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Vesicular Carriers: A novel Approach for a Transdermal Drug Delivery System

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Abstract: Skin acts as a major target as well as a principle barrier for topical/transdermal drug delivery. Many attempts have been exploited by scientists over a long time to overcome the strong barrier property of the stratum corneum, the top most layer of human skin. These attempts include physically-aided methods, chemical penetration enhancers and vesicular carrier technology such as liposomes, niosomes, transferosomes, ethosomes and so forth. Vesicular drug delivery systems are highly structured assemblies composed of one or more concentric bilayers formed by the self-assembly of amphiphilic building blocks in the presence of water. The goal of this article is to give a brief overview of the different types of transdermal vesicular drug delivery systems in particular ethosomes.

Keywords: Vesicular Carrier, Transferosomes, Sphingosomes, Invasomes, Ufasomes.

1. INTRODUCTION

Human skin is composed of three distinct layers: epidermis, dermis and hypodermis. With a weight of 20% of an adult's total body weight and a surface area of around 2 m^2, the skin is the largest organ in the body. Being located at the point where the human body and the environment converge, it serves as a crucial barrier that protects against microbiological, physical, and chemical harm in both a passive and active manner. A basement membrane separates the upper layer of skin, called the epidermis, from the lower layer, called the dermis (Fig. 1). The epidermis is made up of five layers: the stratum corneum, which is the outermost layer, the stratum granulosum, the stratum spinosum, and the stratum basale, which is the innermost layer. Fingertips, palms, and soles are the only places on a human body where the stratum lucidum is present. The stratum corneum is the "formidable" physical barrier because it is so thick and composed of so many dead keratinocytes and intercellular lipids.

However, because it has numerous openings for skin appendages like sweat ducts and hair follicles, its barrier function is flawed. The body can benefit from skin appendages to protect it from mechanical harm, UV light, temperature changes, and dryness [11].

Transdermal drug delivery system (TDDS) has proven to be a very effective substitute for the oral mode of administration due to its numerous advantages. A TDD system aids in reducing the gastrointestinal adverse effects of some medications, also, it is beneficial to new-born who have difficulty taking some oral medications due to their bitter taste, having difficulty in swallowing tablets or abstaining from a patient phobia, risk factors, and inconvenience associated with parenteral delivery. Moreover, TDDS enhances the bioavailability of some oral drugs suffering from first-pass metabolism and decreases the administered dose. A TDDS patch could be easily terminated in case of a mistaken drug accident or a toxic dose occurrence. Therefore, the application of transdermal technology in a variety of therapeutic and cosmetic fields is very crucial and attractive nowadays [29]. The public's interest in transdermal delivery generally increased once nicotine patches became the first transdermal blockbuster a decade later. Fentanyl, isosorbide dinitrate, estradiol, nitroglycerin, methylphenidate,

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lidocaine, testosterone, rivastigmine, and many other transdermal medication preparations are available today, as well as combination patches that combine multiple drugs for both hormone replacement and contraception[51].

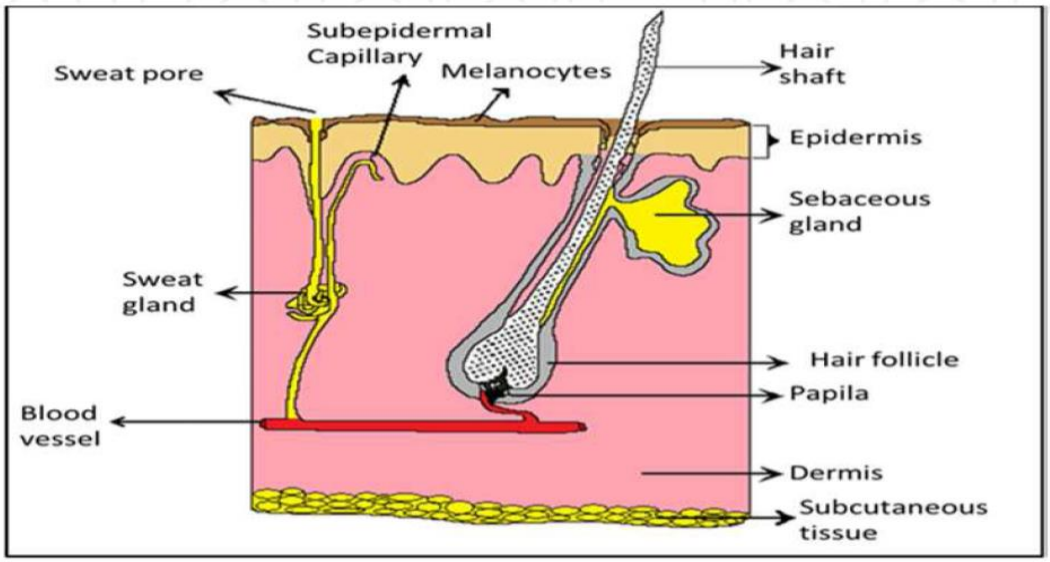

Figure 1. Human skin anatomy[28]

The challenging problem facing transdermal delivery system (TDS) is the permeation of active moiety through skin. Several approaches were developed for enhancing permeability of drug molecules percutaneously. These approaches comprise passive and active penetration enhancement and novel vesicular carriers:

1-physical and chemical means of improving percutaneous medication delivery:

There are several electrical techniques for improving skin penetration. Electroporation uses micro- to millisecond electrical pulses (approximately 100-1000 V/cm) to create transient aqueous pores in lipid bilayers, phonophoresis uses low frequency ultrasound energy to increase lipid fluidity, and iontophoresis uses a small direct current as the driving force for charged drugs through the skin [7]. The complexity of these techniques limited their use by patients and contributed in poor patient compliance.

The agents that make the skin more permeable or the compounds that make the skin less impermeable are known as penetration enhancers[51]. The toxicity related to numerous chemical penetration enhancers has limited their therapeutic utility. In recent years, researchers have begun to shift their focus to potential enhancers recognised as GRAS (Generally Regarded As Safe) by the Food and Drug Administration, such as essential oils and terpenes [7].

An ideal chemical penetration enhancer must possess the following characteristics [9]:

a) It should possess no allergenic, toxic or irritating action on human skin.

b) It should be inert i.e: not pharmacologically active inside the human body.

c) It should act rapidly and uni-directionally.

d) It should be compatible with both excipients and drug, cosmetically acceptable, odourless and colourless.

Examples of famous penetration enhancers include: azones, transcutol, terpenes (e.g.: menthol), urea, alcohol (e.g.: ethanol, methanol, caprylic alcohol), pyrrolidones, glycol (propylene glycol), fatty acids (oleic acid, lauric acid, linoleic acid), surfactants (anionic surfactants, non-ionic surfactants) and esstential oils [21, 27, 45].

2-Topical and Transdermal vesicle-aided drug delivery:

The application of nanotechnology in the development of a suitable drug carrier system that may enable regulated and localised administration of the active drug in accordance with the particular requirements of the therapy is a potential technique for overcoming the challenges associated with drug delivery.

Nanoparticles have advantages as topical carriers because of their unique characteristics. Nanoparticles have various benefits over conventional drug delivery methods. These nanoparticles have many applications, including (i) improve the bioavailability of poorly water-soluble medications, (ii) enable the continuous and regulated release of encapsulated drug, (iii) increasing the

chemical or physical stability of therapeutics, and (iv) delivering both hydrophobic and hydrophilic drugs in a controlled, sustained fashion over an extended period of time; (v) Enhanced Permeation and Retention (EPR) allows for larger drug concentrations at the site of action, and (vi) cell-specific ligand modifications allow for more precise drug delivery.

Drug-loaded nanoparticles are maintained and stored in hair follicles, where they are easily absorbed and released into the dermis and deeper layers of skin. Solid lipid nanocarriers, nanostructured lipid carriers, nanoemulsions, and vesicular systems are some of the most popular types of nanoparticles utilised for topical and/or transdermal drug administration.

3-Vesicular Drug delivery systems:

Vesicular drug delivery systems (VDDS) are established from self-assembling amphiphilic building units that enclose in the form of concentric bilayer (s) when dispersed in water forming an aqueous core space (Fig. 2,3). They serve as an effective drug delivery system for both hydrophilic and hydrophobic medicines[4].

Liposomes were the first generation of VDDS. They were discovered by the british hematologist Dr Alec D. Bangham in 1961, thereby earning the term "Bingham Bodies[4]. Liposomes are vesicles made of natural or manufactured phospholipids, whereas niosomes are non-ionic surfactant vesicles made of non-ionic surfactants (e.g. alkyl ethers and alkyl esters) and cholesterol[25]. Liposomes are generally costy and have short shelf life period. Transferosomes, which contain a single chain surfactant as an edge activator in addition to phospholipids, and ethosomes, which are flexible vesicles composed of phospholipids, ethanol, and water, are examples of modified liposomal systems. Vesicular drug carriers delay elimination of rapidly metabolizable drugs and thus function as sustained release systems.

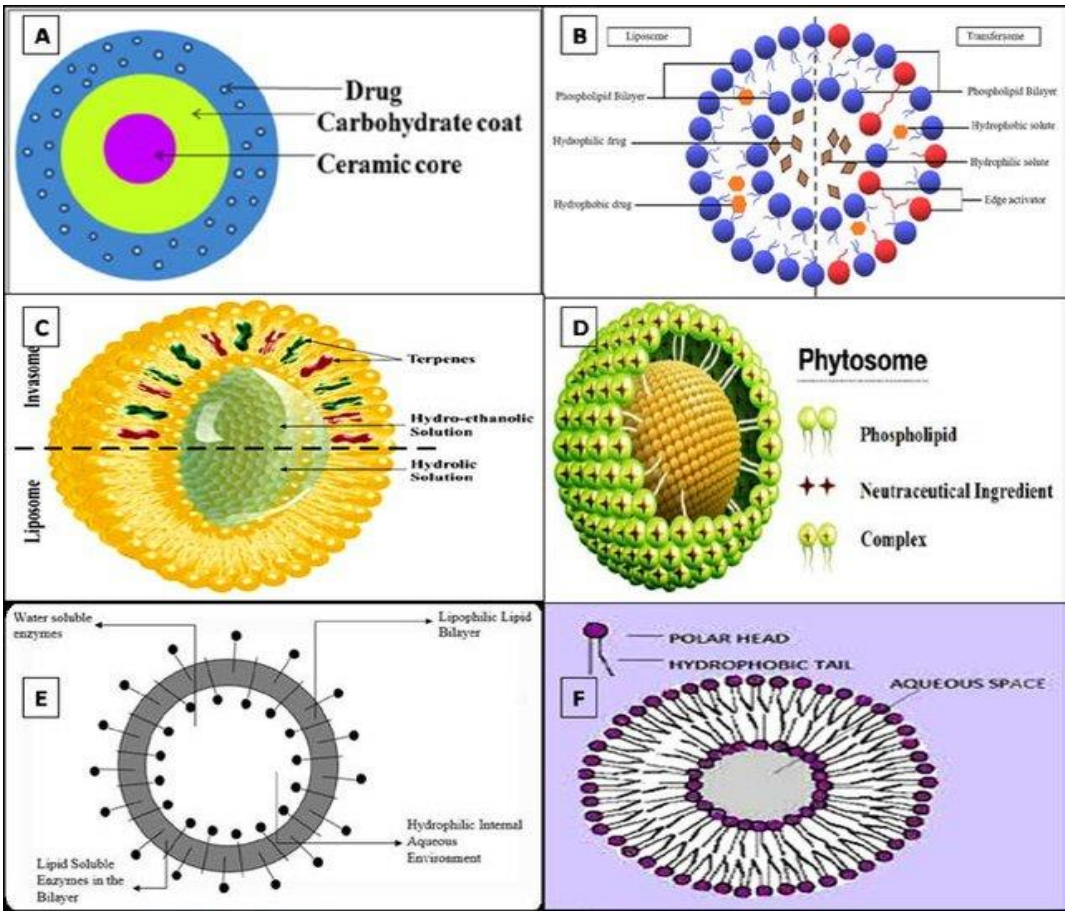

Figure 2: A) structure of aquasome (B) structure of transferosome and in comparison with liposome; (C) structure of invasome and it comparison with liposome; (D) structure of phytosomes; (E) structure of enzymosomes ; (F) structure of sphingosomes [36]

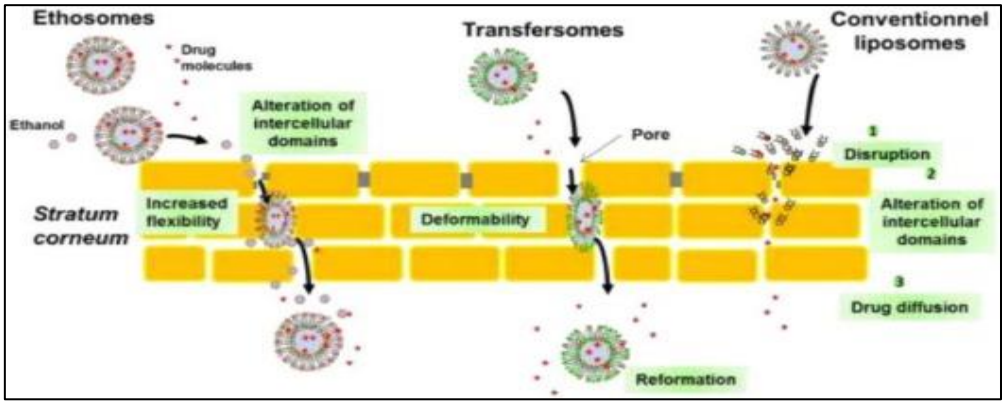

Figure 3. Diagram illustrates the different mechanisms of skin permeation of ethosomes, transferosomes and conventional liposomes [53]

3.1. Liposomes:

Liposomes are spherical lipid bilayer structures comprised of cholesterol and phospholipids.

Because of their hydrophilic head and hydrophobic tail, phospholipids are classified as amphiphilic compounds. The hydrophobic component is two fatty acid chains with 10-24 carbon atoms and 0-6 double bonds, whereas the hydrophilic component is largely phosphoric acid connected to a water-soluble molecule. When phospholipids are dispersed in water, they form liposomes, which are spherical, vesicle-like structures with the polar head group towards the aqueous region and the fatty acid groups facing each other [58].

Liposomes are a distinctive drug delivery system because they are safe, biodegradable and can encapsulate both hydrophobic moieties between the bilayer(s) and water-soluble moieties inside the core [58].

Most liposomes are unilamellar and have a diameter between 50 and 150 nm. Most of the larger liposomes are quickly cleared out of the bloodstream. Liposomes, unlike other delivery systems, may accommodate drug molecules with widely varied physicochemical features such as polarity, charge, and size.

Because of their ability to carry drugs and/or genetic material into the targeted cells, liposomes, were a revolutionary tool in the field of chemotherapeutics that necessitates the presence of drug moiety within the targeted (diseased) cells at a therapeutic level (with minimal drug loss)[4].

Liposomes have a multitude of therapeutic applications, e.g: chemotherapy (tumor cells, antiviral, antifungal eradication), gene delivery (e.g: vaccination), immunology(immunodiagnostics), artificial blood surrogates, radiopharmaceuticals and radio diagnostic carriers[4].

Besides, liposomes have versatile implementations in other criteria like mathematics, physics, biophysics, chemistry, biochemistry and many other fields.

Liposomes as topical drug carriers:

In terms of lipid content, liposomes are similar to the lipid bilayer in the stratum corneum, which allows lipid transfer proteins in the cell membrane to easily recognize liposomes and cause lipid exchange. Subsequently, liposomes will pass through the epidermal barrier more effectively than traditional dosage forms [58]. It has been found that most topically applied liposomal medications concentrate in the stratum corneum's upper layers, acting more like a "reservoir" to deliver a more localised impact. [17, 26, 31]. Liposomes can be neutral or negatively or positively charged on the surface, depending on the pH medium and the present functional groups [58]. Liposomes have different sizes depending on their preparation technique[3]. Liposomes can be multilamellar (>0.5 µm), oligolamellar (0.1-1 µm), unilamellar (small unilamellar, medium sized unilamellar, large unilamellar and gigantic unilamellar) and multivesicular vesicles (1 µm) [4]. As a result, the size of liposomes can range from 25 nm to 100 nm for small unilamellar vesicles all the way up to 500 nm or more for large uni-lamellar vesicles [58].

Melatonin, amphotericin B, ketoprofen, methotrexate, estradiol and indinavir and cyclosporine are just a few examples of medications that have been successfully given by liposomes applied directly to the skin[22-24]. Liposomes have a high cost with a short shelf-life due to the presence of polyunsaturated fatty acids in natural / animal or plant based phospholipids. (polyunsaturated fatty acids can be easily oxidized upon storage)[4]. In order to overcome the drawbacks of liposomes, modifications were made to the composition of traditional liposomes, creating new types of lipid vesicles known as niosomes, transfersomes, and

ethosomes, all of which are more flexible and ultra-deformable and hence better able to carry medications through the deeper skin layers.

3.2. Niosomes:

Niosomes are non-ionic surfactant vesicles that contain cholesterol and single-chain surfactant molecules. These nanoparticles are very similar to liposomes in structure i.e, both are amphiphilic and both function as a drug reservoir for local skin disorders[3]. Niosomes can be taken orally, intravenously, or applied topically, and come in a variety of dose forms, including suspensions and semisolids. Studies were done using niosomes for transdermal drug delivery such as propranolol hydrochloride, capsaicin, ketoprofen, baclofen, salidroside, , resveratrol, alpha-tocopherol, curcumin, diclofenac sodium, rofecoxib, simvastatin, sulfadiazine sodium and tyrosol[40].

Cholesterol concentration, surfactant used, and ionic charge are all variables that affect vesicle entrapment and in vitro release [40]. To improve the horny layer qualities, niosomes were considered to do two things: decrease trans epidermal water loss which will enhance stratum corneum hydration and loosens its tightly packed cellular structure and by increasing smoothness through replenishment of lost skin lipids following fusion to corneocyts [40]. Since niosomes have surfactant capabilities, they can alter the stratum corneum's structure to make it more loose and permeable [26]. Niosomes are cheaper, easier to prepare and store and have higher stability in comparison to liposomes[4].

3.3. Transferosomes:

They are a novel vesicular system made up of phospholipids and an edge activator. Single chain surfactants (such as sodium cholate, Span 60/65/80, and Tween 20/60/80) are commonly used as edge activators because of their propensity to destabilise lipid bilayers, hence improving vesicle deformability and lowering interfacial tension. With this property, transferosomes can conform to and squeeze through pores only 5-10 times their own diameter when subjected to the transdermal water gradient [12].

They have been reported to penetrate intact skin when applied non-occlusively on skin. This feature allows transferosomes to behave like a depot (reservoir) for topical drugs and facilitates systemic delivery in case of transdermal drugs for low and high molecular weight drugs e.g: proteins, peptides, hormones, insulin and interferons which cannot be administered orally since they undergo quick degradation in the harsh environment of the gastro intestinal system[12]. Besides, their huge molecular weight, restricts their penetration into deep skin layers. However, transferosomes successfully allowed simple transportation of these substances through skin[33]. Additionally, tetanus toxoid and other vaccines e.g: hepatitis-B vaccine have been administered using transdermal transferosomes. Transferosomal formulations have high entrapment efficiency approximately 90 % for lipophilic drugs, however are less stable and more expensive than niosomes. Studies on Diclofenac, Ketotifen and corticosteroids as well have been carried out. The Swiss regulatory body (SwissMedic) granted marketing authorisation for ketoprofen in a transfersome formulation in 2007; the medicine is planned to be commercialised under the brand Diractin.

When compared to oral administration of plain ethinylestradiol medication and topical application of conventional liposomes, ethinylestradiol flexible vesicles exhibited significantly greater anti-ovulatory effects. Lidocaine and tetracaine transferosome-based formulations achieved penetration levels equivalent to those achieved via subcutaneous injections. To treat skin cancer, transfersome technology was used to provide anti-cancer medications such methotrexate transdermally. Numerous studies have been conducted on additional medications, such as hormones and peptides like estradiol, low molecular weight heparin, retinol, melatonin, raloxifene HCL, and lisinopril dehydrate, among others[34, 35].

3.4. Herbosomes or phytosomes:

The term herbo is short for "herb," which signifies plant, and "some" is short for "cell-like." In the last century, researchers in the fields of phytochemistry and phytopharmacology have characterised the chemical compositions, biological activity, and health effects of countless plant products.

The majority of physiologically active substances in plants are either polar or water-soluble. However, water-soluble phytoconstituents (such as flavonoids, tannins, glycosidic aglycones, etc.) are inefficiently absorbed due to either their large molecular size, which prevents passive diffusion, or their poor lipid solubility, which severely restricts their ability to cross lipid-rich biological membranes. Both of these factors contribute to the low bioavailability of water-soluble phytoconstituents.

Herbosomes are also sometimes called phytosomes, which is a more scientific name. Phytosomes are the vesicular drug transport system that makes it easier for low-soluble drugs to be absorbed and used by the body. Phytosomes are made up of phospholipids and natural active phytochemicals that are linked in their structures.

They are made by reacting phosphatidylcholine (or any hydrophilic polar head groups) with plant extracts in an alkalotic solvent [6].

Phytosomes are complex structures composed of phospholipids and naturally active phytochemicals. They are produced via the combination of botanical extracts with phosphatidylcholine (or other hydrophilic polar head groups).

Traditional herbal extracts don't compare to the pharmacokinetics and pharmacodynamics profiles of herbosomes, which are far superior. Herbosomes can be taken orally or topically[35]. Herbosomes enhance the oral absorption of the non-lipophilic botanical extract through the intestinal lumen. Herbosomes have a more favourable stability profile than liposomes because, unlike liposomes, chemical linkages are established between the phosphatidylcholine molecules and the phytoconstituents [4].

Phosphatidylcholine, which is employed in the manufacture of herbosomes, serves not only as a carrier but also as a hepatoprotective. As a result, the use of hepatoprotective phytochemical substances produces a synergistic action with along with the hepatoprotective effect of phosphatidylcholine. To date, vesicular drug delivery systems are proven to be the most popular nanocarrier for phytochemicals [6].(Table 1).

Table 1: illustrates various vesicular systems carring some phytochemical constituents.

Name of vesicular system	Name of phytochemical constituents
Liposomes	*Aphanamixis polystachya leaf* *Curcumin* *Eleusine coracana* *Anthocyanins*
Niosomes	Carum Carvi Lawsone *Fumaria officinalis*
Transferosomes	*Mulberry leaves* Apigenin *Epigallocatechin-3-gallate (EGCG)* *Emodin*
Ethosomes	*Thymoquinone* *Capsaicin* *Paeonol*

3.5. Sphingosomes:

Sphingolipid is a type of cellular lipid (Figure 4). J.L.W. Thudichum gave them their name in 1884 due to their unusual nature. Sphingolipids are characterised by having a hydrophobic body that is joined to a polar head. Sphingolipid is a type of polar lipid that has been related to the composition as well as the structure of the lipids found in human skin, particularly in the epidermis layer. Sphingosomes can be delivered via subcutaneous, intravenous, intraarterial, intramuscular, oral, transdermal, and a variety of other drug delivery methods.

Sphingosomes have an intracellular pH ratio of 75 to 25 mol%/mol% (but preferably 55 to 45 mol%/mol%) and are composed of sphingolipids (sphingomyelin) and cholesterol [48].

Sphingolipids can be found in a variety of natural sources, such as mammalian milk, and more specifically bovine milk, brain, egg yolk, and erythrocytes animal blood, mainly sheep's blood.

Natural sources of sphingolipids include mammalian milk, especially bovine milk, brain, egg yolk, and erythrocytes derived from animal blood. Also, sphingolipids may be synthetic or semi-synthetic. Sphingosine and ceramide are the simplest sphingolipids whereas the most complex sphingolipids include sphingomyelin (SM) and glycosphingolipid. Sphingosome membrane lipids have features that allow them to penetrate since they belong to the same chemical compound family as epidermal lipids[48].

Sphingosomes can be made from various kinds of sphingolipids, such as sphinganines, hexadecasphinganines, lysosphingomyelins, lysoglycosphingolipids, N-acylsphingosines, gangliosides, Glucuronosphingolipids, Phosphoglycosphingolipids, and phosphoglycosphingolipids [32].

Sphingolipids are solely linked together by amide and ether bonds. In comparison to the ester linkage of lecithin, they are more resistant to hydrolysis. Additionally, they have fewer double bonds than lecithin, making them more resistant to rancidity. Because of the aforementioned factors, sphingosomes are more stable than phospholipid liposomes[32, 48]. Sphingosomes were utilized in cosmetic industry to prepare beclomethasone sphingosomes for skin inflammation and MOIST™, a skin product used for skin cleansing and make-up removal[48].

3.6. Invasomes:

Invasomes are a form of liposomes that are flexible and consist of phospholipids, ethanol, and either one terpene or a combination of terpenes.

Ethanol improves the fluidity of lipids within the vesicles' structure, providing a soft and flexible shape that is less rigid than typical liposomes and, as a result, enhancing their skin permeability [32]. It has been reported that terpenes increase penetration by disrupting the compact structure of the SC lipids.

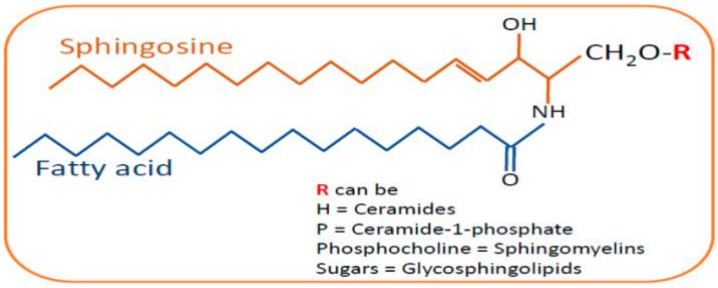

Figure 4. Structure of Sphingolipid

These two important constsituents: terpenes and ethanol lead to enhancement of the permeability of the invasomes through their role as penetration enhancers. Also, terpenes and ethanol, make the invasomes very soft and flexible and also, break up the SC bilayer skeleton. 2-methoxyestradiol. The efficacy of 2-methoxyestradiol invasomes as a lung cancer treatment fortified with apamin (2ME-INVA-APA) was tested and showed significant better apoptotic activity compared to plain formula or 2ME alone. Thus fortunately, 2ME-INVA-APA could easily seep through the cell membrane and induce apoptosis in relatively low doses[5].

3.7. Ufasomes:

The potential of unsaturated fatty acids like oleic acid to form vesicles was first reported by Gebicki and Hicks[57]. Ufasomes, which are also known as unsaturated fatty acid vesicles, are a type of closed lipid bilayer comprised of fatty acids and ionized soaps forming a colloidal suspension. When compared to liposomes, ufasomes are superior to liposomes in terms of both stability and cost because fatty acids are cheaper than other lipids [10, 32]. Several drugs have been incorporated in ufasomal topical gel such as etodolac, a non-steroidal anti-inflammatory drug for local pain relieving effect on desired area of the body as well as roxithromycin, a newer antibiotic for acne treatment[19 37]. Due to the lipophilic nature of fatty acids , ufasomes can enhance penetration of lipophilic drugs through stratum corneum such as clotrimazole ufasomes that were prepared by Bolla *et al.*[10].

3.8. Ethosomes:

Ethosomes are nanovesicles made of phospholipids and rich in ethanol (20 %–45 %). Ethosomes were designed and characterized extensively by Touitou *et al.*[55]. When studying the impact of different system components on the size of ethosomal vesicles, researchers found that increasing the amount of lipid in the system produces larger vesicles having multilamellar structure, while increasing the concentration of ethanol at a constant lipid concentration leads to a decrease in vesicular size.

3.8.1. Application studies on ethosomes:

Ethosomes are non-invasive delivery systems. The capacity of ethosomes to enhance drug penetration through the skin and entry into the circulatory system of the body has led to their use in the transdermal delivery of medications in recent years [2, 38]. Ethosomal carrier can entrap and deliver both hydrophilic and hydrophobic moieties.

Trihexyphenidyl hydrochloride (THP) is a pyscoactive drug used in parkinsonism. According to one investigation, the steady-state transdermal rate of THP ethosomes consisting of phospholipid, ethanol, and water rose by 87-fold compared with normal liposomes [47].

The presence of high % ethanol encourages the development of lamellar-shaped vesicles, which allow better solubility and entrapment of a wide variety of drugs such as nitroglycerine, estradiol, testosterone, methotrexate, progesterone, nifedipine, minoxidil, tetrahydrocannabinol (THC) or other cannabinoids, xanthines, anxiolytics (diazepam and others), diclofenac (and other non-steroidal anti-inflammatory drugs), antiepileptic (valnoctamide and others), antibiotics, tocopherol, corticosteroids, 5-Fluorouracil, acyclovir, zidovudine, colchicine, prazosin, papaverine, peptides, insulin, ketoconazole and other antifungals [42, 46, 55].

Many reports about the effectiveness of ethosomal drug systems both in-vitro and in-vivo were published [13, 42].

Unlike transfersomes, ethosomes can enhance skin delivery of drugs under both occlusive and non-occlusive situations. The key to facilitating the transport of vesicles into the skin is a transepidermal osmotic gradient, which can only be established through a non-occlusive application[54].

Lipoduction, a modern ethosomal cellulite treatment marketed by the U.S. company Osmotics

Inc., decreased the appearance of cellulite by up to 80 percent in less than 60 days (Table 2).

3.8.2. Mechanism of drug penetration by ethosomes:

Ethanol is an excellent ***permeation enhancer***. Yet, previous studies showed that percutaneous penetration from ethosomal systems was more efficient than hydroethanolic solutions or from ethanol alone.

1-Ethanol effect:

At body temperature, the stratum corneum's lipidic multilayers are tightly packed and well-organized. Ethanol acts as a fluidizer because it interacts with the polar head group area of lipid molecules in the stratum corneum which will cause a decrease in the phase transition temperature (Tm) of these lipids and consequently promotes permeability and elasticity of the skin (Figure 5).

2-Ethosomes effect:

Ethanol imparts softness and flexibility to the vesicles, enabling them to penetrate more easily, fuse with intercellular lipids, and release medicines along the penetration pathway.

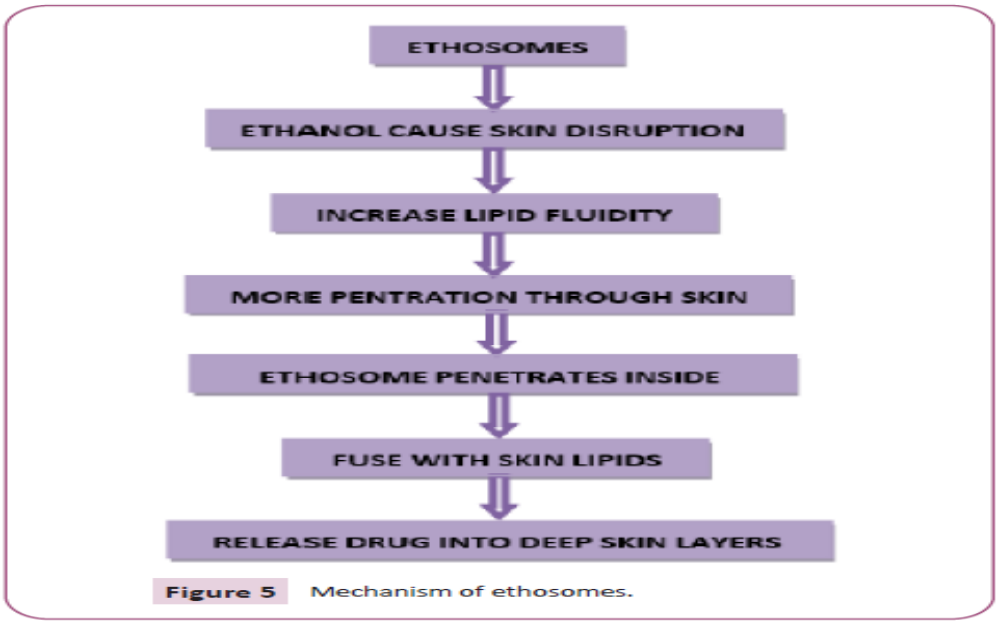

Figure 5. Schematic representation of ethosomes skin permeation mechanisms

3.8.3. Methods of ethosomal preparation:

There are primarily two common methods for preparing ethosomes. Both are quite simple and convenient, and neither requires any complicated instruments. [50].

1. Hot Method:

Colloidal phospholipid solutions are produced by heating phospholipids in water to 40 degrees Celsius in a water bath. Propylene glycol and ethanol are combined in a different vessel and heated to 40 °C. The organic phase is introduced to the aqueous phase once both solutions have reached 40 °C. Depending on its solubility, the drug may be dissolved in ethanol or water. The vesicle size of the ethosomal formulation can be reduced to the desired extent by probe sonication or extrusion method[8].

2. Cold Method:

This is the most reasonable and most common approach for preparing ethosomal systems [44]. It was introduced by Touitou and her co-workers in 1996. In a closed vessel, both phospholipid and drug are dissolved in ethanol or mixture of solvents (ethanol /propylene glycol) at room temperature or at 30 °C until an organic phase is obtained [16, 23]. The aqueous phase can be water or buffer solution or normal saline solution [15, 20, 23, 24, 52]. The aqueous phase is introduced to the organic phase in a dropwise manner [14, 44, 55]. The mixture is stirred at a speed of 700–2,000 rpm using a magnetic stirrer [14]. Ethosomal dispersion is achieved through mixing for 5-30 minutes [9, 14]. Depending on its solubility, the used medication can be dissolved in either the aqueous or organic phase [2, 18, 49]. Sonication or extrusion can be used to reduce the ethosomal vesicle size to the desired level [1]. Afterwards, the formulation should be stored in the fridge [41, 43].

3.8.4. Characterisation of ethosomes[39]:

3.8.4.1. Vesicle size and Zeta potential**:** Particle size and zeta potential are assessed by dynamic light scattering (DLS).

3.8.4.2. Entrapment Efficiency: The entrapment efficiency of ethosomes is evaluated using the ultracentrifugation technique. The vesicles are separated in a high-speed cooling centrifuge for 90 minutes at 20,000 rpm while maintaining a 4°C temperature. The supernatant is collected and amount of drug in the supernatant is assessed by UV spectrophotometry. The entrapment efficiency can be calculated by the following equation:

$$Entrapment\ efficiency\ \% = \frac{Total\ drug\ content - free\ drug\ content}{Total\ drug\ content} * 100$$

3.8.4.3. Vesicle shape: The surface morphology of the ethosomal vesicles is examined using scanning electron microscopy (SEM) and transmission electron microscopy (TEM).

3.8.4.4. Stability studies: The ability of ethosomal formulations to maintain the medication was tested by storing the vesicular preparations at different temperatures, namely 25±2°C, 37±2°C and 45±2°C for varying lengths of time. Using DLS and TEM, the size and morphology of the vesicles are monitored in order to evaluate the stability of ethosomes.

3.8.4.5. Skin permeation studies: By employing confocal laser scanning microscopy (CLSM), the capability of ethosomal preparation in penetration into the various layers of the skin can be ascertained.

3.8.4.6. Transition Temperature: By means of differential scanning calorimetry, the transition temperature of the vesicular lipid in question can be identified

3.8.4.7. Surface tension measurement: The ring method can be used for measuring the surface tension activity of a drug in aqueous solution with the use of a Du Nouy ring tensiometer.

Safety of ethosomes:

Ethosomes are composed of components that are GRAS, which stands for "generally regarded as safe." Extensive research, both in vitro and in vivo, has been conducted to investigate the safety of ethosomal systems that are applied topically to the skin. Research conducted using in-vitro cell cultures demonstrated that ethosomal systems do not cause any damage to human skin cells [30, 56]. After single and chronic application of ethosomal systems containing various molecules (such as buspirone HCl, ibuprofen, testosterone, cannabidiol, etc.), histological examination of the skin at the treatment site revealed no changes in the structure and thickness of the horny layer, as well as no infiltration of inflammatory cells into the skin.

In a study, when comparing the ibuprofen ethosomal gel group with the control group using blood biochemical analysis of rat liver, kidney, and muscle function tests, there were no statistically significant differences found [54]. In another investigation conducted by Paolino et al., reflectance spectrophotometry was employed to evaluate the skin tolerability of ethosomal systems in healthy human participants [44]. The ethosomal systems did not cause skin erythema 12, 24, or 48 hours after application, in contrast to the hydroethanolic solution with the same water-to-ethanol ratio.

Table 2: Some marketed ethosomal products:

Product name	Active ingredient	Application	Manufacturing country
Cellutight EF	Combination of ingredients	Topical anti-cellulite cream	USA
Nanominox	Minoxidil (4%)	Hair tonic for alopecia	Germany
Body shape	Combination of ingredients	Reduces the cellulite and stretches the skin	Israel
Skin Genuity	Combination of active anticellulite agents	Reduces the cellulite	UK
Decorine cream	Combination of ingredients	Antiaging and in hyperpigmentation	USA
Osmotic Lipoduction Cellulite cream	Pure grape seed extract (antioxidant)	Reduce the cellulite and burn fat on skin application	Israel
Noicellex	Combination of ingredients	Topical anti-cellulite cream	Israel
Supravir cream	Acyclovir	Treatment of herpes infection	Israel

5. CONCLUSIONS

Transdermal drug delivery is a crucial tool for certain drugs like proteins, hormones which undergo destruction in the GIT or drugs that cause undesirable side effects to organs like hepatotoxicity and damage to normal cells like anticancer, anti-psoriatic and anti-fungal drugs. Many

pharmaceutical, veterinary and food applications have utilised vesicular systems to boost the transdermal passage of hydrophilic, high molecular weight and potent drugs. Today, ethosomal and non ethosomal drug systems are gaining more attention by researchers due to their cost-effective, easy preparation and high entrapment as well as moderate shelf-life to successfully launch new products in the transdermal market.

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