Review Article

Ethosomes as Novel Drug Delivery System: A Review

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ABSTRACT

Oral route is the normal route of drug delivery, which has many benefits such as easy delivery but has drawbacks such as low bioavailability and a propensity to produce rapid spikes in blood levels, thereby being a requirement for higher dose or repeated dosing, which is difficult for the patient and also high cost. With all these disadvantages in mind, there is a need for novel drug delivery technology with increased therapeutic efficacy and safety with controlled delivery to minimize the size and number of doses. This can be achieved by transdermal delivery which possesses several advantages such as avoids first-pass metabolism, eliminates gastrointestinal irritation reduces frequency of dosing, and rapid termination of drug action. Skin serves as a significant target and a key barrier to the delivery of topical drugs. The major obstacle of this system is the low diffusion rate of drugs across the stratum corneum. Ethosomes are non invasive delivery enable drugs to reach deep skin layers and to the systemic circulation. Ethosomes are flexible, malleable vesicles that are designed to improve active agent delivery. Ethosomes are able to encapsulate and distribute extremely lipophilic molecules through the skin, as well as cationic drugs, due to their unique structure. Ethosomal systems are novel vesicular lipid carriers, which have a relatively high ethanol content. Such nanocarriers are specifically developed for the efficient delivery of therapeutic agents in deep skin layers and through the skin with various physicochemical properties. In this article reviews various aspect of ethosomes including their advantages, disadvantages, ethosomal system types, composition of ethosomes, mechanism of penetration, physicochemical characterization, evaluation & applications of ethosomes.

Keywords: Ethosomes, penetration enhancers, phospholipids, skin interaction, ethanol.

INTRODUCTION

The skin is the largest and most easily accessible organ of the body; it serves as a potential route of drug administration for systemic effects. The skin is an external multilayered organ that functions as a protective tissue and as a permeability barrier, preventing penetration of foreign molecules from the exterior environment. Represents the most resistant barrier to drug penetration through the skin, which restricts drug bioavailability in transdermal form. Unique carriers are therefore required to overcome the natural skin barrier to deliver drug molecules with various physicochemical properties to the systemic circulation. Transdermal drug delivery systems provide many benefits, such as preventing first pass absorption by the liver, managed drug delivery, decreased dose duration, and increased patient compliance, because they are non-invasive and self-administerable. The body's skin barrier against influences from the environment is essentially formed by its uppermost layer, the stratum corneum (SC). The SC is the final result of epidermal differentiation and is approximately 10 – 20 µm in size, and is inactive metabolically. It consists of 10 - 25 layers of dead, elongated, entirely keratinized corneocytes, trapped in a lipid bilayer matrix. This structure is called structure of the "Brick and Mortar." The extracellular lipid contains predominant crystalline phase and liquid lipid phase subpopulation. Such substances must also move through the SC lipid regions when adding substances onto the skin in order to enter the underlying viable epidermis. Therefore, the lipid matrix creates the skin's principal barrier. Lipid dosage types for transdermal delivery are mainly Liposomes and their derivatives such as transfersomes, niosomes and ethosomes. Standard Liposomes are capable of creating large drug reservoirs in the skin's surface strata without transmission to its deeper layers. More recently, vesicles which are able to be reach the SC barrier facilitating delivery of actives to the site of their action in the deep skin strata and systemic circulation have been designed. The transfersomes and niosomes are mainly transported in the pores between keratinocytes and the liposomes are mainly transported through hair follicles. Ethosomes, by their structure, mode of application and mechanism of action are different from classic liposomes, transfersomes and other lipid dispersions. Ethosomes have demonstrated excellent efficacy in percutaneous drug delivery, as a lipid carrier. They also have superior pharmaceutical properties, including room temperature stability, high trap performance, and enhanced compatibility with the SC, thus facilitating the penetration of both hydrophilic and lipophilic drugs through the stratum corneum (SC) into the skin's deep layers more effectively than typical liposoms.

ethosomes contain higher concentrations of ethanol and lipids, it is important to understand its effects on the skin. It has been documented that the presence of ethanol in the formulation has allowed for drug solubilization and has created deformable lipid structures that could easily pass between skin corneocytes, resulting in drug skin retention and improvement of permeation. The role of ethosomes supporting transdermal permeation and the effect on skin is not well understood though. The key techniques used to research the transdermal process currently include Attenuated total Reflectance, Fourier Transform Infrared Spectroscopy (ATR-FTIR), Confocal Laser Scanning Microscopy, Differential Scanning Calorimeter (DSC), Raman, Scanning Electronic Microscopy (SEM), Transmission Electron Microscopy (TEM), X-ray Photoelectron Spectroscopy (XPS) and Electron Spin Resonance (ESR). These techniques facilitated transdermal System study. Nonetheless, these methods were not used extensively for the study of ethosomal transdermal processes, except for fluorescent labeling and ATR-FTIR. Ethosomes are structurally fragile nanocarrier structures, with a high content of ethanol, phospholipids, and water in them. Ethosomes may contain 2%–5% content of phospholipids and 20%–40% concentration of ethanol. Ethosomes skin penetration potential is higher than liposomes, due to ethanol's ability to fluidize various intercellular lipids found in the skin's stratum corneum. It has been reported that as the amount of ethanol increases size of ethosomes decreases by keeping concentration of phospholipids constant. Nearness of ethanol in ethosome additionally gives a negative charge to its surface improving its colloidal security. Be that as it may, ethosomes show high spillage of hydrophilic/ionized medications contrasted with liposomes because of disturbance of close pressing of phospholipid bilayer by the nearness of high ethanol sum. Ethosomes having convergence of ethanol over 30% reason extreme arrival of captured material and disturbance of skin. Ethosomes are phospholipids-based elastic nanovesicles having high content of ethanol (20%-45%). Ethanol is known as a productive pervasion enhancer and has been accounted for to be included the vesicular framework to set up the flexible nano-vesicles. Ethosomes were created as novel lipid transporters made out of ethanol, phospholipids and water and to improve the conveyance of different medications to the skin. It empowers drugs to arrive at the profound skin layers or potentially fundamental flow. Because of high substance of ethanol, the lipid film is stuffed less firmly in correlation with traditional vesicles, however it has comparable security. Forth delivery of diverse group of proteins and peptides molecules ethosomes are preferable. Drug is delivered in the form of gel, a cream for patient convenience, by ethosomes. Ethanol is known as an efficient permeation enhancer. However, due to the inter digitation effect of ethanol on lipid bilayers, it was commonly believed that vesicles cannot coexist with high concentrations of ethanol. At present ethanol can only be contained in the liposome formulations at very low concentrations, if at all. Ethosomal systems contain soft phospholipid vesicles in a hydro ethanolic milieu. The system is able to interfere with skin barrier and penetrate the SC lipid bilayers allowing for enhanced delivery of drugs by passive transport to the low skin strata and transdermally. Ethosomes are another form of flexible liposome, with alcohol incorporated into the lipid bilayer to give the structure their flexibility. Hydrophilic, lipophilic or amphiphilic drugs can be incorporated in ethosomes and these are able to reach the deeper skin layers and the systemic circulation. Ethosomal systems differ from liposomes as they contain fairly high concentrations of ethanol, in addition to phospholipids and water. Since then new generations of ethosomal systems have been developed in an effort to improve vesicular characteristics and skin permeation by incorporating certain compounds to the basic ethosomal formula. Structure of ethosomes is shown in fig 1.

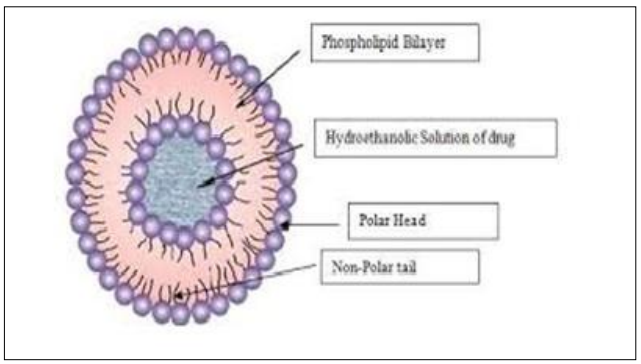


Figure 1: Structure of ethosomes

Advantages of ethosomes:

1. Ethosome enhance permeation of drugs through skin for dermal, transdermal and intracellular delivery.
2. Deliver various molecules with different physicochemical properties, hydrophilic and lipophilic molecules, peptides, proteins and other macromolecules.
3. The components of the ethosomes are generally recognized as safe (GRAS), non-toxic and approved for pharmaceutical and cosmetic use.
4. Low risk profile- Ethosome structure has no large-scale drug development risk as the ethosome feature toxicology profiles are well established in the scientific literature.
5. The ethosomal system is passive and non-invasive, and is suitable for immediate marketing.
6. Ethosomal drug delivery system can be applied widely in Pharmaceutical, Biotechnology, Veterinary, Cosmetic & Nutraceutical fields.
7. High patient compliance: The ethosomal drug is delivered in a semi-solid form (gel or cream) with high patient compliance ensuing.

8. Simple method for drug delivery in comparison to Iontophoresis and sonophoresis and other complicated methods.
9. Ease of industrial scale-up: Relatively simple to manufacture with no complicated technical investments required for production of ethosomes. Multiliter amounts can be conveniently prepared for ethosomal formulation.
10. Ethosomes enhance permeation of drugs across/through the skin in an efficient manner, thereby enabling the drug to reach the desired site in the skin or to the blood.
11. Higher entrapment efficiencies of drugs when compared to liposomes can be observed.
12. Excellent stability over long periods can be observed.
13. Alcohol in the ethosomes acts as natural preservative, and hence there is no necessity to add any other preservative.
14. The cost of manufacturing ethosomes is very cheap.
15. The transport of drugs across the skin is not concentration dependent.[3,4]

Disadvantages of ethosomes:

1. Allergic reaction can be identified if the patients are allergic to ethanol or any of the ethosomal components.
2. Unlike other carriers (solid lipid nanoparticles, polymeric nanoparticles, etc.) which can be used for multiple routes, ethosomal carriers are important only for transdermal use.
3. Due to the fact that ethanol is inflammable, sufficient care should be taken during planning, application, transport and storage.
4. Very poor yield so may not be economical.
5. Loss of product during transfer from organic to water media.
6. It is limited only to potent molecules, those requiring a daily dose of long or less.
7. Ethosomal administration is not a means of achieving rapid drug input of the form of bolus, but is typically intended to provide steady, continuous drug delivery.
8. Adequate drug solubility in both lipophilic and aqueous conditions to achieve microcirculation of the dermal and to obtain access to circulation.
9. The drug's molecular size should be appropriate for it to be absorbed percutaneously.
10. Adhesive may not adhere well to all types of skin.
11. Skin irritation or dermatitis due to excipients and penetration enhancers of drug delivery systems.
12. If shell locking is ineffective, ethosomes can coalesce and fall apart when transferred to water[3,4].

ETHOSOMAL SYSTEM TYPES

1. Classical ethosomes

Classical ethosomes are a variation of classical liposomes, consisting of phospholipids, high ethanol concentrations of up to 45 % w / w, and water. Classical ethosomes for transdermal drug delivery were stated to be superior to classical liposomes because they were smaller and had negative ζ -Potential for greater efficiency without clogging. Moreover, in contrast with classical liposomes, classical ethosomes displayed improved skin permeation and stability profiles. The molecular weights of drugs caught in traditional ethosomes ranged from 130.077 Da to 24 kda.

2. Binary ethosomes

Binary ethosomes were introduced by Zhou et al. We were created essentially by adding a different form of alcohol to the classical ethosomes. Propylene glycol (PG) and isopropyl alcohol (IPA) are the most widely used ethosomes in binary alcohols.

3. Transethosomes

Transethosomes are the latest generation of ethosomal systems and were first recorded in 2012 by Song et al. This ethosomal system includes the basic components of classical ethosomes and an additional compound such as a penetration enhancer or an edge activator (surfactant) in its formula. In an attempt to combine the advantages of classical ethosomes with deformable liposomes (transfersomes) in one formula to generate transethosomes, these novel vesicles were formed. Several researchers have reported superior transethosomal properties over traditional ethosomes. Different forms of edge activators and penetration enhancers were investigated in order to achieve better characteristic ethosomal systems. Transethosomes with molecular weights ranging from 130.077 Da to 200–325 kda have been reported to entrap drugs. [2]

COMPOSITION OF ETHOSOMES

Ethanol

Ethanol is an efficient penetration enhancer. It plays an important role in ethosomal systems by giving the vesicles special dimensional characteristics size, ζ-Potential, stability, prevention of clogging and increased permeability of the skin. Concentrations of ethanol in ethosomal systems have been reported to be ~10%–50%.Many researchers concluded that when the

concentration of ethanol is increased, the size of the ethosomes would decrease. Increasing ethanol concentration above the optimum amount, however, would cause the bilayer to be leaky, leading to a small increase in vesicular size and a significant decrease in the

efficacy of trapping, and would solubilize the vesicles by further raising the ethanol concentration.

Vesicular load is an important parameter which can affect vesicular properties such as stability and skin vesicle interaction. The high concentration of ethanol in ethosomes has moved the vesicular load from positive to negative. Ethanol serves as a negative charge supplier for ethosomal surfaces, thereby preventing accumulation of the vesicular network as a result of electrostatic repulsion. In fact, ethanol had stabilizing effects, too. Ethanol also has a direct effect on the efficiency of trapping in ethosomal systems, and typically increasing concentrations in ethanol would increase the efficiency of trapping.

Phospholipids

Phospholipids from different sources were used in formulation of the ethosomal scheme. The selection of phospholipid type and concentration for formulation are important factors during the production of ethosomal system since they will affect the scale, the effectiveness of the trapping,ζ -Potential vesicular properties, stability, and penetration. Highly negatively charged vesicles were produced by the incorporation of DPPG (1,2-dipalmitoyl-sn-glycero-3-phosphatidylglycerol) in the ethosomal formulation, while cationic ethosomal vesicles were produced by using a cationic lipid, such as DOTAP (1,2-dioleoyl-3- trimethylammonium-propane [chloride salt]). In general, in an ethosomal formulation, the concentration range of phospholipids is 0.5%–5%. Rising phospholipid concentration can increase vesicular size marginally or moderately, but will greatly improve the efficiency of trapping. The relationship, however, is only valid until there is a certain concentration.

Cholesterol

Cholesterol is a stable steroid molecule, and its integration into ethosomal structures increases medication stability and clogging effectiveness. This avoids leakage and decreases permeability of the vesicles and vesicular fusion. Generally, it is used at a concentration of 3% but in some formulations, it was used up to 70% of the total phospholipid concentration in the formulation. Several studies have recorded that the vesicular size of ethosomal systems increased with cholesterol.

Dicetyl phosphate

Dicetyl phosphate is widely used to avoid vesicle aggregation and to improve formulation stability. It is used at concentrations between 8% and 20% of the total phospholipid concentration in the ethosomal formulation. However; the impact of dicetyl phosphate on other properties of the ethosomal system remain uncertain.

Stearylamine

Stearylamine is a positive-charge agent. The addition of stearylamine to the ethosomal formulation caused a great increase in vesicular size and decrease in entrapment. Stearylamine readily penetrates the skin because of its lower molecular weight (296.5 Da).

Other alcohols

Along with ethanol, certain alcohols such as PG and IPA are also used in the preparation of binary ethosomes. efficiency, and change in the ζ-potential charge from negative to positive which lead to aggregation of the vesicles within 1 week.

Propylene glycol

PG is a widely used penetration enhancer. This is used at a concentration range of 5%-20% in the preparation of binary ethosomes and has been found to influence the ethosomal properties of size, trapping capacity, permeation and stability. PG integration into ethosomal systems will result in more reduction of particle size relative to systems without PG . A substantial reduction in particle size was achieved from 103.7±0.9 nm to 76.3±0.5 nm when the PG concentration raise from 0% to 20 % v / v. It is suggested that PG enhances ethosome stability by increasing the viscosity and antihydrolysis property.

Isopropyl alcohol

Dave et al studied the influence of IPA on the entrapment efficiency and skin permeation of a diclofenac-loaded ethosomal system. Three types of formulations have been prepared: classical ethosomes containing 40% ethanol, binary ethosomes containing approximately 20% IPA and 20% ethanol, and a vesicular system containing 40% IPA. The vesicular device containing 40 % IPA was found to have higher trapping performance (95 %) than the binary ethosomes (83.8 %).

Edge activators or penetration enhancers

The selection of a proper edge activator or penetration enhancer is a critical step in the formulation of transethosomes, as they have profound effects on the properties of the ethosomal system.

Tweens and spans

In the ethosomal scheme, Tween 80 is used at concentrations of 10% -50% of the total phospholipid concentration. It has been stated that Tween 80 has been integrated in ethosomal systems to minimize vesicular size and improve the stability of the system and skin-permeation properties. Mainly due to its solubilizing properties and the prevention of vesicle fusion, the impact of Tween 80 on the ethosomal system. Tween 20 formed an unstable formulation. Spans 80, 60, and 40 did not manage to generate homogeneous and stable transethosomes. Only Span 20 was used successfully in the preparation of transethosomes of caffeine and vitamin E.30.

oleic acid

Oleic acid affects vesicular scale and elasticity, ζ- Potential, and skin-permeating properties by increasing stratum corneum fluidity.

l-menthol

L-Menthol was applied to transethosomes of ascorbic acid at a concentration of 5 % as a penetration enhancer.

Cremophor

Cremophor is the brand name of a range of nonionic polyethoxylated detergents. Cremophor EL-35 was used in an ethosomal system of testosterone propionate at concentrations of 0.5%–1.5% w/w. Reducing vesicular size and increasing the drug's solubility and efficacy in trapping was found.

Skin-penetrating and cell-entering peptide

Skin-penetrating and cell-entering peptide (SPACE) is a skin-penetration enhancer discovered by phage display and shown to deliver short RNA (sirna) and streptavidin to the skin after direct chemical conjugation. This penetration enhancer was incorporated in transethosomes for the delivery of hyaluronic acid.

Sodium dodecyl sulfate

Sodium dodecyl sulfate is an anionic surfactant and has been used at a concentration of 0.8% w/v in the preparation of transethosomes of ketoconazole and imiquimod.The results showed that sodium dodecyl sulfate significantly reduced the size, increased the entrapment efficiency and the ζ potential and enhanced the in vitro and in vivo skin-permeation properties of ethosomal systems.

METHODS OF PREPARATION

Preparation of ethosomes grounds on quick and easy scale up techniques without requiring any complex pilot- and industrial-level instruments. Ethosome preparation includes two simple "cold" and "hot" methods.

Cold method

This is one of the most commonly used ethosome preparation methods, consisting of two basic and simple setups. In the first setup, phospholipid and other lipid material is dissolved by intense stirring in ethanol at room temperature with the use of mixer such as Heidolph mixer with continuous addition of polyols such as propylene glycol etc. With constant stirring followed by heating at 30 ^{0}C in water bath. In the second setup, water is to be heated at 30 ^{0}C in a separate vessel, both mixtures (obtained from first and second setup) are to be blended together following 5 min stirring in a covered vessel. Using sonication or extrusion process, the vesicle size of ethosomal formulation can be reduced to desire extend. Finally, the formulation is stored under refrigeration.

Hot method

This method consists of dispersion of phospholipid in water by heating in a water bath at 40 ^{0}C until a formation of a colloidal solution. In a separate vessel, ethanol and propylene glycol are mixed and heated to 40 ^{0}C. If both mixtures exceed 40^{0}C the aqueous phase is added to the organic phase. Depending on their hydrophilic / hydrophobic properties the drug is dissolved in water or ethanol. Using probe sonication or extrusion process, the vesicle size of ethosomal formulation can be diminished to the extent of desire.

Classic mechanical dispersion method

Dissolve phospholipid in an organic solvent, or in a round bottom flask (RBF) mixture of organic solvents. Using a rotary vacuum evaporator above lipid transition temperature to remove the organic solvent to create a thin lipid film on the RBF wall; Traces of the solvent should be separated from the accumulated lipid film by leaving overnight in vacuum. Hydrate the lipid film with the drug's hydroethanol solution by spinning the flask with or without periodic sonication at the correct temperature and eventually cool the resulting ethosomal suspension at room temperature. The formulation should be stored under refrigeration.

The ethanol injection–sonication method

In this process, the organic phase containing the dissolved phospholipid in ethanol is injected into the aqueous phase using a 200-flow syringe system38 µl / min, then homogenized for 5 minutes with an ultrasonic probe.

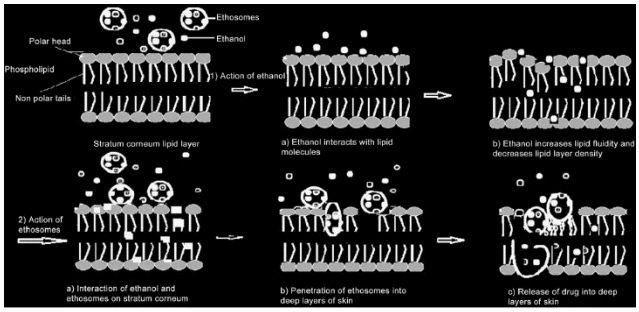
Mechanism of penetration

Although the exact mechanism of ethosomal drug delivery remains a matter of debate, a combination of processes most likely contributes to the enhancing effect. At physiological temperature the stratum corneum lipid multilayer is tightly packed and strongly conformationally ordered. The high concentration of ethanol makes ethosomes special because ethanol is responsible for disrupting the organization of skin lipid bilayers; thus, when incorporated into a vesicle membrane, vesicles are capable of penetrating the stratum corneum. The lipid membrane is also packed less tightly than traditional vesicles due to its high concentration of ethanol but has similar stability, enabling a more malevolent structure, giving it more flexibility and the ability to squeeze through small places such as openings created to disrupt the corneum lipid stratum. Ethanol interacts with lipid molecules in the area of the polar hard group, thereby reducing the rigidity of the corneum stratum lipids and increasing their fluidity. The intercalation of ethanol into the environment of the polar head group will result in an increased permeability of the membrane. The ethosome itself can interact with the stratum corneum barrier, in addition to the effect of ethanol on the structure of the stratum corneum. Although encapsulated drug remained predominantly on the skin surface in classic liposomes, the

ethosomal system was shown to be highly effective carrier for increased drug delivery through the skin. The successful drug delivery shown along with the long-term ethosomal stability makes this device a promising candidate for transdermal drug delivery.

1. Ethanol Effect: Ethanol works through the skin as a penetration enhancer. The mechanism of its enhancing effect on penetration is well known. Ethanol penetrates into intercellular lipids and increases the fluidity of cell membrane lipids and decrease the density of lipid multilayer of cell membrane.

2. Ethosomes Effect: Increased lipid fluidity in the cell membrane caused by ethosomal ethanol results in increased permeability of the skin. The ethosomes thus penetrate very quickly into the deep layers of the skin, where it has been fused with skin lipids and releases the drugs into the deep layer of the blood. Mechanism of permeation of ethosomes is explained in Fig 2.



"Fig 2: mechanism of penetration of ethosomal drug delivery system"

Physicochemical characterization:

Vesicle morphology

Vesicular morphology of ethosomal systems may be exposed by transmission electron microscopy (TEM) and scanning electron microscopy (SEM), which consists of negative staining of the formulation with aqueous solution of agents such as phosphotungstic acid etc.

Vesicle size and size distribution

Dynamic light scattering (DLS), size ranges between nanometers and microns determined by the composition of the formulation will decide the vesicular size of the ethosomal framework.

Configuration of the vesicular bilayer

Since the efficacy of the ethosomal trapping system depends on its vesicle bilayer, performing the investigative analysis of optimum bilayer formation is needed. This can be done by the execution of research on Nuclear Magnetic Resonance (NMR).

Drug entrapment efficiency

Efficiency of drug trapping Once the investigative studies of the configuration of the vesicular bilayer of ethosomal systems are affirmatively verified, the measurement of the efficiency of the trapping of ethosomes becomes the next essential characterization parameter because it endows the ethosomal system with sustained release characteristics. It is usually achieved using two methods as described below.

Ultracentrifugation

This process consists of two segments in the vesicle preparation of the first segment, which are held overnight and subjected to ultracentrifugation for measured time and RPM. In the second chapter, every advanced approach is used to check pure drugs, e.g. High-performance liquid chromatography (HPLC) then finally the entrapment efficiency is calculated by applying following relationship:

$$EE = \frac{Dt - Ds}{Dt} \times 100$$

Where EE is the entrapment efficiency, Dt is the theoretical amount of drug added and Ds is the amount of drug detected only in the supernatant.

Dialysis

Bags were prepared using polymers in this dialysis e.g. Cellulose acetate, which was held in a saline solution for 1 h before dialysis to ensure complete wetting of the membrane after that measured amount of drug-loaded vesicles or free drug in aqueous solution was inserted in the dialysis bag and then moved to 500ml of phosphate buffer saline (PBS) pH 7.0. A magnetic stirrer was used to stir the receiver mediums. Aliquots of the same quantity were withdrawn from the receiver medium at set time ranges and replaced with equivalent quantities of PBS solution to maintain optimal sink conditions. Samples for the drug content were further analyzed using HPLC methods.

Permeation distinctiveness

Ethanol has long been considered to possess permeation enhancement properties. Nevertheless, the permeation enhancement from ethosomes was much higher than expected from ethanol alone, indicating some form of synergistic mechanism between ethanol, vesicles and skin lipids that provides versatile characteristics for ethosomes that generate enhanced penetration capabilities due to two results. (A) an increase in thermodynamic activity due to ethanol evaporation known as "pressure effect" and (b) an increase in the penetration of the drug molecule due to a decrease in the barrier properties of subcutaneous tissue due to ethanol.

Physical stability

The freeze-drying technique possibly ensured long-term storage stability of ethosome suspension. Cakes of freeze-dried ethosomes were found to be lightweight, glassy and distinguished by low viscosity and rapid rehydration. Nevertheless, the percentage of drug encapsulation within ethosome was somewhat affected by the storage period that showed a drug leakage after rehydration of about 10 %. The lipid part of ethosomes is produced from natural phospholipid and/or synthetic sources. Oxidative reactions are known to occur in phospholipids containing

unsaturated fatty acids. The products of the reaction can cause changes in permeability of the bilayer ethosomes. Oxidative lipid degradation in general can be minimized by the addition of antioxidants such as α-tocopherol to protect the lipid preparation from light. Additionally, lipid hydrolysis leads to the formation of lyso- PC. The presence of lyso-PC improves the permeability of ethosomes and, thus, it is necessary in a given preparation to keep its level to minimum.

Transition temperature

Vesicular lipid transition temperature (T) can be determined in duplicate by DSC in an aluminum pan at a heating rate of 10 ° C per min, under a steady stream of nitrogen.

Confocal laser scanning microscopy (CSLM)

CSLM can be used to examine the extent and function of the ethosomal preparation skin penetration. The thickness of the skin can be optically scanned through the z axis of a confocal laser scanning microscope at different increments.

Drug content

Using the UV spectrophotometer, ethosome quality can be calculated. It can also be quantified using a updated chromatographic high-performance liquid process.

Surface tension measurement

Drug surface tension activity in aqueous solution can be measured in a Du Nouy ring tensiometer using the ring process.

Phospholipid-ethanol interaction

The interaction between phospholipid-ethanol was tested using 31P-NMR decoupled protons and calorimetry differential scanning.

Degree of Degradability and turbidity

Extrusion method may perform the degree of deformability of the ethosomal preparation, and the turbidity of the preparation can be performed with the use of Nephalometer.

Drug entrapment efficiency

Differential calorimetry scanning thermograms and anisotropy analysis of AVPC (a fluorescent analog of phosphatidylcholine), showed that ethosomes had lower Tm compared to standard liposomes and that the bilayers had a high degree of fluidity. This imparted the vesicles a gentle and maleable quality. Godin and Touitou used confocal laser scanning microscopy (CLSM) to demonstrate that ethosomes can trap both hydrophobic and hydrophilic fluorescent samples efficiently. Similar findings were obtained using the method of ultracentrifugation to test trapping of different drugs. Hydrophobic and hydrophilic medicines have been verified effectively by the use of hydrophilic 6- carboxy fluorescein and hydrophobic Rhodamine 123 fluorescence markers. Ethosomes 'capacity to efficiently clog lipophilic and hydrophilic drugs can be explained by the high degree of lamellarity and the presence of ethanol in the vesicles. Furthermore, ethosomal formulations have greater capability of trapping than liposomes. Dayan and Touitou have shown that trihexyphenidyl hydrochloride trapping efficiency increased from 36% for liposomes to 75% for ethosomes.[1,6]

EVALUATION OF ETHOSOMES

Vesicle skin interaction study

Different visualization strategies e.g. for assessing the process of improved skin permeation of ethosomal formulations. Transmission electron microscopy, eosin-hematoxyl staining, fluorescence microscopy, and laser microscopy (CSLM) confocal scanning were used. Such visualization methods also provided a better understanding about modulation of the structure and vesicle penetration pathways when used in combination. It was only to the upper layer of skin (stratum corneum) that traditional liposome penetrated. Deep penetration of liposomes free from alcohol was nearly negligible. In comparison, the ethosomal carrier was used to observe improved distribution of 6-CF and Rhodamine 123 in terms of depth and quantity (dermis-layer).

1. **Filter membrane-vesicle interaction study by scanning Electron microscopy**

This requires adding vesicle suspension (0.2 ml) to filter membranes with a 50 nm pore size, and positioning them in diffusion cells. The upper side of the filter was exposed to the air, whereas the lower side was in contact with phosphate buffer saline solution, (having pH6.5).The filters were removed after 1 hour and were prepared for SEM studies by fixation at 4°C in Karnovsky's fixative overnight followed by dehydration with graded ethanol solutions (30%, 50%, 70%, 90%, 95%, and 100% v/v in water).

2. Skin permeation studies

The hair of test animals (rats) was carefully cut short (< 2 mm) with a pair of scissors, and a scalpel separated the abdominal skin from the underlying connective tissue. For any adhering fat and/or subcutaneous tissue, the excised skin was put on aluminum foil, and the dermal side of the skin was gently teased off. The effective difusion cell and receptor cell volume permeation area was 1.0 cm^2 and 10 ml, respectively. The temperature was kept to 32 ° C ± 1 ° C. The receptor compartment contained saline solution with phosphate buffer (10 ml pH 6.5). It placed excised skin between the donor and the receptor compartment. Applied to the epidermal surface of the skin was ethosomal formulation (1.0 ml). Samples (0.5 ml) were taken at 1, 2, 4, 8, 12, 16, 20 & 24 hour time intervals via the sampling port of the diffusion cell and analyzed using a high-performance liquid chromatography assay.

3. Stability study

The stability of the vesicles was determined by the vesicles being held at 4 ° C ± 0.5 ° C. The vesicle size, zeta potential,

and trapping efficiency were calculated after 180 days using the method previously stated.

4. Drug uptake studies

Drug absorption into MT-2 cells (1,1106 cells / ml) took place in 24-well plates (Corning Inc) where 100 µl RPMI medium was applied. In phosphate buffer saline solution (pH7.4), ethosomal formulation, or advertised formulation, cells were incubated with 100 µl of the drug solution, and then drug absorption was calculated by HPLC assay analysis of the drug material.

5. HPLC assay

During in vitro skin permeation experiments and in MT-2 cell, the amount of drug permeated in the receptor compartment was determined by HPLC assay using methanol: distilled water: acetonitrile mixture (70:20:10 v / v) as a mobile step.

6. Statistical analysis

The statistical significance of all the produced data was evaluated using ANOVA followed by studenized range testing. Using the PRISM program, a confidence limit of P<.05 was set for interpreting the results.[4]

APPLICATIONS OF ETHOSOMES

Ethosomes, the high ethanol derived vesicles are capable of penetrating deeper layers of the skin and thus tend to be vesicles of choice for transdermal drug delivery via the skin of hydrophilic and impermeable drugs.

Hormone delivery

Oral hormone delivery is related to numerous issues, such as high first-pass metabolism, poor oral bioavailability and many dose-dependent side effects . In addition, oral hormonal preparations which depend heavily on patient compliance with these side effects. The risk of treatment failure is known to rise with every missed pill. Touitou et al. Revealed ability of ethosomes in hormonal delivery by performing a comparative analysis of transdermal delivery of testosterone loaded ethosomes (Testosome), as compared to transdermal testosterone patch (Testoderm patch, Alza) through rabbit pinna skin, which showed approximately 30-times higher skin permeation of testosterone from ethosomal formulation. For ethosomal formulation, the volume of drug deposited was substantially (p50.05) higher (130.76 ± 18.14 and 18.32 ± 4.05 mg at the end of 7 h for Testosome and Testoderm, respectively. The area under the curve (AUC) and Cmax of testosterone significantly improved after the application of Testosome as compared to Testoderm. Thus, both in vitro and in vivo studies have shown increased skin permeation and testosterone bioavailability from ethosomal formulation.

Transcellular delivery

Ethosomes have been shown to be an effective penetration enhancer and carrier device for the transcellular delivery of various therapeutic agents in active clinical trials. In contrast, almost no fluorescence was observed when integrated in a hydroethanolic solution or classic liposomes. After 3 min of incubation, the intracellular existence of each of the three tested probes was evident.

Pilosebaceous targeting

The percutaneous drug delivery of hair follicles and sebaceous glands is increasingly recognized as potentially significant elements. The interest in pilosebaceous units was directed to their use as depots for localized therapy, particularly for the treatment of follicle-related disorders such as acne or alopecia. In addition, extensive attention has also been paid to using the follicles as transportation shunts for systemic drug delivery.

Delivery of anti-parkinsonism agent

Dayan and Touitou prepared ethosomal formulations of the psychoactive drug trihexyphenidyl hydrochloride (THP) and contrasted their delivery from traditional liposomal formulations. THP is an antagonist of M1 muscarinic receptors and used to treat Parkinson's disease. The transdermal flux value of THP from ethosomes via the nude mouse skin was 87, 51 and 4.5 times higher than that of liposome, phosphate buffer, and hydroethanol solution, respectively. After application of ethosomes, the amount of THP remaining in the skin at the end of 18 hr was substantially higher than after application of liposome or hydroethanolic (control) solution. Such findings revealed a greater potential for skin permeation of ethosomal-THP formulation and its use to help treat Parkinson disease.

Topical delivery of DNA

A lot of environmental pathogens are trying to get into the body through the skin and skin has developed into an outstanding defensive barrier that is both immunologically active and capable of expressing the gene. The important use of ethosomes on the basis of the above facts is to use them for the topical delivery of DNA molecules to express genes in skin cells. It has been proposed that ethosomes may be used as carriers for applications for gene therapy that require transient gene expression. The findings also suggested the ability to use ethosomes for successful transdermal immunization. Therefore improved ethosomal skin permeation capacity opens the possibility of using these dosage types to deliver immunizing agents.

Delivery of anti-arthritis drug

Topical delivery of anti-arthritis medication is a better alternative for site-specific delivery and overcomes traditional oral therapy-related problems. Cannabidol (CBD) is a drug candidate recently discovered to treat rheumatoid arthritis. His oral administration is associated with a variety of issues such as low bioavailability, first pass metabolism, and degradation of GIT. Significantly increased in CBD-ethosomal formulation biological anti-inflammatory activity was observed when examined by the carrageenan mediated rat paw edema model. Thus, it was concluded that encapsulation of CBD in ethosomes greatly

increased its permeation of the skin, its accumulation and thus its biological activities.

Delivery of antibiotics

Topical antibiotic delivery is a safer option to improve the therapeutic efficacy of those drugs. Conventional oral therapy and other side effects cause many allergic reactions. Conventional outer formulations have poor permeability to deep layers of skin and subdermal tissues. Ethosomes can get around this issue by delivering sufficiently antibiotic into deeper layers of skin. Ethosomes penetrate easily through the epidermis and carry substantial amounts of drugs into the deeper layer of the skin and kill infection at its core. The findings of this study showed that antibiotic ethosomal formulation could be highly effective and would solve the problems associated with traditional therapy.

Anti-viral drug delivery

Zidovudine is a potent antiviral agent that acts on the acquired immunodeficiency virus. Fast side effects link oral administration of zidovudine. So an appropriate zero-order delivery of zidovudine is needed to maintain the anti-AIDS effect predicted. It was concluded from various studies that ethosomes could increase the transdermal flux, prolong the release and pose an attractive route for sustained zidovudine delivery. Acyclovir is another anti-viral drug which is commonly used topically for Herpes labialis treatment. The traditional external formulation of the marketed acyclovir is associated with low skin penetration of hydrophilic acyclovir to the dermal layer resulting in inadequate therapeutic efficacy. Scientists have devised the acyclovir ethosomal formulation for dermal delivery to solve the problem associated with traditional topical acyclovir preparation.

Delivery of problematic drug molecules

It is difficult to transmit large biogenic molecules such as peptides or proteins orally, because they are fully degraded in the GI tract. Non-invasive protein delivery is a safer choice for addressing the oral delivery problems. Researchers have been investigating the effect of ethosomal insulin delivery in normal and diabetic SDI rats on reducing blood glucose levels in vivo. The result showed that insulin administered from this patch in both normal and diabetic rats induced a substantial decrease (up to 60 %) in BGL. At the other hand, an injection of insulin from a control formulation does not reduce the BGL.

Cosmaceutical application of ethosomes

The benefit of applying ethosomes in cosmaceuticals is not only to enhance cosmetic chemicals 'stability and decrease skin irritation from irritating cosmetic chemicals, but also to enhance transdermal permeation, particularly in elastic types. Furthermore, the compositions and sizes of the vesicles are the key considerations that need to be addressed in order to achieve these benefits of the elastic vesicles for cosmaceuticals.[6,7]

CONCLUSION

Ethosomes provides a good opportunity to deliver small, medium and large drug molecules with no intrusive effect. Ethosomes have been found to be much more efficient at delivering drug to the skin, than either liposomes or hydroalcoholic solutions. It can therefore be a fair inference that ethosomal formulations have promising future in the successful delivery of bioactive agents to the dermal / transdermal.

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