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Review Article

Ethosomes: A Vesicular Carrier as a Novel Tool for Transdermal Drug Delivery System

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Abstract

In recent years, nanotechnology has a new era of drug delivery for many drug systems. Transdermal drug delivery is one of the few systems that have recently become a major focal point of research. When compared to traditional drug delivery systems, the Transdermal Drug Delivery System (TDDS) has various advantages. Due to the barrier properties of the Stratum Corneum, TDDS has had limited market success. The introduction of ethosomes, which are soft, malleable vesicular carriers containing ethanol and are tailored for enhanced delivery of active agents, has opened up a new area of vesicular research for transdermal drug delivery. According to various reports, ethosomes have a promising future in improving the efficacy of transdermal delivery of various agents. Ethosomes are also useful for the non-invasive delivery of small, medium and large drug molecules. Ethosome preparation is simple, requires no complicated equipment, and can thus be scaled up to industrial levels. The review emphasises introduction to ethosomes, their composition and preparation methods, evaluation, and their efficiency in penetrating the skin and the use of ethosomes in transdermal medication delivery as being more effective than liposomes or hydroalcoholic solution in terms of quantity and depth.

Keywords: Ethosomes, Novel carriers, Transdermal Drug Delivery System

Introduction:

The most broad and flexible method for administering both systemic and topical medications is through the skin. The skin's, outermost layer stratum corneum acts as the skin's most reliable barrier against drug penetration, reducing the bioavailability of medications when administered topically. Hence, in order to overcome the natural skin barrier, it is essential to investigate and evaluate the numerous carriers required for systemic medicine delivery. Transdermal drug delivery is a less invasive approach to medication administration that provides controlled drug distribution, less frequent dosing, patient compliance, and prevention of first pass metabolism [1–3] Transdermal delivery can provide a number of advantage including enhanced efficacy increased safety, improved patient compliance. This route of drug administration avoids the hazards and discomfort associated with parenteral therapy and improves patient compliance[3]. Transdermal route is an interesting option in this respect because transdermal route is convenient and safe[4]

Transdermal drug delivery system encounters the barrier properties of the horny layer (Stratum Corneum) and hence only the lipophilic drugs that have molecular weight <500 Da can pass through it. TDD has some other therapeutic benefits such as sustained drug delivery to provide a steady state plasma profile and hence reduced systemic side effect, thus generating the potential for improved patient compliance, the bypass of first pass metabolism effect for drug with poor oral bioavailability[5,6].

Vesicles are small structures with a bilayer arrangement similar to the natural lipid bilayer structure of our body membrane. They are extremely effective at encapsulating drugs with a wide range of physicochemical properties. The stratum corneum is generally regarded as the major impediment to good drug penetration through the skin, and these vesicular structures are able to easily overcome it [1]. The amphiphillic nature of vesicles aids in the delivery of both hydrophilic and lipophilic drugs to their respective targets[2]. Liposomes were previously developed as a pioneer model in the vesicular delivery system. Vesicles play an important role in both cellular communication and particle transport. Researchers came to the conclusion that vesicular morphology aids in drug delivery and that vesicles can be tagged for cell specificity, resulting in a targeted action. The liposomes were further modified for improved properties, leading to the discovery of ethosomes, which many consider to be a major advancement and advantage in vesicular research [3].

Ethosomes are highly malleable, soft lipid vesicles and has an enhanced chance to attain deeper penetration into the skin as well as systemic circulation. Their size can vary from 10 nanometres to a very few microns and contain a very high concentration of ethanol (20-45%) and a lower content of water[4,5]. They are considered as the modified successors to classical liposome with increased ethanol content[6]. The action

of ethosomal permeation is mainly by lipid bilayer disturbance in skin caused by the ethanol which enhances their penetrability[7]. Fluidized lipids in the membrane and high flexibility of the vesicular membrane together help them to even squeeze through the pores in stratum corneum which are smaller than them[8]. The major constituents are phospholipids(phosphatidylcholine, phosphatidyl serine, phosphatidic acid)generally used at 0.5-10% concentration along with high concentration of alcohol(ethanol or isopropyl alcohol),glycols such as transcutol and propylene glycol enhance permeation or act as edge activators. Cholesterol is generally used at 0.1-1% range. These advancements have helped in producing several marketed formulations as well as newer types of ethosomes such as transethosomes for enhanced therapy.

Vesicular systems

Liposomes: They are microscopic sized water containing vesicles similar in structure to skin phospholipid bilayer structure .The phospholipid chain from soya or egg yolk and cholesterol in some cases [9]. Mezei was a lead in the use of liposomes as delivery agents. It only helped in delivering the medication to the reservoir in the top layer of skin; no direct absorption was achieved, addressing the need for sophisticated features. Studies with liposomes showed increased miconazole nitrate deposition with limited penetrability in the top skin strata. [10].

Niosomes: They are similar to classical liposomes in their composition except for the use of non-ionic surfactants thus giving them greater stability and lower cost. The mechanisms depends on physico-chemical properties of drug, vesicle type as well as lipids used [11]. Fluconazole niosomes with span 60, span 40 and brij 72 prepared by thin film hydration method exhibited sustained drug release and higher cutaneous retention [12]. Cyclopirox is another drug delivered with better efficiency in a different study.

Transferosomes: They are also known as ultra-deformable vesicles or liposomes due to greater elasticity and deformability. Phospholipids along with surfactants of different types provide the flexibility and is used as an efficient delivery system for transdermal as well as topical delivery of drugs, genetic materials and vaccines study of clotrimazole loaded ethosomes lead to the finding that the drug flux was found to be more in the system than normal transferosomes which ultimately proved the higher efficiency of ethosomes as a vesicular delivery system[12].

Spingosomes: They are concentric bilayered vesicles where the aqueous compartment is completely enclosed by bilayered membrane composed of natural or synthetic spingolipids and has a size range of 0.05 to 0.45 microns. They are more stable and have increased circulation time compared to normal vesicular systems because they are made of only amide and ether linkages with lesser number of double bonds than lecithin. They are ideal in targeting tumours and gene delivery and immunology studies. It was used in cancer therapy according to saraf et al in 2001. Spingosomes are prepared using spingomyelin based cholesterol that impart them characteristics like resistance against oxidation and acid hydrolysis giving them greater stability in plasma as well as increased circulation time leading to better bioavailability. The oral as well as transdermal mode of use was coined by Webb et al in 1996.Major limitations of the system are limited use because of highly expensive spingolipids and also decreased entrapment efficiency.

Pharmacosomes: Pharmacosomes are the potential alternatives for conventional vesicular systems. Pharamcon means drug and some means carrier and thus they constitute of colloidal dispersion of drug covalently bound to lipids and may exhibit ultra-fine vesicular, micellar or hexagonal aggregate forms depending drug- lipid complex structure. Certain drugs with a carboxyl group or an active hydrogen group can be esterified producing an ampiphilic prodrug containing system. It is a self-assembled nanoparticle system and can be used to load more amount of drug and can have a low interfacial tension, higher contact area leading to enhanced bioavailability.

Virosomes: Virosomes are spherical, unilamellar phospholipid bilayered vesicles incorporating virus derived proteins so that it can fuse with target cells. The nucleocapsid and genetic material of source virus is incorporated within the envelope and for influenza virus resistance, lipids are intercalated with membrane proteins like haemagglutinin and neuraminidase thus enabling them to transfer the drug to target cell cytoplasm. The viral surface glycoprotein is contained in the vesicles and size ranges from 120-180 nanometres.

Colloidosomes: They are hollow shelled microcapsules consisting of coagulated or fused particles at the interface of the emulsion droplets. They have highly versatile and flexible application as their membrane offers greater potential in controlling permeability of entrapped species ensuring selective and timed drug release. They system is only at its developmental stage as wider utility is not prevalent.

Aquasomes: It is a three layered self-assembled nanoparticle system with ceramic carbon nanocrystalline particulate core coated with glassy cellobiose that helps in specific targeting and molecular shielding.

Cubosomes: These are systems which have been experimentally put into use for herbal medicine delivery for the KIOM-MA 128 drug used in atopic dermatitis treatment. The permeation feature of M-A 128 was enhanced using cubosomes compared to suspension form.

Ethosome: Ethosome is another novel lipid carrier, recently developed by Touitou et al. (2000), showing enhanced skin delivery. The ethosomal system is composed of phospholipid, ethanol and water. The size of ethosomes varies from few nanometres' to micrometres depending on method of preparation and application of techniques like sonication.

Major types of ethosomes based on composition

Classical ethosomes: They are actually modification of classical liposomes with high alcohol content(45%w/w).they have enhanced entrapment efficiency and higher negative zeta potential compared to classical ethosomes. Molecular weight ranges from 130.07Da to 24kDa.Thus have greater stability as well as increased permeation.

Binary ethosomes: They were introduced first by zhou et al .They are binary because they are made by adding another alcohol in to the formulation for enhancement of ideal properties. The commonly added alcohols include propylene glycol (PG) and isopropyl alcohol (IPA).

Transethosomes: The new generation of vesicular systems developed by song et al in 2012. They are similar to classical preparations but contains an additional component in the form of an edge activator(surfactant mostly) and/or penetration enhancer. The novel delivery system combines the ideal properties of classical ethosomes as well as the elasticity and deformability of transferosomes as in one formulation known as transethosomes. They were reported to have superior and beneficial characteristics compared to classical ethosomes. They are capable of entrapping drug which have a molecular weight ranging from 130.077Da to 200-235kDa.

Impact of major constituents on ethosomal properties

Table 1:

PARAMETER	CLASSICAL ETHOSOMES	BINARY ETHOSOMES	TRANSETHOSOMES
Composition	Phospholipids Ethanol Stabilizer Charge inducer Water Drug	Phospholipids Ethanol Propylene glycol other alcohol Charge inducer Water Drug	1. Phospholipids 2. Ethanol 3.Edge activator (surfactants) 4. Charge inducer 5. Water 6. Drug
Skin Permeation	Higher than classical liposomes	Equal to or higher to classical ethosomes	Typically higher than classical ethosomes
Entrapment efficacy	Higher than classical liposomes	Usually higher than classical ethosomes	Mostly higher than classical ethosomes
Potential	Negatively charged	Negatively charged	Positively or Negatively charged
Size	Smaller than the classical liposomes	Equal to or smaller than classical ethosomes	Based on edge activator permeation enhancer concentration

Ethanol: Ethanol is considered to be of great use as an ideal penetration enhancer. The amount of ethanol used was found to range between the concentrations of 10%to50%in most of the studies Various researches over the years have given enough evidence stating that increasing the ethanol concentration has an impact on vesicular size thus the size of vesicles are decreased to some extend with increased ethanol concentration. It should be noted that the increase in concentration above a specific limit which is considered as optimum after which further increase may lead to a slight increase in the vesicular size. This case also causes a very high reduction in the entrapment efficiency also. The actual reason behind the decreasing vesicular size is due to the interpenetration of the ethanol hydrocarbon chain that leads to a reduction in vesicular membrane thickness that reduces the size of vesicles significantly .But the very high content may not give fruitful results as it causes the solubilisation of the vesicle as such and the ideal process of drug delivery is not attained .The net charge on the system is also affected by ethanol as the surface charge which is expressed as the zeta potential helps to build some steric stability due to negative charge given by the ethanol. Bendas and Tadros are two researchers who in their studies revealed that the vesicular size decreased around 44.6% compared to classical liposomes when ethanol concentration of 40% was used in the ethosomal preparations [13]. The vesicular zeta potential has a great impact on the properties of the system. It produces effects on stability as well as skin interaction of the vesicle. Dayan and Touitou found that net negative charge was imparted to the vesicles by ethanol. Thus this further was found to enhance the vesicle stability as it makes use of the principle of electrostatic repulsion between the components. Increasing ethanol content increases the entrapment efficiency. Another important aspect that is greatly improved by ethanol is the solubility of lipophilic as well as amphiphillic drugs which shows a greater dissolution profile or solubility when the ideal concentration of ethanol which is 20-40% is made use of in the formulation. Entrapment efficiency shows an increasing trend until a very high concentration of alcohol is reached where it predominantly leads to dissolution of the vesicles itself where the entrapment efficiency is diminished [14].

Phospholipids: The amount as well as the type of phospholipids used can have an influence on the vesicular structure and other properties .Soya and egg phosphatidyl choline mostly lecithins are used in general. The variations arising in the source can also affect the vesicular size, zeta potential, stability as well as penetration power. Phospholipon 90H, Phospholipon 80H and soya phosphatidyl choline were used by Prasanthi Lakshmi et al so as to reach the conclusion that revealed the major impact of source of the phospholipids on ethosomal size as well as vesicular entrapment efficiency[15]. Shen in his research found out that higher phospholipid content enhanced vesicular stability greatly. The optimal range of the component is found to be 0.5% to5% .It was found that there was a moderate or slight increase in the size with increased concentration .This trend of increasing size is only observed till a point is reached. Entrapment efficiency increases with increase in phospholipid concentration till a particular level after which no more effect is observed on the entrapment efficiency [16].

Cholesterol: The component finds lesser use in the preparation process and is steroidal moiety having a very complex structure on the whole. It is capable of imparting good rigidity which further leads to reduced leakage and higher stability of the system. It also enhances entrapment efficiency and is used at concentrations less than 3% [17]. It can also have a very high concentration in certain cases that is around 70%.most studies reveal that they cause a slight or moderate increment in the vesicular size. The above mentioned idea is made clear by the results of a certain study wherein increase in the concentration from 0% to0.15% caused a great increment of the vesicular size from 102±13 nanometres to 152±12 nanometres. Very high concentrations of cholesterol may often have a negative impact as the rigidity exceeds the required limit and the deformability of the vesicles may be reduced greatly further causing a hindrance to penetration through stratum corneum as it is commonly seen in case of certain multi lamellar vesicles.33nn. Such rigidity related problems were addressed by various other researchers [18].

Propylene glycol: The use of propylene glycol as the component in a binary ethosomes showed a significant decrease in particle size from 103.7±0.9 nm to 76.3±0.5 nm when 0% to 20% v/v of propylene glycol was used. Many researches also identified propylene glycol as an agent that greatly improves drug stability as well as drug distribution. Higher entrapment efficiency was also noted in many cases. Enhancement of drug permeation is based on the relative ratios of ethanol and propylene glycol. Terbinafine hydrochloride showed a higher skin deposition when ratio used was 7:3.They increased stability by enhancing viscosity as well as by preventing hydrolysis Classical ethosomes have lower stability than binary ethosomes when stored at 40C[19]

Isopropyl alcohol: It was clear from the studies conducted by Dave et al that isopropyl alcohol had an influence over enhancing drug entrapment efficiency in the system. The study was based on finding out various properties of 3 different formulations namely a) classical ethosomes containing 40% alcohol in the form of ethanol, b) binary ethosome containing both ethanol and isopropyl alcohol in 20% each concentration, c) vesicular system which contain 40% of isopropyl alcohol. Further the comparison studies revealed that the vesicular system had the highest entrapment efficiency at 95% .The in vitro release studies carried out showed the vesicular system showed the lowest release rate in 8hrs.It also had least transdermal flux than other preparations. It was concluded that isopropyl alcohol had an enhanced effect on entrapment efficiency but a reduced effect on drug release.[20]

Dicetyl phosphate: This component was found to have minimal influence on the prevention of aggregation of the ethosomes and thus increases stability of system. It is usually used at a range of 8-10% of the total phospholipids used in the formulation. They are capable of producing vesicles with sharp negative zeta potential [21].

Penetration enhancers/edge activator: Penetrability of drug is an important aspect of the topical drug delivery systems. Several ethosomal systems are available but it is the transethosomal preparation which has a very high success rate in such delivery .They makes use of certain extra components compared to the classical ethosomes called penetration enhancers or edge activators.

Tweens and spans: Tween 80 was generally used at 10-50% concentration of the total phospholipids used. It was attributed to have a positive impact on the system by reducing the vesicular size while increasing stability and skin permeation characteristics. The major action has been explained as a direct outcome of their solubilising property as well as their innate ability to prevent fusion of the vesicles. A study where Tween 20 was found to be used to prepare transethosomes produced ethosomes with smaller size, higher entrapment efficiency and enhanced ex vivo skin permeation through human skin than Tween 80.Span 20 was found as ideal component in preparing transethosomes containing caffeine and vitamin E [22].

Oleic acid: Vesicle size, elasticity, zeta potential and skin permeability are enhanced by oleic acid as it alters the stratum corneum properties to improve the fluidity of the layer. It is usually a penetration enhancer when used at a low concentration of 0.5%.The oleic acid containing transethosomes have a negative zeta potential, higher skin permeation and greater drug disposition in rat dermis/epidermis under the studies conducted.

Polyethylene glycol 4000: The studies that were conducted using transethosomes containing mycophenolic acid revealed a major result that they have a very positive impact on essentially increasing the vesicular size while it produced no visible effect on other properties like entrapment efficiency, permeability and vesicular stability [23].

L-menthol: They are penetration enhances that were reportedly used in transethosomes containing 5%ascorpic acid .It enhanced the release rate and showed increased release pattern of the drug through human skin cadaver. A higher release of (36.5%) was obtained which was higher than that observed in case of classical ethosomes after 24 hrs. The process that is responsible for the higher release is the formation eutectic mixture of drug and L-menthol leading to enhanced solubility of the drug and also leads to alteration of the barrier characteristics of stratum corneum layer

Ethosome Preparation techniques:

The first two techniques that are most widely used are relatively simpler than others as it lacks the use of highly sophisticated technology.

Hot method: The phospholipids are dispersed on the whole into water taken and the colloidal solution is formed by heating at 400C.ethanol and propylene glycol is heated up to 400C in a separate vessel. The organic phase is added to aqueous phase .further the drug is dissolved either in alcohol or water based on its hydrophilicity/lipophilicity, which in turn determines its solubility in either of the solvents. Size of the obtained vesicles is reduced by subjecting to probe sonicator or by extrusion technique. The storage was properly done afterwards [24].

Cold method: This is the method having the greatest popularity among all the methods, a covered vessel is used to dissolve phospholipids, drug and other lipid constituents in alcohol mostly ethanol at room temperature, this process is followed by vigorous stirring. Polyols like poly ethylene glycol is added during the stirring process at 40○C.The arrangement is allowed to be heated in a water bath at 300C.Water that is separately heated at 300C. Is added and mixed for 5 minutes in a covered vessel. The process of size reduction of vesicles to the desirable levels done by sonication and extrusion. The use of proper temperature that fulfils the demands of the preparation is essential. It implies the necessity of a refrigerated storage. The aqueous phase used can be water, buffer solution or normal saline [25].

Mechanical dispersion or thin film hydration method: Phospholipids namely soya phosphatidyl choline and organic solvents namely chloroform and methanol in the ratio 3:1 is taken in a round bottom flask. The organic solvents are further removed from the contents by use of a rotary evaporator above the transition temperature of the lipid molecules present. This leads to the formation of a thin lipid film on the walls .It is further kept in vacuum overnight for the removal of the traces of solvent remaining .The hydration of the film by a hydro ethanolic solution of drug at an ambient temperature is done with or without the use of sonicator for sonication process. The product is cooled to room temperature and the suspension is stored under refrigeration. The lipid is heated and rotated during hydration process and these parameters are set depending on the properties of phospholipids.30 minutes process .1hr or 6hr [26].

Classic method: The phospholipids, drug and ethanol were mixed till the drug dissolution at a temperature of 30OC using a water bath .The fine stream of double distilled water were added to the mixture with continuous stirring at 700 rpm in a closed vessel. Homogenization was carried out by passing the resultant suspension through polycarbonate membrane with a hand extruder for 3 cycle.

Reverse phase evaporation technique: This is the rarest method and finds very less use. Production of large unilamellar vesicles are its major use. Diethyl ether is used as the organic phase to dissolve the phospholipids. It is mixed with aqueous phase at 3:1 ratio in ultrasonic bath at for 5 minutes which leads to the formation of water in oil emulsion. The pressure is reduced to a minimal to bring about the removal of the organic phase which in turn leads to the formation of colloidal dispersion on mechanical agitation in a vigorous manner [27].

Ethanol injection-sonication method: Organic phase made use of is the ethanolic solution of phospholipids which is further injected into the aqueous phase by using a syringe system. The flow rate is maintained at 200 µL/minutes.

Homogenization is further done using ultra sonic probe in 5 minutes.

Ethosomal drug penetration mechanism:

The mechanism of absorption of drug from ethosomal vesicles is still not clear in idea, but the proposed mechanism of penetration involves 2 steps in concise. Ethanol effect and the ethosomal effect together bring about the high level penetration of the system into deeper skin layers.

Ethanol effect: Ethanol is an important constituent that improves penetrability of the drug to a great extent. Generally it acts by interacting with the lipid bilayer membrane by increasing its fluidity and subsequently decreasing the thickness and density of the multilayer membrane. Further stratum corneum is the major layer that hinders the permeation but in this case a much greater permeability and reduced blockage due to ethanol interaction is guaranteed.

Ethosomal effect: Ethosomes as such has its own merits and acts by fusing with the skin lipid bilayer structurally and due to already increased membrane fluidity are delivered into deeper layers of the skin and their ability to fuse with skin lipids enables them to achieve better release characteristic [28]. Permeation studies were conducted using fluorescent probes with different physicochemical properties like rhodamine red , rhodamine B,B-carotene , rhodamine 6G for vesicle filling. On the whole the vesicular softness depends on the transition temperature of lipids in the system. The amount of ethanol as well as phospholipids play an important part in determining the drug entrapment efficiency and particle size which is pivotal in determining the leakage parameters as well as penetration.

Advantages of ethosomal drug delivery:

Ethosomes have a greater impact on enhancing the permeability through skin which is ideal in transdermal and intercellular drug delivery. They help in better and efficient delivery of larger constituent groups (proteins and peptides. The technique is relatively simpler compared to other physical methods like iontophoresis and sonophoresis. The use of non-toxic raw materials has its own benefits and being a non-invasive and passive technique it has immediate marketability and commercial utility. It provides a better patient compliance along with greater stability and solubility than many other systems. Particle size is also smaller to acceptable limits. Ease of industrial scale up is another important feature as large quantities of ethosomes can be easily prepared without the use of rather sophisticated technology or equipments, Thus proprietary technology boosts market attractiveness on the whole .They thus find wider domain of usefulness in the fields of biotechnology, pharmaceutical, cosmetics and veterinary fields. Lipophilic and hydrophilic drugs can be incorporated into the vesicle so that most of the drugs that lack permeation and solubility can be easily delivered .As the vesicle mimics the skin and its phospholipid bilayer structure, it can further enhance the availability of potent drug at the site of action and thus bring about targeted and better therapy.

Limitations of ethosomal drug delivery

The yield from ethosomal formulations can be poor at times; if not properly prepared. The loss of drug while transfer from organic to aqueous media is of considerable importance Ineffective shell locking in certain cases may lead to coalescence as well as loss of much needed stability. Molecular size of the drug to be loaded should be reasonable so all types of drugs cannot be delivered by the method .In certain cases it may be uncomfortable to wear or irritation causing rendering them less useful. The uneconomical aspect involved in their pricing is also a great deal of hindrance.

Characterization Studies of Ethosomes

SEM/TEM Imaging: Visualization of ethosomes can be done using transmission electron microscopy (TEM) and by scanning electron microscopy (SEM). This provides visual information about the size, shape, surface morphology, lammelarity etc. Different lipid types might influence the surface morphology or shape of the particles. **Vesicle size distribution and Zeta potential:** Particle size and zeta potential can be determined by a zeta-sizer or dynamic light scattering (DLS) using a computerized inspection system and photon correlation spectroscopy (PCS). The size of ethosomes ranges between tens of nanometers to microns and is influenced by the composition of the formulation.

Zeta potential is an important and useful indicator of particle surface charge, which can be used to predict and control the stability. In general, particles could be dispersed stably when the absolute value of zeta potential was above 30 mV due to the electric repulsion between particles.

Entrapment Efficiency: The entrapment efficiency of drug by ethosomes can be measured by the ultracentrifugation technique. The vesicles are separated in a high speed cooling centrifuge at temperature maintained at 4°C. The sediment and supernatant liquids are separated; amount of drug in the supernatant is determined by UV-Visible spectrophotometry. From this, the entrapment efficiency is determined by the following equation,

$$EE\% = \frac{\text{actual drug loading}}{\text{theoretical drug loading}} \times 100\%$$

In-Vitro Skin Permeation Study: In-vitro skin permeation of drugs is studied using Franz diffusion cell. The excised skin from abdomen of male nude rats (Touitou et. al., 2000, Mustafa M.A. Elsayed et. al., 2005), rabbit pinnae (Giuseppe Lucania et. al. 2005), human cadaver (Zhen Zhang et. al. 2011) etc. is separated from the adhering fat and/or subcutaneous tissue. The skin is mounted between donor and receptor compartment with the stratum corneum side facing upward into the donor compartment. Phosphate buffer saline pH 7.4 was taken in the receptor compartment. The formulation was applied on the skin in donor compartment which was then covered with aluminum foil to avoid any evaporation process. Samples were withdrawn at predetermined time intervals over 12/24 hours, and suitably diluted to analyze the drug content. The receptor medium was immediately replenished with equal volume of fresh medium to maintain the sink conditions throughout the experiment. The percentage of drug release was plotted against time to find the drug release pattern.

Skin Retention Study: The amount of drug retained in the skin is determined at the end of the 12 hours in-vitro permeation studies. The formulation remain in the in-vitro permeation experiment is removed by washing with distilled water. The receptor content is replaced by 50% v/v ethanol and kept for further 12 hours with stirring and the drug content was estimated. This receiver solution diffused through the skin, disrupting any liposome and ethosome structure and extracting deposited drug from the skin.

Vesicular stability: The abilities of the formulations to retain the drug content and shape are analyzed at different temperatures I.E., 25 ± 2°C (Room Temperature, Rt), 37 ± 2°C And 45 ± 2°C For Different Periods Of Time (1, 20, 40, 60, 80 And 120 Days).Nitrogen gas was flushed and was kept in sealed vials. Stability can be found by analyzing the size and structure of vesicles over time and the mean size is measured

by DLS while structural changes are estimated by making use of transmission electron and nanosystems for biomedical applications microscopy.

Patented and marketed formulation of ethosome:

Prof. Elka Touitou and her students from the Department of Pharmaceutics at the Hebrew University Faculty of Pharmacy created and copyrighted the ethosome. 19,20. Hebrew University's Novel Therapeutic Technologies Inc (NTT) has been successful in launching a number of products based on the ethosome delivery technology.

1. Noicellex TM an anti –cellulite formulation of ethosome is currently marketed in Japan.

2. Lipoduction TM another formulation is currently used in treatment of cellulite containing pure grape seed extracts (antioxidant) is marketed in USA. Similarly

3. Physonics is marketing anti – cellulite gel Skin Genuity in London.

4. Nanominox© containing monoxidil is used as hair tonic to promote hair growth. It is marketed by Sinere.

References

1. Cal K. Skin disposition of menthol after its application in the presence of drug substances. Biopharmaceutics & Drug Disposition. 2008 Nov; 29(8):449-54. https://doi.org/10.1002/bdd.631

2. Cevc, Gregor.Lipid vesicles and other colloids as drug carriers on the skin. Adv. Drug Deliv. Rev. 2004; 56:675-711. https://doi.org/10.1016/j.addr.2003.10.028

3. Nair SS, Chitosan-based transdermal drug delivery systems to overcome skin barrier functions, Journal of Drug Delivery and Therapeutics. 2019; 9(1):266-270 https://doi.org/10.22270/jddt.v9i1.2180

4. Verma DD, Fahr A. Synergistic penetration enhancement effect of ethanol and phospholipids on the topical delivery of cyclosporin A. Journal of controlled release. 2004 May 31; 97(1):55-66. https://doi.org/10.1016/j.jconrel.2004.02.028

5. Dhadwal A, Sharma DR, Pandit V, Ashawat MS, Kumar P, Cubosomes: A novel carrier for transdermal drug delivery, Journal of drug delivery and therapeutics 2010; 10(1):123-130. https://doi.org/10.22270/jddt.v10i1.3814

6. Touitou E, Dayan N, Bergelson L, Godin B, Eliaz M. Ethosomes-novel vesicular carriers for enhanced delivery: characterization and skin penetration properties. Journal of controlled release. 2000 Apr 3; 65(3):403-18. https://doi.org/10.1016/S0168-3659(99)00222-9

7.] Rao Y, Zheng F, Zhang X, et al. In vitro percutaneous permeation and skin accumulation of finasteride using vesicular Ethosomal carrier. AAPS PharmSciTech.2008; 9:8605. https://doi.org/10.1208/s12249-008-9124-y

8. Honeywell-Nguyen PL, Bouwstra JA. Vesicles as a tool for transdermal and dermal delivery. Drug discovery today: technologies. 2005 Mar 1; 2(1):67-74. https://doi.org/10.1016/j.ddtec.2005.05.003

9. Barry BW. Novel mechanisms and devices to enable successful transdermal drug delivery. European journal of pharmaceutical sciences. 2001 Sep 1; 14(2):101-14. https://doi.org/10.1016/S0928-0987(01)00167-1

10. Patel S. Ethosomes: A promising tool for transdermal delivery of drug. Pharma Info. Net. 2007; 5(3).

11. Sahin NO. Niosomes as nanocarrier systems. Nanomaterials and nanosystems for biomedical applications. 2007:67-81. https://doi.org/10.1007/978-1-4020-6289-6_4

12. Chauhan P, Tyagi BK, Herbal novel drug delivery systems and transfersomes. Journal of Drug Delivery and Therapeutics, 2018; 8(3):162-168 https://doi.org/10.22270/jddt.v8i3.1772

13. Elsayed MM, Abdallah OY, Naggar VF, Khalafallah NM. Lipid vesicles for skin delivery of drugs: reviewing three decades of research. International journal of pharmaceutics. 2007 Mar 6; 332(1-2):1-6. https://doi.org/10.1016/j.ijpharm.2006.12.005

14. Fang YP, Tsai YH, Wu PC, Huang YB. Comparison of 5-aminolevulinic acid-encapsulated liposome versus ethosome for skin delivery for photodynamic therapy. International journal of pharmaceutics. 2008 May 22; 356(1-2):144-52. https://doi.org/10.1016/j.ijpharm.2008.01.020

15. Verma P, Ram A. Effect of different penetration enhancers on skin permeation of drug using ethosomal carrier systems. J Curr Pharm Res. 2011; 5:42-44.

16. Limsuwan T, Amnuaikit T. Development of ethosomes containing mycophenolic acid. Procedia Chem. 2012; 4:328-335. https://doi.org/10.1016/j.proche.2012.06.046

17. Liu X, Liu H, Liu J, He Z, Ding C, Huang G, Zhou W, Zhou L. Preparation of a ligustrazine ethosome patch and its evaluation in vitro and in vivo. International journal of nanomedicine. 2011 Jan 2; :241-7. https://doi.org/10.2147/IJN.S16044

18. Prasanthi D, Lakshmi PK. Development of ethosomes with taguchi robust design-based studies for transdermal delivery of alfuzosin hydrochloride. International current pharmaceutical journal. 2012 Oct 3; 1(11):370-5. https://doi.org/10.3329/icpj.v1i11.12063

19. Akhtar N, Pathak K. Cavamax W7 composite ethosomal gel of clotrimazole for improved topical delivery: development and comparison with ethosomal gel. AAPS pharmscitech. 2012 Mar; 13: 344-55. https://doi.org/10.1208/s12249-012-9754-y

20. Zhou Y, Wei Y, Liu H, Zhang G, Wu XA. Preparation and in vitro evaluation of ethosomal total alkaloids of Sophora alopecuroides loaded by a transmembrane pH-gradient method. Aaps Pharmscitech. 2010 Sep; 11:1350-8. https://doi.org/10.1208/s12249-010-9509-6

21. Zhou Y, Wei Y, Liu H, Zhang G, Wu XA. Preparation and in vitro evaluation of ethosomal total alkaloids of Sophora alopecuroides loaded by a transmembrane pH-gradient method. Aaps Pharmscitech. 2010 Sep; 11:1350-8. https://doi.org/10.1208/s12249-010-9509-6

22. Ma M, Wang J, Guo F, Lei M, Tan F, Li N. Development of nanovesicular systems for dermal imiquimod delivery: physicochemical characterization and in vitro/in vivo evaluation. Journal of materials science: materials in medicine. 2015 Jun; 26:1-1. https://doi.org/10.1007/s10856-015-5524-1

23. Daddona P. Macroflux® transdermal technology development for the delivery of therapeutic peptides and proteins. Drug Deliv Tech. 2002 Jun; 2(5).

24. Touitou E, Godin B, Dayan N, Weiss C, Piliponsky A, Levi-Schaffer F. Intracellular delivery mediated by an ethosomal carrier. Biomaterials. 2001 Nov 15; 22(22):3053-9. https://doi.org/10.1016/S0142-9612(01)00052-7

25. Cortesi R, Ravani LA, Zaid AN, Menegatti EA, Romagnoli RA, Drechsler MA, Esposito EA. Ethosomes for the delivery of anti-HSV-1 molecules: preparation, characterization and in vitro activity. Die Pharmazie-An International Journal of Pharmaceutical Sciences. 2010 Oct 1; 65(10):743-9.

26. Raza K, Singh B, Lohan S, Sharma G, Negi P, Yachha Y, Katare OP. Nano-lipoidal carriers of tretinoin with enhanced percutaneous absorption, photostability, biocompatibility and anti-psoriatic activity. International journal of pharmaceutics. 2013 Nov 1; 456(1):65-72. https://doi.org/10.1016/j.ijpharm.2013.08.019

27. Maestrelli F, Capasso G, González-Rodríguez ML, Rabasco AM, Ghelardini C, Mura P. Effect of preparation technique on the properties and in vivo efficacy of benzocaine-loaded ethosomes. Journal of liposome research. 2009 Dec 1; 19(4):253-60. https://doi.org/10.3109/08982100902788408

28. Patil R, Patil S, PatilS. Ethosomes a versatile tool for novel drug delivery system. J. Current. Pharm res. 2014; 4:1176-1177