



Revolutionary Approach towards Transdermal Drug Delivery: Ethosomal Gels

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Authors' contributions

This work was carried out in collaboration among all authors. Author EOK designed the study and wrote the first draft of the manuscript. Author SA managed the literature survey. Author JP managed the literature searches, guided authors for drafting the manuscript according to the author guidelines, publishing the review article and being corresponding author for the manuscript. All authors read and approved the final manuscript.

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ABSTRACT

The human body is up of the skin which is the largest organ in the body and hence acts as a biological barrier that obstructs drug movement across the stratum corneum into the systemic circulation. The topical drug delivery system serves as a delivery system in which drugs are delivered for systemic circulation through the skin. Low diffusion rate across the stratum corneum is the main disadvantage of this system and for this limitation to be overcome, an Ethosomal formulation can be formulated which acts as a delivery system for the drug to be delivered across the biological barrier of the skin into the body. In ethosomal gel formulation, The prepared Ethosome is converted into a gel that can be applied to the skin what makes ethosomal gel formulation unique which enables drugs to reach the deep skin layers and/or the systemic circulation and enhance the delivery of active agents. In addition to this, it is also a malleable vesicular delivery carrier, soft and non-invasive. There is also a higher influx of drugs transdermally into the skin as ethosomal gel formulation allows drug to penetrate deep into the skin and enters systemic circulation. There is the development of new and novel therapies for the treatment of

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disease through the ethosomal drug delivery system as it is safe and effective and also easy to prepare. Topics ranging from preparation of ethosomes, Ethosomal gel, advantages and disadvantages, and characterization techniques are focused on in this review article.

Keywords: Transdermal; ethosomes; ethosomal gels; phospholipids; ethanol.

1. INTRODUCTION

Due to its easy accessibility, the transdermal route is one of the most attractive routes for the delivery of drugs. When compared with other routes of drug delivery such as the oral route, there is a promising result as there is the elimination of associated problems or barriers such as first-pass metabolism and Gastrointestinal tract interference. The transdermal drug delivery system (TDDS) showed good results when compared to the oral drug delivery system as there are no gastrointestinal interferences and first-pass metabolism of the drug. Problems associated with drug delivery not only related to drugs but also those of barriers like the skin has been overcome through the development of vesicular formulations [1]. A formulation that is conventional like liposomes cannot cross intercellular channels of the stratum corneum. Ethosomes are been formulated to overcome these limitations which are associated with the conventional lipid-based system.

2. ETHOSOMES

“Ethosomes are delivery carriers which enable drugs to reach the deeper layer of the skin such as stratum corneum and enter system circulation. They are soft and malleable vesicles and they enhance the delivery of drugs [2].

Ethosomes are mainly composed of phospholipids, (phosphatidylserine, phosphatidylcholine, phosphatidic acid), ethyl alcohol (ethanol) in high concentration, and

water. Ethosomes are termed as unique because of the ethanol content which disrupts the layer of the skin making it leaky and permeable and thereby making the drug cross the stratum corneum and enters systemic circulation. It also enhances drug delivery [1]. The structure of ethosomes is illustrated in Fig. 1.

2.1 Advantages of Ethosomal Drug Delivery [3]

1. It is non-toxic
2. Delivery of large molecules is possible using ethosomes
3. Permeation of drugs through the skin is enhanced.
4. Pharmaceutical, Veterinary, Cosmetic fields can apply the Ethosomal drug delivery system.
5. High patient compliance: There is high patient compliance an ethosomal drug delivery can be administered in semisolid dosage form(cream or gel)
6. Simple technique as compared to Iontophoresis and Phonophoresis.
7. Passive, noninvasive and is available for immediate commercialization.

2.2 Limitations of Ethosomes [4]

1. There is a Poor yield.
2. Where shell locking is not effective then the ethosomes may coalesce and fall apart on transfer into the water.
3. During the transfer from organic to water media, loss of product may occur.

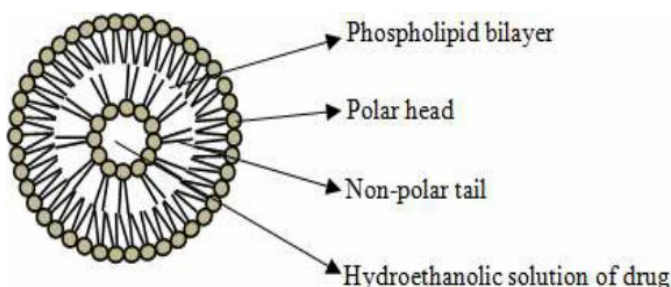


Fig. 1. Ethosomes structure

2.3 Mechanism of Penetration of Ethosomes

2.3.1 Effect of ethanol

Ethanol is present in a concentration of 20-50% in ethosomes and act as an effective penetration enhancer. Nevertheless, due to the interlocking effect of ethyl alcohol on supermolecule (lipid) bilayers, it was widely assumed that vesicles do not exist in high ethyl alcohol concentration. Touitou studied supermolecule sac systems and discovered that they contain ethyl alcohol in their composition and named them ethosomes. The structure of liposomes and ethosomes varies. The higher skin penetration potential of ethosomes was the key explanation for the suggestion of a synergistic effect of a combination of comparatively high concentrations of ethyl alcohol (20-50 percent) in sac form. The high concentration of ethanol (20-50%) will disrupt the skin lipid bilayer organization. The skin lipid bilayer organization can be disturbed by the high concentration of ethanol (20-50%) in ethosomal formulation. They reduce the thickness and compactness of the lipid multilayer of the cell membrane and also boost up the cell membrane fluidity as ethanol is known as a good penetration enhancer, they can disrupt the stratum corneum of the skin making it leaky, thereby making it possible for the drug to be delivered into the body [5].

2.3.2 Ethosomes effect

As the stratum corneum is been disrupted due to the effect of ethanol which acts as a good permeation enhancer and also known to boost up the cell membrane lipid fluidity, thereby making it easy for the ethosomes to permeate into the skin layers and bind with skin lipids to release the drug into the deeper layer of the skin [6].

2.4 Preparation of Ethosomes

There are different method which is been utilized for the preparation of Ethosomes. The three most common method include:

1. Cold method
2. Hot method
3. Classic mechanical dispersion method

2.4.1 Cold method

One of the commonly used methods for the preparation of Ethosomes. Firstly in this method,

Phospholipid in addition to cholesterol and drug is solubilized in ethanol with continuous stirring. This process is simultaneously carried out in a covered vessel and also at room temperature.

Next, with continuous stirring propylene glycol is been added at 40C. This mixture is further heated up to 30C. The mixture is further stirred in a covered vessel for 5 minutes.

Size reduction of the particles or mixture can be done or carried out through the process which is known as sonication or extrusion. Finally, It is stored under refrigeration [8]. The Fig. 2 shows the cold method process for the preparation of ethosomes.

2.4.2 Hot method

For the preparation of ethosomes using the Hot method, Firstly, at 40C, a colloidal solution is obtained by dispersing phospholipid in water and heating on a water bath.

Furthermore, propylene glycol and ethanol are mixed together at 40C, which act as the organic phase. This is then incorporated into the aqueous phase and mixed together at a temperature of 40C.

In the next step, the drug is solubilized by dissolving in a suitable solvent either in water or ethanol (depends on solubility) and then simultaneously added to the above mixtures.

Further size reduction process can be carried out using sonication or extrusion method [9]. Fig. 3 illustrate the hot method for the preparation of ethosome.

2.4.3 Classic mechanical dispersion method [5-10]

In a round bottom flask, soya phosphatidylcholine is solubilized in chloroform: methanol at a ratio of 3:1.

Using an evaporator known as a Rotary vacuum evaporator, the solvents are withdrawn above the transition temperature of lipid to form on the wall of the flask a thin lipid film. Lastly, by keeping the mixture under a vacuum for a day, the residue of the solvent mixture is eliminated from the lipid film deposit.

Varying concentration of hydroethanolic mixture which contains drugs completes the hydration process by simultaneously rotating the flask at a temperature that is appropriate.

Table 1. Various additives incorporated in ethosomes formulation [7]

Sl. No.	Class	Examples	Uses
1.	Phospholipids	Soya phosphatidylcholine and Egg phosphatidylcholine	Vesicles forming component
2.	Polyglycols	Propylene glycol, Transcutol RTM	Increase Skin penetration of drugs
3.	Alcohol	Ethanol	For vesicle softness and as a permeation enhancer
4.	Dye	Rhodamine red and Fluorescence Isothiocyanate	Characterization study
5.	Cholesterol	Cholesterol	To make the vesicle membrane stable i.e for stability
6.	Vehicles	Carbopol D934	Gel forming agent

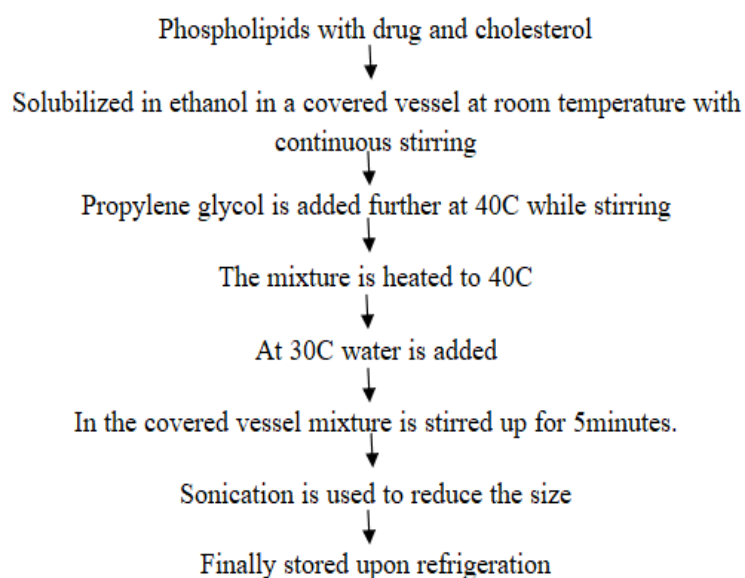


Fig. 2. Cold method process for the preparation of ethosomes

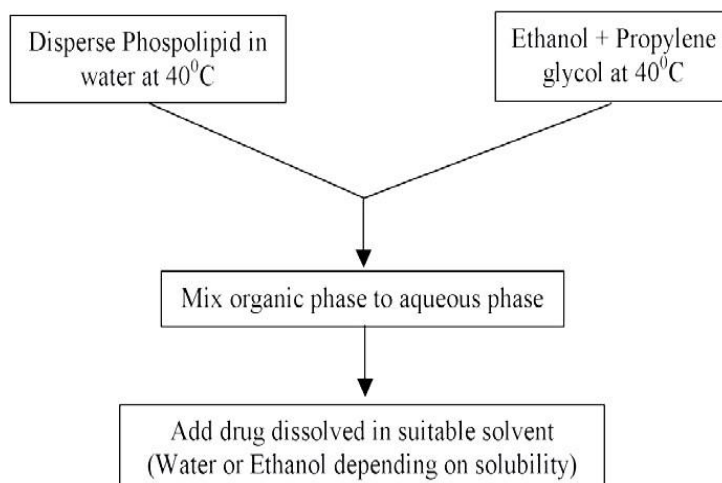


Fig. 3. Hot method for the preparation of ethosomes

2.5 Preparation of Ethosomal Gel [11]

Ethosomes which show ideal entrapment efficiency are added to carbopol D934 (which acts as the Gel former).

1. Carbopol D934 powder is incorporated into the water at 100C.
2. Slowly and in a drop-wise manner, triethanolamine is added.
3. Drug is then added into the gel base and finally, water is added with vigorous stirring until a homogenous formulation is obtained.

2.6 Techniques of Characterization of Ethosomal Formulation

2.6.1 Morphology of vesicles

This process can be done using a scanning electron microscope or by using the transmission electron microscope as they aid the visualization of the vesicles [12]. Electron microscope reveals an ethosomal formulation with a vesicular structure 300-400nm in diameter. The vesicles tend to be bendable as shown by their crooked circular form.

2.6.2 Size of vesicles and zeta potential

Photon correlation spectroscopy is used for determining the size of the vesicles and dynamic light scattering can also be used to determine size distribution of ethosome [13].

2.6.3 Drug entrapment efficiency

The difference between the initial drug quantity and the free or untrapped quantity of drug in the supernatant with respect to the total quantity incorporated in the ethosomes preparation. This can be carried out using the ultra-centrifugation method [14]. The formulation will be centrifuged at high speeds, and the amount of free drug in the supernatant will be calculated using an appropriate analytical process.

The amount of entrapped drug can be calculated from this equation:

$$\% \text{ Drug entrapment} = \left(\frac{\text{Total amount of drug} - \text{Free drug}}{\text{Total amount of drug}} \right) \times 100$$

2.7 Transition Temperature

It is the temperature at which sudden change of physical properties of the formulation occurs.

Differential scanning calorimetry can be used to determine the transition temperature of vesicular lipid systems [15].

2.8 Content of drug or Drug Content

An analytical instrument known as a UV spectrophotometer is used for determining the drug content, high performance liquid chromatographic (HPLC) method can be used as well to quantify the drug [15,16].

2.9 Surface Tension

For the measurement or determination of the surface tension, an instrument known as Tensiometer is used [17]. The ring method in a Du Nouy ring tensiometer can be used to calculate the surface tension of the drug in aqueous solution.

2.10 Turbidity

A nephelometer is used to determine the turbidity. Turbidity is the cloudiness of liquid caused by suspended particles, turbidity meters work by shining a light (laser) through a fluid sample and observing the amount of scattering or reflection. Turbidity, a measure of the cloudiness of a fluid, is recorded in Nephelometric Turbidity Units (NTUs) [1].

2.11 Skin Permeation Studies

The permeation studies are used to determine the capacity of the formulation to penetrate into the skin. The extent of penetration of ethosomes on the skin can be visualised and measured using a confocal laser scanning microscope. Ethosomes containing a dye can be applied to the skin of an animal and the extent of penetration can be measured [18].

2.12 Stability Studies

The ability of a specific formulation, in a specific container, to remain within its physical, chemical, therapeutic, and toxicological specifications is defined as drug stability. The aim of stability testing is to provide data on how the nature of a drug material or drug product changes over time as a result of environmental factors such as temperature, humidity and light, allowing recommended storage conditions, re-test periods, and shelf lives to be determined.

The ICH defines the period of the analysis as well as the storage conditions:

Long term testing at $3^{\circ}\text{C} \pm 2^{\circ}\text{C} / 60 \pm 5 \%$ for 12 months.

Accelerated testing at $40^{\circ}\text{C} \pm 2^{\circ}\text{C} / 75 \pm 5 \%$ for 6 months.

Can be determined by observing the change in morphological characteristics or properties after a period of time. Dynamic light scattering is used to determine the mean size and a transmission electron microscope is used to determine structural changes [19].

2.13 For Ethosomal Gel Formulation

2.13.1 Organoleptic properties

It should be free from grittiness.
Should be non-greasy in nature.
There should be ease of application.
Should not cause skin irritation [20].

2.13.2 Spreadability

This can be determined by slip and drug basis. The gel is sandwiched within the slides and 100 g weight is placed on the upper slide for 5 min. The time taken for the two slides to separate is noted [21]. It is the term expressed to denote the extent of area to which gel readily spreads on application to skin or affected part. The therapeutic efficacy of a formulation also depends upon its spreading value. Lesser the time taken for separation of two slides, better the spreadability.

It is calculated by using the formula:

$$S = M \cdot L / T$$

Where

M = wt. tied to upper slide
L = length of glass slides
T = time taken to separate the slides

2.13.3 Washability

It should be easily washable without leaving residue skin surface.

2.13.4 pH of ethosomal gel

The pH measurements of the gel are evaluated using a digital pH meter by dipping the glass electrode completely into the semisolid formulation to cover the electrode [22].

2.13.5 Viscosity of the ethosomal gel

Brookfield viscometer is used for measurement of viscosity of ethosomal gel formulation [23]. This can be done by selecting suitable spindle number and rpm. Suitable amount of formulation is kept in a beaker which is set till spindle groove is dipped and rpm is set and dial reading is been measured after a certain time interval.

2.13.6 In-vitro drug release study

Franz diffusion cells are used for the in-vitro drug release study. Treated egg membrane is mounted upward into the donor compartment and drug release is determined. On the compartment of the donor, ethosomal gel formulation of 1gm is applied and covered with petroleum jelly. 250 ml of pH 7.4 phosphate buffer is filled in the reservoir compartment. At a speed of 50 rpm and a duration of 12 hr at $37 \pm 0.5^{\circ}\text{C}$, the study is been carried out. By the use of a magnetic stirrer, the receptor side solution is been stirred up at 50 rpm. After every 1hr interval, a 5ml sample is removed and replaced by fresh saline PBS 7.4 pH in order to still maintain the simulated condition [24].

2.14 Application of Ethosomes Therapeutically

Ethosomes may be used for a variety of drug delivery applications. Ethosomes are primarily used to replace liposomes. The transdermal route of drug delivery is generally preferred. Ethosomes can be used to deliver hydrophilic and impermeable drugs through the skin.

Various drugs have been used in conjunction with ethosomal carrier.

2.15 Antibiotics Delivery

Topical antibiotic delivery is a better option for increasing the antibiotic efficacy of these agents. Oral tradition therapy causes a number of allergic reactions as well as a number of other side effects. The permeability of traditional external preparations to deep skin layers and subdermal tissues is low. This problem can be avoided by using ethosomes to deliver a sufficient amount of antibiotic into the skin's deeper layers. Ethosomes pass quickly through the epidermis, bringing with them a significant number of drugs into the skin's deeper layers and suppress infection at their root.

2.16 Transcellular Delivery

Touitou et al. demonstrated improved intracellular uptake of bacitracin, DNA, and erythromycin in different cell lines using CLSM and FACS techniques. Superior cellular absorption of zidovudine and lamivudine, two anti-HIV drugs in MT-2 cell line derived from ethosomes when compared to commercially available formulation suggested ethosomes as a promising clinical candidate alternative for anti-HIV therapy alternative.

2.17 Problematic Drug Molecule Delivery

Since large biogenic molecules like peptides or proteins, as well as insulin, are totally degraded in the GIT tract, oral delivery is difficult, so transdermal delivery is a viable option. However, traditional transdermal formulations of biogenic molecules like peptides or protein, as well as insulin, have low permeation. The integration of these molecules into ethosomes greatly increases permeation and therapeutic efficacy.

2.18 Topical Delivery of DNA

Many pathogens from the atmosphere try to penetrate the body through the skin. As a result, skin has developed into a highly effective protective barrier that is both immunologically active and capable of expressing genes. Another significant ethosomes application, based on the

facts above, is topical delivery of DNA molecules to express genes in skin cells.

Touitou et al. encapsulated the GFP-CMV-driven transfecting construct in an ethosomal formulation in their analysis. This formulation was applied to the dorsal skin of 5-week male CD1 nude mice for 48 hours. The treated skin was extracted after 48 hours, and CLSM observed the penetration of the green fluorescent protein (GFP) formulation. Topically applied ethosomes-GFP-CMV-driven transfecting constructs were found to allow efficient gene delivery and expression in skin cells. It has been proposed that ethosomes could be used as carriers for gene therapy applications requiring transient gene expression. These findings have suggested that ethosomes may be used for successful transdermal immunisation. Gupta et al. recently published a paper describing the immunisation potential of a 361 transfersomal formulation. As a consequence, ethosomes' enhanced skin permeation capacity opens up the possibility of using these dosage forms to deliver immunising agents.

2.19 Marketed Product of Ethosomes

The ethosomes technology started to be commercialised in the year 2000. Only two companies have produced ethosomes-related goods (Table 2).

Table 2. Products marketed based on ethosomal drug delivery system

S. no	Product name	Uses	Manufacturer
1.	Decorin cream	Wrinkles, sagging, age wrinkles, loss of elasticity, and hyperpigmentation are some of the noticeable ageing symptoms of the skin that can be treated, healed, and delayed with an anti-aging cream.	Genome Cosmetics, Pennsylvania, US
2.	Noicellex	Anti-cellulite cream for the skin	Novel Therapeutic Technologies, Israel
3.	Cellutight EF	A effective combination of ingredients in a topical cellulite cream helps to boost metabolism and breakdown fat.	Hampden Health, USA
4.	Nanominox	The ethosomes are used for the first time in a minoxidil containing product. Contains 4% Minoxidil, a well-known hair growth promoter that must be metabolised to the active compound through sulfation.	Sinere, Germany
5.	Supravir cream	In order to cure the herpes virus	Trima, Israel
6.	Skin genuity	Cellulite-busting agent that also decreases the appearance of orange peel.	Physonics, Nottingham, UK

Table 3. Various drug molecules used in ethosomal drug delivery system

Drug	Application	Remark
Erythromycin	Cure of f <i>S. aureus</i> - induced deep dermal infections	Enhancement of systemic effect and drug penetration
Zidovudine	AID treatment	Transdermal flux is enhanced
Acyclovir	For treatment of herpetic infection	Improveent of drug delivery
Insulin	Diabetes treatment	Therapeutic efficacy of drug is improved
Trihexypenidyl HCL	Parkinsonian syndrome treatment	Drug entrapment efficacy is improved, side effects are minimised, and the systemic level remains constant.
Bacitracin	Dermal infection treatment	Drug toxicity reduced
Minodixil	Promote hair growth	Skin retention is high
Cannabidol	Prevention of edema and inflammation	Significant amount of drug accumulation in the skin

3. CONCLUSION

It has been nearly two decades since the discovery of ethosomes, and during that time, these nanocarriers have demonstrated their unique ability to deliver therapeutic agents with varying physicochemical properties through the skin for both local and systemic use. Ethosomal gel are a new generation of ethosomal systems that have been developed as a result of comprehensive research. which have been discovered to have better vesicular properties and skin-permeation abilities than conventional ethosomes. Ethosomal gel give the formulator the most freedom to change according to the required research, the ethosomal properties by modifying the edge activators and / or penetration enhancer and these system also improve the stability of the formulation. The incorporation of ethosomal systems into appropriate vehicles such as gels is an important step toward improving skin permeation and therapeutic results and serves as a revolutionary approach towards transdermal drug delivery.

CONSENT

It is not applicable.

ETHICAL APPROVAL

It is not applicable.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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