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Therapeutic Potential of Herbal Ethosome in Applied Nanotechnology

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Review Article

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Abstract: Phytomedicines are becoming more popular in the world for their ability to cure diseases with less toxicity and better therapeutic efficacy. Herbal medicines may also have disadvantages of poor bioavailability, toxicity, stability issues and patient compliance. In order to minimize these problems various drug delivery systems such as liposomes, phytosomes, niosomes, ethosomes and trasferosomes etc are being developed for phytomedicines. Novel drug delivery systems can improve bioavailability of drug that refers to the existence of drugs in the body part where they are actually needed. Ethosomes are noninvasive flexible vesicular carriers that enable the drugs to permeate through the deeper layers of skin and systemic circulation. They are mainly composed of phospholipids, high concentration of ethanol and water. As ethanol is known for its disturbance of skin lipid bilayer arrangement; therefore, inclusion of ethanol into a vesicular membrane provides the ability of vesicle to permeate through the stratum corneum. The high flexibility of ethosomal carrier from the added ethanol allows the elastic carrier to squeeze through the skin pores. Herbal ethosomal formulation has been effectively used to enhance bioavailability of many herbs including *Glycyrrhiza glabra, Sophora alopercuroides, Cannabis sativa, Sesbania grandiflora* and *Podophyllum hexandrum.*

Keywords: Phytomedicines, Ethosomes, Ethanol, Phospholipids, Vesicular carrier.

INTRODUCTION

Human skin is the outer covering and largest organ of the integumentary system. It has always been a challenge to efficiently administer drugs by topical application.

To overcome the barrier properties of skin, which has numerous advantages; many approaches have been considered, including chemically assisted methods and chemical permeation promoters. Though skin is the most easily accessible organ of the body, the outer layer of the skin which is called as stratum corneum, is also the most resistible barrier to drug penetration across the skin. Therefore, special carriers are required to combat skin barrier to administer drug molecules into the circulation system. Ethosomal system is a system of drug administration across skin [1, 2].

Over the past few years several ideas have came into view which has given tremendous popularity and rapid progress to transdermal delivery formulations over conventional formulations like, steady permeation of drugs across the skin, allowing consistent serum drug level, similar to iv infusion, it also achieves consistent plasma levels, but noninvasive in nature, toxicity develops from a drug administered transdermally could be moderated by removing the patch, it can be used as an alternative delivery system for patients who cannot tolerate oral dosage forms, drugs that causes gastrointestinal upsets can be a good candidates for transdermal delivery because it avoids direct effects on stomach and intestine.

Ethosomal systems are novel lipid vesicular carriers prepared using phospholipids (phosphatidylcholine, phosphatidylserine, phosphatitidic acid), ethanol (in high concentration) and water. High concentration of ethanol enhances delivery drug to the infected nail plate, skin and prolongs the physical stability of ethosomes. Ethosome transfer active substances more efficiently through the stratum corneum into the deeper layers of the skin. Ethosomes can entrap hydrophilic, lipophilic and amphiphilic drug molecule. Large molecules like proteins, peptide molecule is possible to deliver, increased skin permeation, non toxic in comparison to oral drug delivery system as it eliminates gastrointestinal interference & first pass metabolism of drug. Different preparation techniques (cold, hot and classic method) are used to prepare these carriers. Inclusion of ethosomal dispersion in gels, patches and creams make it easy for application and stability purposes. Ethosomal systems are classified into three categories viz classical ethosomes, binary ethosomes and transethosomes. Difference between them is on account of zeta potential, skin permeation property and stability [3-5].

Ethosomal systems differ from lipsomes because they contain relatively high concentration of

ethanol in addition to water and phospholipids. New generation of ethosome system are developed by adding other compounds to basic classical ethosome to enhance skin permeation and vesicular characteristics. However there has been no clear distinction between the classical and new generation ethosomes [6, 7].

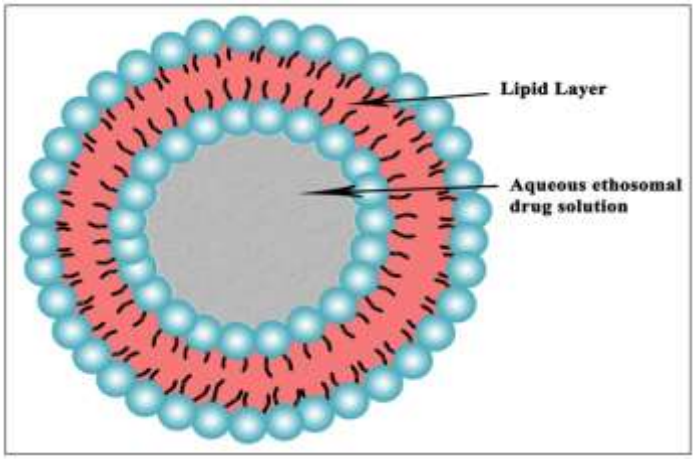


Fig-1: Anatomy of ethosome

DIFFERENT CATEGORIES OF ETHOSOME

Classical ethosomes

They are modified classical liposomes which show better skin permeation. They are composed of phospholipids, a high concentration of ethanol up to 45% w/w and water. Classical ethosomes are found to be excellent over liposomes for transdermal delivery of drug on account of their smaller size, and negative zeta potential and higher entrapment efficiency [8, 9].

Binary ethosomes

They were developed by adding a different type of alcohol to the classical ethosomes. Commonly used alcohols are propylene glycol and isopropyl alcohol [10].

Transethosomes

This is known as the new generation ethosomal system. It consists of basic component of classical ethosomes and an additional component which is either a penetration enhancer or an edge activator i.e. surfactant [11].

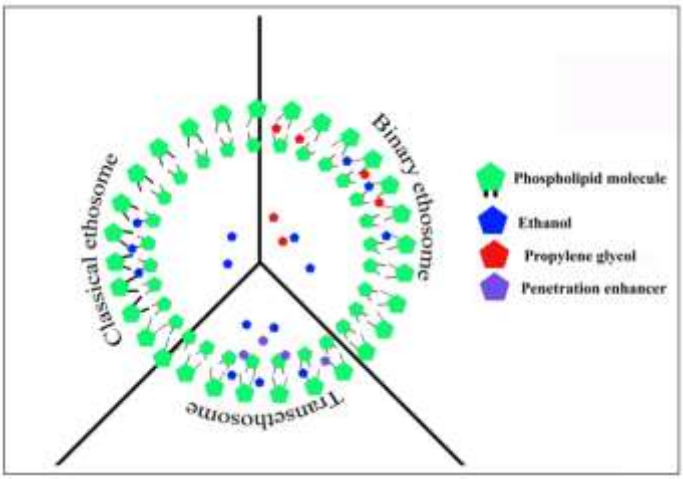


Fig-2: Schematic representation showing different types of ethosome

EFFECTS OF MATERIAL USED FOR FORMUALATION

Ethosomal systems are basically composed of phospholipids, ethanol and water. Ethanol enhances penetration by giving the vesicles unique characteristics in terms of size, stability, entraption efficacy, skin permeability and zeta potential. The effect of ethanol on lipid systems came into existence during early 1990s. The investigative account of phase and packing properties of dipalmitoylphosphotidyl choline vesicles or multi-bilayer in the presence of ethanol, and then employment of high ethanol content was commenced by Touitou. Ethanol basically increases runniness and engorges the rigid lipid multilayer system of the stratum corneum, embodiment of high concentration of ethanol in ethosomes makes lipid vesicular membrane less

densely chock up which render positive attitude and flexibility to them resulting in the arrangement of distort and vesicular system which might get penetrate through minute openings which get formed in the disarranged layer in the corneum lipids more intensely in contrast to other vesicular system. A outrange of inquiry have covered 30-40% as the best range of ethanol in the evolution of balanced and successful ethosomes [12-16].

Phospholipids are one of the eminent vesicles forming component in ethosomes which are made of two layers of lipid molecules having hydrophilic head and a hydrophobic tail. Different phospholipids used in ethosomal formulations are phosphatidyl choline (PC) from soybean (90%), hydrogenated phosphatidyl choline from soybean (90%), hydrogenated phospholipids from soybean with 70% phosphatidyl choline, contained phosphatidyl choline (73%–79%), lysophosphatidylcholine (up to 6%), cephalin (up to 4%), and phosphatidic acid (up to 6%) of the dry residue; natural oils and sterol up to 6%; and ethanol (23%–27%), 1,2-dipalmitoyl-rac-glycero-3-phosphocholine(99%), phosphatidyl choline content (70%–75%) from soybean, phosphatidyl choline content (68%–73%) from soybean, phosphatidyl choline content (81.7%) from egg yolk (agglomerates) etc. Elsayed studied ethosomes by using phosphatidyl choline (PC) from soybean lecithin as carriers for delivery of ketotifen through skin. The ethosomes formed were evaluated in terms of vesicle size, entrapment efficiency, stability, in vitro permeation and skin deposition properties and they finally conclude that delivery of ketotifen through skin is more effective as compare to liposomes [17, 18].

Other ethosomal formulations include: Cholesterol which enhances the stability and entrapment efficiency of drug, dicetyl phosphate used to prevent agglomeration of the vesicles and enhance the stability of the formulation. Edge activators or penetration enhancers such as tween (20, 60, and 80), span (20, 40, 60, and 80), dimethyl sulfoxide and oleic acid etc are also use in ethosomal formulation [12].

Table-1: various additives used in ethosomal formulation

Class of Material	Uses with example
Phospholipid	Used as vesicles forming component Eg: Soya phosphatidyl choline, Egg phosphatidyl choline etc.
Polyglycol	As a skin penetration enhancer Eg: Propylene glycol, Transcutol RTM.
Alcohol	Used for providing the softness of vesicle membrane Eg: Ethanol, Isopropyl alcohol.
Cholesterol	For providing the stability to vesicle membrane Cholesterol.
Vehicle	Used as a gel former Eg: Carbopol 934.

EFFECT OF DRUGS/AGENTS IN THE PROPERTIES OF ETHOSOMAL SYSTEMS

- Physicochemical properties of the drugs/agents going to be incorporated because it may affect the ethosomal properties especially particle size and zeta potential.
- Lodzki noticed when trihexyphenidyl hydrochloride, buspirone hydrochloride, cromolyn sodium, and diclofenac sodium was incorporated in the ethosomal system, its size was decreased. The authors assigned this effect to the surface active properties of the incorporated drug. In contrast, Paolino noticed when paclitaxel was incorporated in ethosomal system; its size was increased [19, 41].
- Shumilov and Touitou described about the consigned frequently bare ethosomal system as pessimistic (-8.8 mV) and moved to certainty (7.16 mV) after consolidating of 30 mg buspirone hydrochloride into ethosomal system. An analogous determination was determined with trihexyphenidyl hydrochloride 0.5% w/w, where the vacuous ethosomes pessimistic charge (-4.5 mV) moved to an optimistic charge (4.8 mV). This effect was relying on the denseness of trihexyphenidyl hydrochloride added. Increasing the percentage of the drug to 1% and 3% w/w resulted in a correlative increase in ζ-potential values of 7.2 mV and 10.4 mV respectively [20, 21].

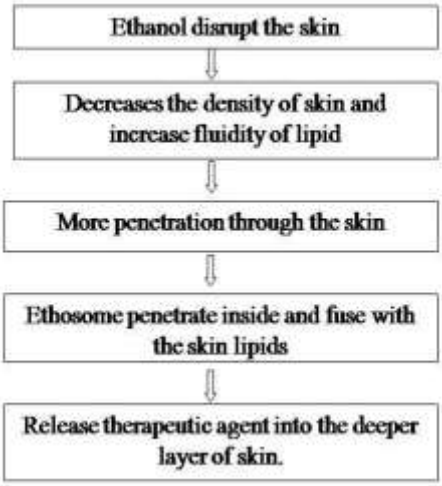
Table-2: recent studies on ethosome

Study	Results/conclusion	References
Ethosomes loaded with Cryptotanshinone (CPT) for the treatment acne.	An *in vivo* study proved that CPT loaded ethosomal gel had better anti-acne effect than a conventional gel and a CPT ethosomal gel may be a viable acne treatment in the future.	Zhenwei Yu *et al*., [53].
Ethosomal formulation containing griseofulvin (GRF) for topical treatment of fungal infections.	GRF-loaded ethosomes showed to be suitable systems for upper skin delivery of GRF, the developed formulation appears to be a potential candidate for further research studies, and to possibly join the expanding market of topical antifungals.	Joana Marto *et al*., [54].
Ethosomes-based hydrogel formulations of methoxsalen for enhanced topical delivery against vitiligo.	The ethosomes-based hydrogel formulation containing methoxsalen was found to be a promising carrier to enhanced percutaneous penetration with reduced phototoxicity and erythema, thus leading to improved patient compliance for the treatment against vitiligo.	Bhawna Jain Garg *et al*., [55].
Compound antimalarial ethosomal cataplasm: preparation, evaluation, and mechanism of penetration enhancement.	They demonstrate that ethosomal cataplasm could make a large quantity of antimalarial drug quickly penetrate through skin after transdermal administration. These could be favorable for drugs to kill *Plasmodium* spp. quickly and prevent the resurgence of *Plasmodium* spp. Antimalarial results showed that drug-loaded ethosomal cataplasm had very good antimalarial efficiency.	Shuo Shen *et al*., [56].
Novel elastic membrane vesicles (EMVs) and ethosomes containing aceclofenac for pain and inflammation.	The phospholipid-based vesicular systems, especially, ethosomes can be a promising tool to enhance the delivery and safety of aceclofenac by topical route.	Gajanand Sharma *et al.*, [57].
Nanoethosomes for dermal delivery of lidocaine.	The developed nanoethosomes are proposed as an efficient carrier for topical delivery of anesthetics such as lidocaine.	Soraya Babai *et al*., [58].
Improved anti-melanoma effect of a transdermal mitoxantrone (MTO) ethosome gel.	The MTO ethosome gel is an effective non-invasive melanoma therapeutic approach without the severe side effects that accompany intravenously injection of anticancer agents. The application of successful transdermal MTO lights up the hope for effective and convenient melanoma treatment.	Xiang Yu *et al*., [59].
Combination of nano-ethosomes and iontophoresis for transdermal delivery of vancomycin hydrochloride.	Combination of nanoethosomes and iontophoresis had succeeded in delivering vancomycin transdermally.	Magdy I. Mohammed *et al.*, [60].
Mechanism of transdermal permeation promotion of lipophilic drugs by ethosomes.	They conclude that ethosomes as a percutaneous drug carrier showed transdermal superiority over liposomes and hydroethanolic solution, due to the synergistic effect of their ingredients with the skin structures. During the percutaneous process, the vesicles might break up in the superficial layer of skin, allowing drugs to permeate into the deeper layer alone, thus allowing the phospholipid to be retained in the upper epidermis.	Li Yang *et al.*, [61]
Poloxamer 407-based intranasal thermoreversible gel of zolmitriptan-loaded nanoethosomes: formulation, optimization, evaluation and permeation studies.	Zolmitriptan-loaded ethosomes were formulated as a thermoreversible gel using thermoreversible polymer (poloxamer 407) and mucoadhesive polymers (carbopol 934 and HPMC K100). Zolmitriptan-loaded ethosomal intranasal gel could serve as a better alternative to existing dosage forms for effective treatment of recurrent migraine.	Santosh Shelke *et al.*, [62].

MECHANISM OF PENETRATION OF ETHOSOME THROUGH SKIN

Two simultaneous mechanism of action have been reported: Ethanol has a fluidization effect on lipid bilayer of ethosome and fluidization effect on the stratum corneum lipid which changes the arrangement and decreases the density of skin lipid. Ethosomes increases the deformability of prepared vesicles. Therefore, the highly flexible and soft ethosome vesicles penetrate the modified structure of the stratum corneum and forge a pathway through the skin. The release of the drug occurs by the fusion of ethosomes into cell membranes in the deepest skin layers. It is reported that transethosomes have more skin permeation capability as compared to classical ethosomes as it contain both ethanol and edge activator or permeation enhancer [22, 23].

Flow chart showing action of ethosome



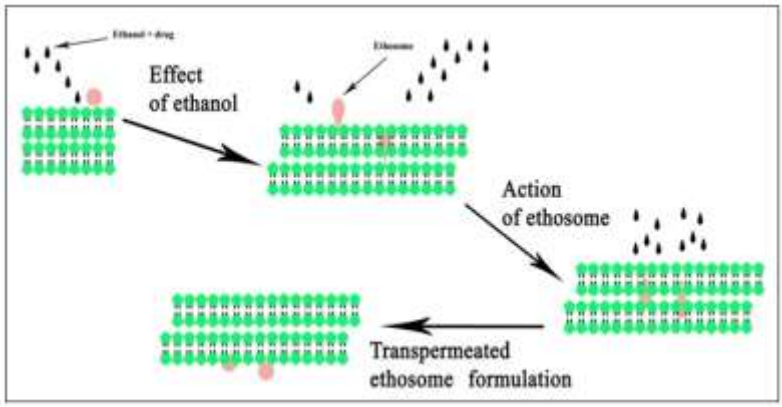


Fig-3: Mechanism of penetration of ethosome

METHOD/TECHNIQUE FOR ETHOSOMES PREPARATION

Ethosomal formulation may be prepared by hot, cold and thin film hydration method. All this methods are convenient and not require any sophisticated equipment for preparation.

Cold method

This is the simplest and most commonly used method for ethosome preparation. It was familiarized by Touitou in 1996 which involves two basic steps. In the first step i.e. organic phase is obtained by dissolving phospholipid and other lipid material in ethanol or mixture of solvents (ethanol/PG) at room temperature by vigorous stirring with the use of mixer with continuous addition of polyols such as propylene glycol etc. with constant stirring followed by heating at 30 ˚C in water bath. In the second step i.e. aqueous phase (water, buffer solution or normal saline solution) is heated at 30 ˚C in a separate vessel. The aqueous phase is added to the organic phase which is then stirred for 5 min in a covered vessel. The drug is dissolved in either the aqueous or the organic phase, depending on its physicochemical properties. The vesicular size of ethosomal formulation can be decreased to desire size by using sonication method. Finally, the formulation is stored at the refrigeration [24].

Hot method

In this method Phospholipid is dispersed in water in a water bath and heated at 40˚C until a colloidal solution is formed. Ethanol and propylene glycol are mixed in a separate vessel and heated to 40˚C.The organic phase is added to the aqueous one which is then stirred for 5 min and cool the suspension at room temperature. The drug can be dissolved in water or ethanol depending on its hydrophilic or hydrophobic properties. The vesicle size of ethosomal formulation can be modified by using probe sonication or extrusion [25].

Thin film hydration method

In this method phospholipids and drug are dissolved in chloroform: methanol in a ratio of 3:1 and kept in a round bottom flask and evaporated in rotary evaporator above lipid transition temperature i.e. above 60˚C until complete evaporation. The thin lipid film in the round bottom flask is then hydrated with phosphate buffer saline (pH 7.4) containing ethanol. The sample is

then sonicated for 5 min and then stored at the refrigerator [26].

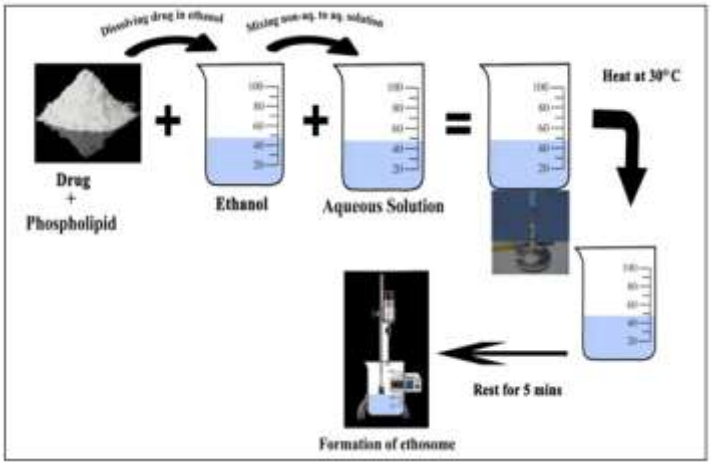


Fig-4: Cold method for preparation of ethosomal system

CHARACTERIZATION OF ETHOSOMAL SYSTEM

Vesicle visualization

Vesicular shape of an ethosomal system can be visualized by transmission electron microscopy (TEM) and scanning electron microscopy. Samples are dried on carbon-coated grid and negatively staining the formulation with aqueous solution of agents such as phosphotungstic acid etc. The vesicular structure of ethosomal formulation exhibited 300-400 nm in diameter [27, 28].

Vesicle size, size distribution and zeta potential

Vesicular size, size distribution and zeta potential of an ethosomal system can be determined by dynamic light scattering (DLS), by using a computerized Malvern Autosizer 5002 inspection system and photon correlation spectroscopy (PCS).The size ranges between nanometers and microns which is influenced by the composition of the formulation e.g. in the ethanol concentration range of 20–45%, the vesicles size increased with decreasing ethanol concentration, with the largest particles in preparations containing 20% ethanol (193±8nm) and the smallest particles in preparations containing 45% ethanol (103±9nm). Touitou *et al.*, [1, 19] reported that ethosome size exhibits a limited dependence on Phospholipid concentration [29, 30].

Bilayer configuration

Since the ability of ethosome to efficiently entrap lipophilic and hydrophilic drugs depend on the high degree of lamellarity, an investigative study of optimum bilayer formation is essential. This can be done by performing Nuclear Magnetic Resonance (NMR) studies. Entrapment efficiency of ethosomal formulation is higher than liposomes. Dayan and Touitou reported that entrapment efficiency of trihexyphenidyl hydrochloride increased from 36% for liposomes to 75% for ethosomes [1, 30].

Entrapment efficiency

This is carried out by two methods as described below-

Ultracentrifugation

This is a two-step method where in the first step ethosome preparation is kept overnight and subjected to ultracentrifugation for a particular period of time. In second step, pure drug is evaluated by any highly developed method, e.g. high-performance liquid chromatography (HPLC) then finally the entrapment efficiency is calculated by using the following relationship:

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

Where,

EE=Entrapment efficiency.
Dt= theoretical amount of drug.
Ds= the amount of drug detected only in the supernatant.

Dialysis

Dialysis bags are prepared by using polymers, e.g. cellulose acetate in which the calculated quantity of the drug-loaded vesicles or free drug in aqueous solution are placed which are transferred into 500 ml of phosphate buffer pH 7.0. The mediums are stirred with a magnetic stirrer. The samples are withdrawn at fixed time interval from the medium and replaced with equal volumes of phosphate buffer saline solution to maintain the sink conditions. Entrapment efficiency can then be finally calculated using equation mentioned below - [1, 31, 36].

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Transition temperature:

The transition temperature of vesicle lipid system can be measured by differential scanning calorimetry in an aluminium pan at 10˚C per min, under a constant nitrogen stream [32].

Confocal scanning laser microscopy:

It is used to check out the depth and mechanism of penetration of ethosomal preparation through the skin. Godin and Touitou showed better entrapment of fluorescent probes by ethosomes after conduction of confocal laser scanning microscopy (CLSM) and fluorescence activated cell sorter (FACS) studies [1,19].

Drug content

UV spectrophotometer is use to determine the drug content of ethosomes. This can also be evaluating by high performance liquid chromatographic technique.

Surface and interfacial tension measurement

It had been reported that the use of water-miscible solvents in the formulation of surfactant-free nanoparticles reduced interfacial tension resulting in increased stability. It had also been reported that lowering interfacial tension is effective for better stability of oil-in-water emulsions. This is important for stability of ethosomes since it is composed of lipid-in-water. The surface and interfacial tension activity of a drug can be measured by using the ring method in Du Nouy ring tensiometer, Wilhelmy plate, drop weight, drop volume and capillary rise methods [33].

Stability studies

Stability study of ethosomal formulation is one of the major factors since it reveals their ability to retain their constitution along with active therapeutic agents. Instabilities in ethosomal formulations are caused by hydrolysis or oxidation of the phospholipid and are assessed by leakage of the encoated drug and alterations in size due to fusion and aggregation. Alteration in size, size distribution, entrapment efficiency and aggregation of vesicles are very important parameters for monitoring the stability. These parameters can be assessed by transmission electron microscopy (TEM) or dynamic light scattering (DLS) [29, 34].

Vesicle fluidity

The fluidization of vesicle membrane influences release of the active pharmaceutical ingredients which can be determined by fluorescent anisotropy by using a suitable probe. The fluorescence anisotropy of a vesicle membrane is inversely proportional to membrane fluidity. The vesicle bilayer loaded with active pharmaceutical ingredients may influence layer aggregation and also increases the fluidity of membrane. Fluorescent anisotropy of both loaded and unloaded vesicles helps to evaluate the influences of presence of active pharmaceutical ingredients in vesicle [35].

APPLICATION OF ETHOSOME CARRIER FORMULATION

Ethosomes can be used for delivery of hydrophilic and impermeable drug through the skin. Ethosomes are mainly used to replace liposomes. Ethosomes are use as a carrier for delivery of various drugs-

Delivery of anti-parkinsonism drug

Mishra AD *et al.* developed an ethosomal formulation containing ropinirole hydrochloride which is an anti-parkinson drug for transdermal delivery. From the results they concluded that ethosomes are capable of the delivery of ropinirole hydrochloride into the systemic circulation [36].

Ethosomes for transdermal delivery of an antidiabetic drug

Siddhodhan S. Bodad *et al.* prepared and evaluated ethosomes for delivery of repaglinide (RPG) transdermally. As ethosomes containing RGP possesses the size of 0.171–1.727mm and entrapment efficiency of 75–92%, they demonstrated a significantly higher permeation rate (64–97% of the administered dose) across excised rat skin when compared to free the drug and its hydro alcoholic solution. This is because the lipid and ethanol concentration of ethosomes affected

the physicochemical attributes and performance of ethosomes. The flexible ethosomes enhances RGP permeation through the stratum corneum and make the availability of RPG for antidiabetic action. They also prolonged the antidiabetic effect of RPG for a longer period of time which reduces the dosing frequency in comparison to the equivalent oral dose. Finally they demonstrated that by using ethosome as a carrier, RPG can be successfully delivered through skin for the treatment of type II diabetes mellitus [37].

Pilosebaceous targeting

For pilosebaceous targeting Maiden *et al.*, prepared and evaluated minoxidil ethosomal formulation used on the scalp for the treatment of baldness. It was found that the amount of minoxidil ethosomal formulation accumulated into skin of nude mice was 2, 7 and 5 fold higher than ethanolic phospholipid dispersion; hydroethanolic solution and ethanolic solution of drug each containing 0.5% of the drug [38].

Ethosomes for delivery of anti-inflammatory drug

Abdul Ahad *et al.* developed nanoethosomes for transdermal delivery of meloxican. They prepared the ethosomes by varying concentration of phospholipid 90G, ethanol and sonication time. They reported that meloxicam formulation showed higher entrapment efficiency, lesser vesicular size and better transdermal flux through skin as compare to liposomes. The optimized formulation was then evaluated for *in vivo* anti-inflammatory activity in rat. They have found optimized formulation possesses vesicle size of 142.3 nm, entrapment efficiency of 78.25% and achieved transdermal flux of 10.42 µg/ cm²/h. Nanoethosomes showed better permeation through skin with an enhancement ratio of 3.77 as compared to rigid liposome. *In vivo* study of carbopol loaded nanoetosomal gel showed better percent of inhibition in rat paw edema as compared to oral administration of meloxicam [39, 40].

Ethosome for skin delivery

Nava Dayan and Elka Touitou prepared ethosome containing trihexiphenidyl HCL (THP) and investigate its delivery against classic liposomes. They have found that the THP concentration was increased from 0 to 3%, the vesicles size decreased from 154 to 90nm which is most likely due to the surface activity of THP as measured in the work (critical micelle concentration of 5.9mg/ml). And also the zeta potential value of ethosome increased from -4.5 to +10.4 when the concentration of THP was increased from 0 to 3%. When they compared with standard liposomes, they have found that ethosomes had a higher entrapment capacity and a more ability to deliver the drug to the deeper layers of the skin. After 18 hours experiment they have found that the quantity of THP remaining in the skin was significantly more from the ethosmoe than from liposome. Finally they have reported that ethosome may be a promising candidate for transdermal delivery of trihexiphenidyl HCL.

Marco Bragagni *et al.*, developed three types of vesicular formulation: liposomes, transferosomes and ethosomes containing celecoxib. This formulations were characterized for particle size, polydispersity index and encapsulation efficiency. They have found that ethosomes containing Tween 20 as edge activator not only showed the best characteristics like vesicle dimensions, homogeneity, and the highest encapsulation efficacy (54.4%) but also enabled the highest drug penetration through the skin, which may be due to the permeation enhancers such as ethanol and Tween 20. Therefore, they reported that among the various vesicular formulations, ethosomes containing Tween 20 could be a promising carrier for topical application of celecoxib to prevent skin cancer development and increase the effectiveness of anticancer drugs against skin tumors.

Yong-Tai Zhang developed a novel psoralen ethosomal formulation which can penetrate the stratum corneum and can target the site of action. An in vitro skin permeation study of psoralen-loaded ethosomes showed better permeability than liposomes. Transdermal flux of psoralen ethosomes and skin deposition were 38.89±0.32 mg/cm²/h and 3.87±1.74 mg/cm² respectively. Ethosomes showed better biocompatibility with human embryonic skin as the phosphatidylcholine present in ethosome vesicles improved their biocompatibility. Finally they reported that ethosomes could be a potential carrier for dermal and transdermal delivery of psoralen and also for other drugs that require deep skin delivery [41-44].

Delivery of anti-arthritic drug

Chao Fan *et al.*, developed ethosomes containing tetrandrine by pH gradient loading method. The transdermal flux of tetrandrine through rat skin and deposition of the drug in the skin from ethosomes was 2.1 and 1.7 fold higher than liposomes respectively. Finally they reported that ethosomes could be a promising candidate for topical delivery of tetrandrine ethosomes for the treatment of arthritis [45].

Delivery of anti-viral drugs

Jain *et al.* formulated ethosomes that could enhance the transdermal flux, prolong release and presented in a charismatic way for sustained delivery of zidovudine. Dubey *et al.*, prepared an ethosomal formulation comprising Indinavir which is an anti-HIV drug, and look into their transdermal delivery potential. Ethosomal formulation showed improved skin deposition ability and shorter lag time for indinavir.

Horwitz *et al.*, developed ethosomal system containing acyclovir and reported that a 5% acyclovir ethosomal preparation compared to the 5% acyclovir

cream showed better improvements in treatment of herpetic infections [46].

Transdermal delivery of hormones

Touitou *et al.* inquire the effectiveness of ethosomal systems for delivery of testosterone hormone and compared the permeability potential of ethosomal formulation containing testosterone across rabbit pinna skin with marketed transdermal patch of testosterone. From tested results they found permeability of testosterone from ethosomal system through skin is nearly 30 times higher than the marketed formulation [47].

Delivery of anti-fungal agent

Rahul G.S. Maheshwari *et al.,* prepared ethosomes and ultra-deformable liposomes for transdermal delivery of clotrimazole. Sarat Chandarn C *et al.* prepared ethosomes containing Ketoconazole [48].

Used in angina pectoris

Xingyan Liu *et al.,* developed a ligustrazine ethosomal patch and its evaluation was carried out in vitro and in vivo. Ligustrazine plays a role in expanding blood vessels, which increase coronary and cerebral blood flow, preventing platelet aggregation, inhibiting thrombosis, and improving the circulation [49].

Used as bronchodilator

Ehab R *et al.,* prepared ethosomal formulation of salbutamol sulphate which is a hydrophilic drug utilized as bronchodilator and equated its transdermal delivery potential with classic liposomes comprising different concentrations of cholesterol and dicetylphosphate. The experiment found that the size of vesicle falls significantly ($p<.05$) by decreasing cholesterol concentration and increasing dicetylphosphate and ethanol concentrations. The entangle effectiveness percentage significantly increased ($p<.05$) by increasing cholesterol, dicetylphosphate and ethanol concentrations. In-vitro permeation studies of prepared gels showed that ethosomal systems were more efficient in delivering salbutamol sulphate through mice skin than from liposomes [50].

Table-3: Various herbal ethosomal formulations [51, 52]

Botanical name	Formulation	Biological activity	Active constituents	Application of ethosomal Formulation
Glycyrrhiza Glabra	Ammonium Glycyrrhiza zinate Ethosomes	Anti inflammatory	Glycrrhizic Acid	Increases of *in vitro* percutaneous permeation and significantly enhanced anti-inflammatory activity.
Tripterygium wilfordi	Triptolide	Anti inflammatory	Diterpene Triepoxide	High entrapment efficiency, good percutaneous permeability
Podophyllum hexandrum	Podophy llotoxin	Purgative, antirheumatic, antiviral and antitumor	Etoposide Teniposide	Higher entrapment efficiency and enhance therapeutic effect
Sesbania Grandiflora	Sesbania ethosome	Anti-microbial	leucocyanidin cyaniding	Enhance transdermal permeation
Cannabis sativa	Cannabis ethosome	Rheumatoid arthritis	Tetrahydrocnn abi-diol(THC)	Improve patient compliance and skin permeation
Curcuma longa	Curcumin ethosome	Anti inflammatory	Curcumin	Improved bioavailability.
Sophora alopecuroides	Sophora ethosome	Antiendotoxic, anticancer, Anti inflammatory	Sophocarpine, matrine, oxymatrine, sophoridine,	Enhance drug delivery and Stability
Sophora Flavescens	Matrine ethosome	Cardio protective, Anti inflammatory	Matrine and oxymatrine alkaloid	Improve percutaneous permeation

CONCLUSION

Even after exhibiting promising therapeutic effects, most of the phytoconstituents fail to achieve bioavailability because of poor absorption. Large molecular sizes and low lipid solubilities are the prominent factors causing poor absorption of phytoconstituents resulting in to reduced bioavailability. Incorporation of these plant actives or extracts into vesicular carriers E.g ethosomes vastly improves their absorption and consequently bioavailability. Ethosomes are uniquely designed and tailored vesicles consisting high concentration of ethanol which makes them extra

malleable and expert enough to respond peripheral hassling by fluidizing and disturbing the stratum corneum lipids. Finally, it results in successful delivery of therapeutic agents deeply across the skin. These systems not only offers a superior prospect for the non-invasive delivery of small, medium and large-sized drug molecules but also provides simplified patient compliance and low-cost treatment. In response to the interest of researchers on ethanol-based vesicles, it is evident that these systems hold immense prospective in future on various grounds like their easy manufacturing, vastness in drug delivery and therapeutic effectiveness. All these perspective makes ethosomes a promising carrier in delivery of bioactive agents.

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