barrier in the skin. In general the highly organized crystalline lipid lamellae play an essential role in the barrier properties of the stratum corneum. 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 or to increase the driving force for permeation of drugs across this skin barrier. The vesicles have been well known for their important in cellular communication and particle transportation for many years. Researchers have understood the properties of vesicle structures for use in better drug delivery within their cavities that would allow for tagging the vesicle for cell specificity. 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. High patient compliance as it is administrated in semisolid form (gel or cream) and various application in Pharmaceutical, Veterinary, Cosmetic field.

Composition of ethosomes [31-32]
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), phosphatidylglycerol (PPG), phosphatidylinositol (PI), hydrogenated PC, alcohol (ethanol or isopropyl alcohol), water and propylene glycol (or other glycols). Such a composition enables delivery of high concentration of active ingredients through skin. Drug delivery can be modulated by altering alcohol: water or alcohol-polyol: water ratio. Some preferred phospholipids are soya phospholipids such as Phospholipon 90 (PL-90). It is usually employed in a range of 0.5-10% w/w. Cholesterol at concentrations ranging between 0.1-1% can also be added to the preparation. Examples of alcohols, which can be used, include ethanol and isopropyl alcohol. Among glycols, propylene glycol and Transcutol are generally used. In addition, non-ionic surfactants (PEG-alkyl ethers) can be combined with the phospholipids in these 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% (Table 1).

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 permeants, 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 corneum, facilitating improved drug partitioning. Ethanol has been employed in vitro to enhance transdermal delivery of levonorgesterol, hydrocortisone and 5- fluorouracil across rodent skin, and estradiol across human skin in vivo. Megrab and collaborators noted that the enhancement effect of ethanol was concentration dependent. The authors investigated the effect of ethanol on skin water content and concluded that formulations containing high levels of alcohol were capable of dehydrating the skin, which may explain the concentration dependant action of ethanol.

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.
Touitou discovered and investigated 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 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. The versatility of ethosomes for systemic delivery is evident from the reports of enhanced delivery of quite a few drugs like acyclovir, minoxidil, triphexyphenidyl, testosterone, cannabidol and zidovudine.

Table 1: Different Additives 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 Transcutol RTM	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 Ð934	As a gel former

Method of preparation [30-35]

There are two methods which can be used for formulation and preparation of ethosomes. Both of these are very simple and convenient and do not involve any sofisticated instrument or complicated process.

Methods are
1. Hot method.
2. Cold method

Hot Method

In this method phospholipid is dispersed in water by heating in a water bath at 400 °C 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.

Cold Method

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 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 de-creased to desire extendusing sonication or extrusion method. Finally, the formulation is stored under refrigeration.

Mechanism of penetration [35-37]

The mechanism of penetration of the ethosomes in and through the skin is not yet completely elucidated. Two simultaneous mechanisms of action have been proposed: ethanol has a fluidization effect on the ethosomal lipids and ethanol has a fluidization effect on the *stratum corneum* lipids. (Figure 2) Because of the use of ethanol in the preparation of the ethosomes, the deformability of the prepared vesicles is increasing. Besides, the high alcohol content is expected to partially extract the *stratum corneum* lipids. These processes are responsible for increasing inter and intracellular permeability of ethosomes. The ultra-deformable vesicles can forge paths in the disordered *stratum corneum* and finally release drug in the deeper layers of the skin. Therefore, a path through the skin can be expected to result, permitting the fusion of ethosomes with the cells from the deepest skin layers. Flow chart of penetration of ethosomes is as depicted in figure 1.
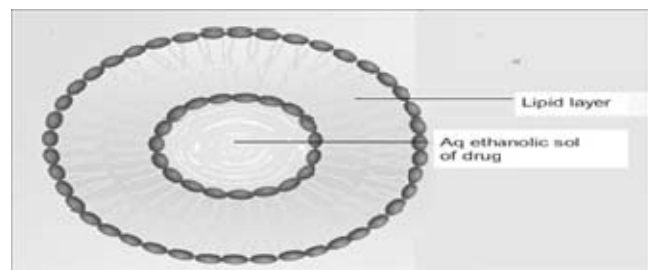

Fig 1: Composition of Ethosome

Mechanism of action of Ethosomes
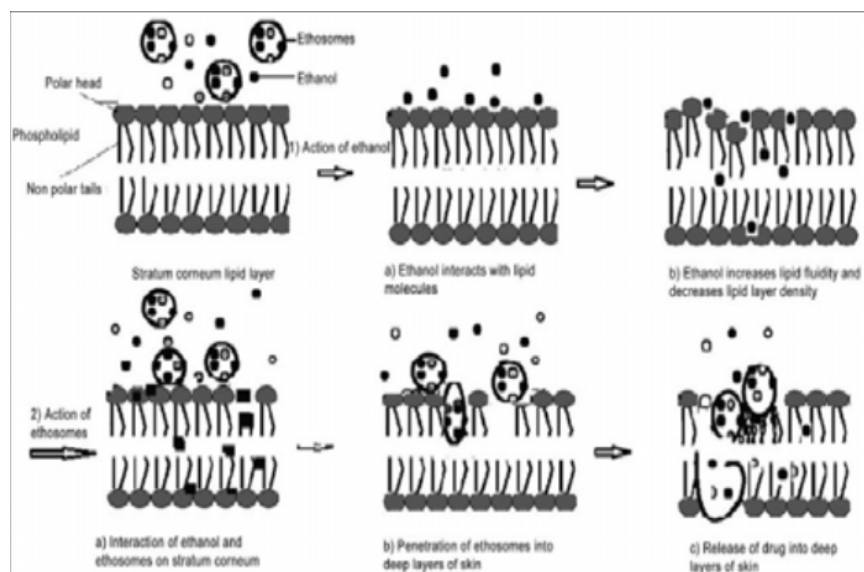
Methods for the Characterization of Ethosomal Formulation

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	^{31}P 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

Flow chart showing action of Ethosome
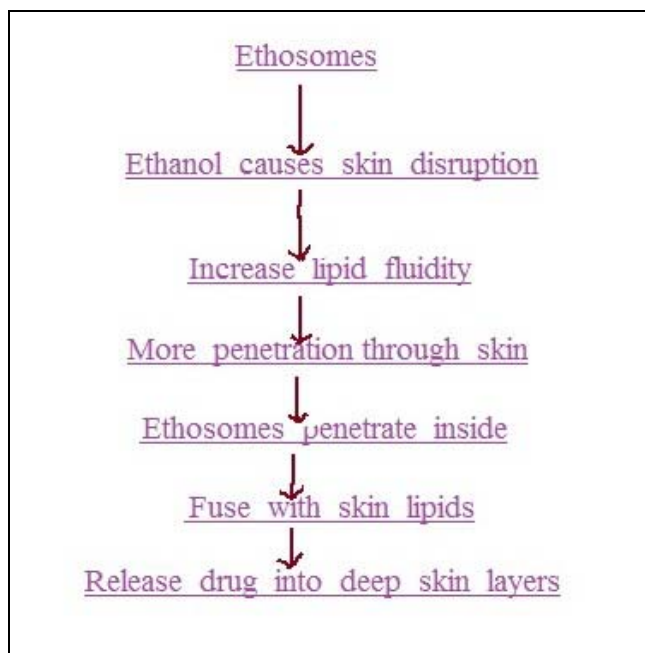
Characterisation of ethosomal system
Visualization of Vesicles by TEM and by SEM
Vesicular shape of the ethosome preparations is assessed by using. Transmission Electron Microscope (TEM). Samples are dried on carbon-coated grid and negatively stained with aqueous solution of phosphotungstic acid. After drying the specimen is viewed under the microscope at 10–100 k-fold enlargements at an accelerating voltage of 100 Kv. The size and shape of the vesicles are observed in the Scanning Electron Microscopy (SEM). One drop of ethosomal suspension is mounted on a clear glass stub. It is then air dried and gold coated using sodium aurothiomalate to visualize under scanning electron microscope at 10,000 magnifications.

Size Distribution and Vesicular Size:-
The size distribution of ethosomal preparation can be measured in a multimodal mode, by Dynamic Light Scattering (DLS) technique using a computerized Malvern Autosizer 5002 inspection system. For vesicle size measurement, ethosomal preparation is mixed with the medium.

Entrapment Efficiency:-
Entrapment efficiency of ethosomal vesicles can be determined by centrifugation method. The vesicles were separated in a high speed cooling centrifuge at 20,000 rpm for 90 minutes in the temperature maintained at 4 °C [15]. The sediment and supernatant liquids were separated amount of drug in the sediment can be determined by lysing the vesicles using methanol. From this, the entrapment efficiency can be determined by the following equation,

Entrapment Efficiency = DE ⁄ DT x 100
Where,
DE - Amount of drug in the ethosomal sediment
DT - Theoretical amount of drug used to prepare the formulation (equal to amount of drug in supernatant liquid and in the sediment)

Transition Temperature
The Transition temperature (T) of vesicular lipids can be measured in duplicate by DSC in an aluminum pan at a heating rate of 10 °C per min, under a constant nitrogen stream.

Confocal Scanning Laser Microscopy (CSLM):-
CSLM can be used to investigate depth and mechanism of skin penetration of ethosomal preparation. The skin thickness can be optically scanned at different increments through the z axis of a confocal laser scanning microscope.

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

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

Phospholipid-ethanol interaction
The Phospholipid-ethanol interaction was studied by using Proton decoupled 31P-NMR and Differential Scanning calorimetry.

Degree of deformability and Turbidity
The Degree of deformability of the ethosomal preparation can be performed by Extrusion Method and the turbidity of the preparation can be performed by using Nephalometer.

In vitro drug release study and Drug Deposition study
In vitro drug release study and Drug Deposition of ethosomal preparation can be performed by Franz diffusion cell with artificial or biological membrane, Dialysis bag diffusion.

Advantages of ethosomal drug delivery
In comparison to other transdermal & dermal delivery systems
- Enhanced permeation of drug through skin for transdermal drug delivery.
- Delivery of large molecules (peptides, protein molecules)

is possible.
- It contains non-toxic raw material in formulation.
- High patient compliance-The ethosomal drug is administrated in semisolid form (gel or cream) hence producing high patient compliance.
- The Ethosomal system is passive, non-invasive and is available for immediate commercialization.
- Ethosomal drug delivery system can be applied widely in Pharmaceutical, Veterinary, Cosmetic fields.
- Simple method for drug delivery in comparison to iontophoresis and phosphophoresis and other complicated methods.

Therapeutic applications
Ethosomes, the high ethanol containing vesicles are able to penetrate the deeper layers of the skin and hence appear to be vesicles of choice for transdermal drug delivery of hydrophilic and impermeable drugs through the skin. Various therapeutic applications of ethosomes are as shown in table 2.

Table 2. Application of Ethosomes as a Drug Carrier

Drug	Results
NSAIDS (Diclofenac)	Sel↓ective 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↓ Reduced drug toxicity↓ Affected the normal histology of skin ↓
Azelaic acid	Prolong drug release ↓
Ammonium glycyrrhizinate	Improved dermal deposition exhibiting sustained release↓ Improved biological anti-inflammatory activity ↓

Drug entrapment efficiency
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. Godin and Touitou 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. Efficient loading of both hydrophobic and hydrophilic drugs was confirmed by using hydrophilic 6-carboxyfluorescein and hydrophobic Rhodamine 123 fluorescence markers. The ability of ethosomes to efficiently entrap lipophilic and hydrophilic drugs can be explained by the high degree of lamellarity and by the presence of ethanol in the vesicles. In addition, ethosomal formulations possess greater entrapment capability than liposomes. Dayan and Touitou have shown that entrapment efficiency of trihexyphenidyl hydrochloride increased from 36% for liposomes to 75% for ethosomes.

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.
No ultrastructural changes were observed in cell layers below the stratum corneum indicating that rigid liposomal formulation did not induce any changes in the ultrastructure of stratum corneum and accumulated only in the top layer of the skin. These results illustrated that liquid state vesicles might act not only in superficial stratum corneum layers, but may also induce liquid perturbations in deeper layers of the SC, while gel state vesicles interacted only with the outermost layers in the SC. This might explain the difference in drug permeation enhancement between ethosomal and conventional liposomal formulation. In addition, fusion of conventional liposomal vesicles on top of the stratum corneum might also act as additional barrier for diffusion of drugs and therefore inhibit skin permeation.
To support the result of TEM study Jain et al. 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 ultrastructure of viable tissue (epidermis or dermis) could be observed after treatment with conventional liposomal formulation.
Fluorescence photomicrographs of the skin after a 6 hr application of Rhodamine 123 (lipophilic probe) or 6-CF (hydrophilic probe) loaded liposomal and ethosomal formulation.
Penetration from conventional liposomes was only to upper layer of skin (stratum corneum). Deep penetration from alcohol free liposomes was almost negligible. In contrast enhanced delivery of 6-CF and Rhodamine 123 in terms of depth and quantity (dermis layer) was observed using the ethosomal carrier. These results supported the results of skin permeation studies and showed the feasibility of using ethosomal formulation for delivering drugs into the deeper layers of skin or across the skin.
Touitou et al. m. 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 reported

better skin permeation of fluoreceine isothiocyanate-bacitracin ethosomal formulation to deeper layer of skin as determined by CLSM (Table [24] 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 hydroalcoholic 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 3).

Table 3. Vesicle Skin Interaction with Rat Skin

S. No.	Formulation	Physical state	Adsorption	Structural changes	
				Intercellular	Intracellular
1	Rigid Liposomes	Gel	+	-	-
2	Ethosomal formulation	Liquid	-	+	+
3	Plain drug	Liquid	ND	-	-

Summary of the interaction between the different formulations and the stratum corneum.
+ = Frequently observed   - = Not observed   ND= Not determined

Proposed mechanism of skin permeation of ethosomes
The stratum corneu showed the schematic representation of mechanism of skin permeation of ethosomes. The stratum corneum lipid multilayers at physiological temperature are densely packed and highly conformationally 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.

4.6 Different Studies Related to the Application of Ethosomes as a Carrier System [36-38]
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

Pilosebaceous targeting
Hair follicles and sebaceous glands are increasingly being recognized as potentially significant elements in the percutaneous drug delivery. Interest in pilosebaceous units has been directed towards their use as depots for localized therapy, particularly for the treatment of follicle-related disorders such as acne or alopecia. Furthermore, considerable attention has also been focused on exploiting the follicles as transport shunts for systemic drug delivery. With the purpose of pilosebaceous targeting, Maiden et al. prepared and evaluated minoxidil ethosomal formulation. Minoxidil is a lipid-soluble drug used topically on the scalp for the treatment of baldness. Conventional topical formulation has very poor skin permeation and retention properties. It was found that the quantity of minoxidil accumulated into nude mice skin after application of its ethosomal formulation was 2.0, 7.0 and 5.0 fold higher as compared to ethanolic phospholipids dispersion, hydroetanolic solution and ethanolic solution of drug each containing 0.5% of the drug. These results showed the possibility of using ethosomes for pilosebaceous targeting of minoxidil to achieve its better clinical efficacy.

Transdermal delivery of hormones [38-40]
Oral administration of hormones is associated with problems like high first pass metabolism, low oral bioavailability and several dose dependent side effects. In addition, along with these side effects oral hormonal preparations relying highly on patient compliance. The risk of failure of treatment is known to increase with each pill missed.
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. The amount of drug deposited was significantly (p <g at the end of 7 hr for Testosome and Testoderm¨, respectively). The AUC and Cmax of testosterone significantly improved after application of Testosome as compared to Testoderm. Hence, both 4.05 18.14 and 18.32 0.05) higher in case of ethosomal formulation (130.76 in vitro and in vivo studies demonstrated improved skin permeation and bioavailability of testosterone from ethosomal formulation. This group in their further study designs the testosterone nonpatch formulation to reduce the area of application [101]. They have found that with ethosomal testosterone formulation area of application required to produce the effective plasma concentration was 10 times less than required by commercially gel (AndroGel¨) formulation.

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. THP has a short biological half-life (3hr) and its oral administration is difficult due to motor disorders and neurogical manifestations associated with parkinsonian syndrome. THP ethosomal formulation when visualized under transmission and scanning electron microscope found to consist of small, phospholipid vesicles. The value of transdermal flux of THP through nude mouse skin from ethosomes was 87, 51 and 4.5-times higher than that from liposome, phosphate buffer and hydroethanolic solution, respectively. The quantity of THP remaining in skin at the end of 18 hr was significantly higher after application of ethosomes than after application of liposome or hydroethanolic solution (control). These results indicated better skin permeation potential of ethosomal-THP formulation and its use for better management of Parkinson disease.

Transcellular delivery [41-42]

Touitou et al. M from ethosomes, hydroethanolic solution and liposomes, respectively. Maximum fluorescence intensities measured for RR delivered from ethosomes, hydroethanolic solution and liposomes were 150, 40 and 20 arbitrary units (AU), respectively. Fibroblasts viability tests showed that the ethosomal carrier was not toxic to the cultured cells. investigated the efficiency of transcellular delivery of ethosomes in Swiss albino mice 3T3 fibroblast. The probes chosen for study were D-289 [4-(4-(diethylamino) styryl-N-methylpyridinum iodide], rhodamine red [dihexadecanoylglycerophosphoethanolamine] and fluorescent phosphatidylcholine. The penetration of these fluorescent probes into fibroblasts and nude mice skin was examined by CLSM (Confocal Laser Scanning Microscopy) and FACS (Fluorescent Activated Cell Sorting) techniques. CLSM micrograph showed that significant quantity of probe was penetrated into the cells when incorporated into ethosomes as evident from the high intensity of fluorescence. In comparison, incorporation into hydroethanolic solution or classic liposomes produced almost negligible fluorescence. The intracellular presence of each of the three probes tested was evident after 3 min. of incubation. Enhanced delivery of the hydrophilic calcein and lipophilic rhodamine red (RR) probe to nude mice skin was also observed when incorporated into ethosomes. Calcein penetrated the skin to a depth of 160, 80 and 60 Touitou et al. in their further 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 be an attractive clinical alternative for anti-HIV therapy.

Topical delivery of DNA [40-42]

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. 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 48 hr. 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-driven transfecting 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 permeation ability of ethosomes opens the possibility of using these dosage forms for delivery of immunizing agents

Delivery of anti-arthritis drug [42]

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. Its oral administration is associated with a number of problems like low bioavailability, first pass metabolism and GIT degradation. To overcome the above mention problem Lodzki et al. prepared CBD-ethosomal formulation for transdermal delivery. Results of the skin deposition study showed significant accumulation of CBD in skin and underlying muscles after application of CBD-ethosomal formulation to the abdomen of ICR mice Plasma concentration study showed that steady state level was reached in 24 hr and maintained through 72 hr. Significantly increased in biological anti-inflammatory activity of CBD-ethosomal formulation was observed when tested by carrageenan induced rat paw edema model. Finally, it was concluded that encapsulation of CBD in ethosomes significantly increased its skin permeation, accumulation and hence it's biological activity.

Delivery of antibiotics

Topical delivery of antibiotics is a better choice for increasing the therapeutic efficacy of these agents. Conventional oral therapy causes several allergic reactions along with several side effects. Conventional external preparations possess low permeability to deep skin layers and subdermal tissues. Ethosomes can circumvent this problem by delivering sufficient quantity of antibiotic into deeper layers of skin. Ethosomes penetrate rapidly through the epidermis and bring appreciable amount of drugs into the deeper layer of skin and suppress infection at their root. With this purpose in mind Godin and Touitou prepared bacitracin and erythromycin loaded ethosomal formulation for dermal and intracellular delivery. CLSM experiments revealed that ethosomes facilitated the co-penetration of antibiotic and phospholipid into cultured 3T3 Swiss albino mice fibroblasts. The data obtained by CLSM experiment was confirmed by FACS techniques and it was found that ethosomes penetrated the cellular membrane and released the entrapped drug molecules within the cells. The results of this study showed that the ethosomal formulation of antibiotic could be highly efficient and would overcome the problems associated with conventional therapy.

Conclusion

All the formulation of ethosomes showed a zero order release forin-vitro release studies. Though the ethosomes are rapidly penetrated through the skin, there is variation between the sonicated and unsonicated products. Stability studies carried out for a period of 8 weeks showed no changes in the charecterisation of ethosomes and further the loss of drug is not more than 3%. When effect of sonication was compared on ethosomal formulation, sonicated formulations are possessed better or suitable characterization (smaller size, uniform size distribution, highest entrapment efficiency and higher transdermal flux) as compared with unsonicated formulation. From the above observation it can be concluded sonication is essential tool for the preparation of ethosomes. An extensive investigation is needed with reference to depth of penetration into the skin, determination of zeta potential and confirmation of configuration of phospholipid in lipid bilayer. There is a need to develop suitable transdermal formulation by using prepared ethosomes for transdermal application and for commercial exploitation. Thus, the specific objective listed in the introduction chapter of this thesis were achieved namely design, characterization and release studies of enalapril maleate ethosomes. Certainly these finding can be applied for transdermal drug delivery of enalapril maleate for treatment of hypertension. Further, these finding may help the industry for development and scaling up a new formulation.

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