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Review Article

A Comprehensive Review on Ethosomal-Loaded Hydrogel for Transdermal Drug Delivery System

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ABSTRACT

Transdermal drug delivery is a forward-looking approach which complements the constraints of standard drug delivery systems such as oral and injectable techniques. This delivery path enables both easy and painless delivery of drugs and a continuous release profile. Ethosomal hydrogel or Nano hydrogel is an innovative approach for transdermal drug delivery system which combines the benefits of both hydrogel as well as ethosome in delivery of drugs. Hydrogels are relatively similar to natural tissue as they possess a degree of flexibility due to their significant high water content and also helps in skin hydration which is one of the most significant factors in determining percutaneous absorption rate of a given drug molecule. Using carriers system, such as ethosomes have demonstrated promising results in transdermal drug delivery capable of delivering drugs to the deeper skin tissues more effectively and efficiently. This review highlights and presents an overview on ethosomal-loaded hydrogel, method of preparation, characterization, evaluation and application ethosomal hydrogel in transdermal drug delivery.

INTRODUCTION

Transdermal drug delivery is a forward-looking drug delivery approach complements the constraints of standard drug delivery systems including oral and injectable techniques.This delivery path enables both easy and painless delivery of drugs and a continuous release profile. It has several benefits, such as extended therapeutic effect, decreased side effects, enhanced bioavailability, enhanced patient compliance, and simple drug therapy termination [1-2]. Transdermal drugs delivery system can be achieved with the help of delivery device such as adhesive device, monolithic device, and reservoir device.Further innovation for transdermal drug delivery system may come from the use of hydrogels [3].

Hydrogels were first reported by Wichterle and Lím in 1960 [4]. These are hydrophilic polymeric materials with a three-dimensional complex structure consisting of hydrophilic polymer chains.They have hydrodynamic properties that are closely related to biological tissues and they can be effectively used as drug reservoir or carrier as the drug are efficiently disperse in the hydrogel's matrix. Since hydrogel possesses high water content, therefore aids in skin hydration and which in-turns promote skin permeation due to moisturizing effect and made it more suitable for topical application [5]. In addition, they are studied as a way to stabilize and enhance the transdermal delivery of other systems such as ethosomes, liposomes, micelles, and nanoparticles. Colloidal lipid-based vesicles, such as ethosomes, liposome, Transferosomes, and Niosome, have demonstrated promising results as carrier systems in transdermal drug delivery. Furthermore, ethosome, a lipid vesicle, has recently emerged as a new delivery system capable of delivering drugs to the deeper skin tissues more effectively and efficiently than other colloidal lipid-based vesicles [6].

Ethosomes are exciting and innovative vesicular systems for pharmaceutical technology and drug delivery. Ethosomes are soft, malleable vesicles customized to improve the delivery of the active constituent. Ethosomes are non-invasive carriers which delivers drugsdeep through the skin layers into the systemic circulation. Ethosomes have certain characteristics that are responsible

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for their tendency to permeate intact human skin due to their good deformability. Ethosome have certain physiological characteristics that allow this vesicular vehicle to deliver active substances more effectively through the stratum corneum into deeper skin layers and/or systemic circulation compared to other vesicular carriers such as liposomes [7-8].

Transdermal drug delivery system (TDDS)

Transdermal drug delivery system is one of the controlled drug delivery systems in which the objective is to deliver the drug at a predetermined and managed pace through the skin. Transdermal drug delivery system demonstrated promising results compared to an oral drug administered method as the transdermal drug delivery system avoids gastrointestinal intervention and first-pass metabolism of the drug, but the main limitation of TDDS is it has to pass through the stratum corneum and encounters its barrier properties. In transdermal permeation the rate limiting barrier for most molecules isthe stratum corneum, i.e. only the lipophilic drugs having molecular weight less than 500 Da can pass through it [9].

To enhance drug permeation through the skin various mechanisms have been explored, including use of chemical or physical enhancers, such as electroporation, iontophoresis, surfactants, alcohols, etc. Colloidal particles such as ethosomes, liposomes, niosomes, and transferosomes also have been reported to enhance permeability of drugthrough the stratum corneum. Permeation enhancers increase the permeability through the skin, so that the drugs can cross the skin easily.

Skin hydration is the one of the key factors in determining the rate of percutaneous absorption of a given solute.The level of hydration is the capacity of the stratum corneum to bind water. Skin penetration enhancer is added into device formulation to facilitate the percutaneous drug absorption by providing suitable skin hydration state [10].

General Requirement for a Transdermal Drug Delivery System

Drug

The drug molecule should have a molecular weight of less than 500 Da and a low melting point of less than 200^{o}c. The drug should also have an optimum partition coefficient to exert good therapeutic action. In past decades only BCS class II drugs are susceptible and preferable to be delivered through transdermal route because they have high permeability through skin, but in recent development class III drugs can also be deliver through transdermal route with the help of carriers [11].

Adhesive & Reservoir

The most important part of transdermal drug delivery device or system isthe drug reservoir, consisting of drug particles dissolved or dispersed in the matrix which provides to adhesion of the patch to the skin together along with the components of the patch. The adhesion property of the adhesive should be sufficient so as to keep the TDDS in place for a long time. Pressure sensitive adhesives are commonly used for transdermal patch to remain attach to the skin. Adhesives commonly used are silicone adhesives, poly-isobutylene adhesives and poly acrylate-based adhesives [12].

Permeation Enhancer

Permeation enhancer is used for enhancing the delivery of drug molecules through the skin. Penetration enhancer improves the absorption of drug by disrupting the structure of stratum corneum, interacts with the intercellular protein, and improves partition of drug into the stratum cornea. Permeation or penetration enhancers are classified in Table 1 [13-16].

Carriers

Lists of carriers are used mainly for transdermal drug delivery system (Table 2).

Advantages of Transdermal Drug Delivery System

Transdermal drug delivery is effectively for controlling drug level in plasma and sustaining the drug therapeutic level in systemic circulation, no longer frequent drug intake and controlling undesirable side effects. With TDDS we can avoid problems in the gastrointestinal absorption of drugs due to gastrointestinal pH, enzymatic behavior, or drug interactions with water, beverage, and other drugs.In the case of drugs which might cause vomiting and diarrhea, we can consider substituting oral medication with transdermal medication and also avoid the first-pass metabolism and drug degradation by liver enzymes. TDDS are non-invasive delivery and the inconvenience of parenteral therapy can be avoided [9-11].

Table 1: Classification of penetration enhancers

Physical penetration enhancers		**Chemical penetration enhancer**	
Types	**Process**	**Types**	**Example**
Electroporation	Application of high voltage pulses to induce skin perturbation. The generation of transient pores during electroporation leads to increase in skin permeability**.**	Sulphoxides and similar chemicals	Dimethyl sulphoxides (DMSO)
Iontophoresis	Application of a low-level electric current, either directly to the skin or indirectly via the dosage form	Azone	1-dodecyl-azacycloheptan-2-one
Sonophoresis	Use of ultrasonic energy to enhance the transdermal delivery of solutes either simultaneously or through pretreatment.	Pyrrolidones	N-methyl-2-pyrolidone
Suction ablation	A suction blister is produced by vacuum or negative pressure to remove the epidermis and leave the basal membrane intact.	Fatty acids	Lauric acid,Oleic acid
Skin abrasion	Direct removal or disruption of the upper layers of the skin.	Essential oil	Eucalyptus oil, chenopodium oil
		Alcohols, fatty alcohols, and glycols	Ethanol, Propylene glycol
		Surfactants	Sodium lauryl sulphate

Table 2: Various types of carriers for TDDS

Liposome	Liposomes are small artificial vesicles of spherical shape that can be created from cholesterol and natural non-toxic phospholipids. Due to their size and hydrophobic and hydrophilic character, liposomes are promising systems for transdermal drug delivery [17].
Niosome	Niosomes is a vesicular system which is composed of nonionic surfactants (NIS). In the aqueous media, these NIS self-assemble to produce bilayer structures. Thus, it can encapsulate both hydrophilic and hydrophobic drugs into this bilayer structure. Niosomes is a very beneficial drug delivery system with several applications [18].
Solid lipid nanoparticles (SLN)	SLNs are colloidal carrier systems for controlled drug delivery. The nanoparticles are colloidal carrier systems providing controlled release profiles for many substances. These carriers are composed of physiological and biodegradable lipids exhibiting low cytotoxicity and systemic toxicity [19].
Nano emulsion	Nano-emulsion is an isotropic, transparent/translucent, heterogeneous system of two immiscible liquids consisting of a fine dispersion of drugs in nano-droplets. It is stabilized by an interfacial layer of emulsifiers and co-emulsifiers. They are thermo-dynamically and kinetically stable systems (without any apparent flocculation or coalescence during long-term storage) with extremely small droplet size (20 to 400 nm), uniform size distribution and different physicochemical and biological properties than that of other emulsions (>500 nm) [20].
Micro- emulsion	Micro-emulsions are clear, stable, isotropic mixtures of oil, water, and surfactant, frequently in combination with a co-surfactant. Micro-emulsions is an effective vehicle of the solubilization of certain drugs and as protecting medium for the entrapped of drugs from degradation, hydrolysis, and oxidation. It can also provide prolonged release of the drug and prevent irritation despite the toxicity of the drug [21].
Transferosome	Transferosomes are a special type of liposomes, consisting of Phosphatidylcholine and an edge activator. They are soft malleable vesicles tailored for enhanced delivery of active agents. A transferosome carrier is an artificial vesicle designed to be like a cell vesicle or a cell engaged in exocytosis and thus suitable for controlled and potentially targeted drug delivery [22].
Ethosome	**"**Ethosomes are ethanolic liposomes". Ethosomes can be defined as noninvasive delivery carriers that enable drugs to reach deep into the skin layers and/or the systemic circulation. These are soft, malleable vesicles tailored for enhanced delivery of active agents. Ethosomes are lipid vesicles containing phospholipids, alcohol (ethanol and isopropyl alcohol) in relatively high concentration and water. Ethosomes are soft vesicles made of phospholipids and ethanol (in higher quantity) and water [23].
Aquasome	Aquasomes are like "bodies of water" and can be well-defined as tri-layered self-assembled nanostructures consisting of a solid-phase nano-crystalline core coated with an oligomeric film (made up of carbohydrate) to which biochemically active molecules are adsorbed with or without modification. Aquasomes are also called as ceramic nanoparticles. The solid core provides the structural steadiness, protects bio molecules against dehydration, and stabilizes them. The nanocrystalline core encompasses polymers such as gelatin, albumin, or acrylate or ceramic such as diamond particles, tin oxide, and brushite (calcium phosphate) [24].

Limitations of Transdermal Drug Delivery System

The limitations of the delivery of transdermal drugs are specifically related to the layers of the skin, which acts as a barrier function which is limited only to potent molecules. Another limitation includes skin irritation or contact dermatitis which may cause by drug, excipients and enhancers.The challenging aspects of this system are barrier property of skin to prevent the entry of drug molecule and enzyme metabolism of micro flora on skin surface. Therefore, the optimal physicochemical properties, biological properties and suitable permeation enhancer are factors that are taken into a consideration [10-11].

Factors Affecting Transdermal Permeation

The potential possible rate-controlling factors of skin permeation are diffusion through the delivery device/ formulation, diffusion through the stratum corneum due to complexity of membrane and micro flora enzyme on skin membrane, diffusion through the dermis into blood circulation.

Therefore, the candidate drug passing through skin should have optimal lipophilicity and hydrophilicity in order to overcome all these possible factors.The uses of carriers system, such as ethosomes, liposome, transferosomes, and niosome, have demonstrated promising results in transdermal drug delivery capable of delivering drugs to the deeper skin tissues more effectively and efficiently.

Skin hydration is one of the most significant factors that determine the rate of percutaneous absorption of a drug molecule. The level of hydration is ability of the stratum corneum to bind water. Skin penetration enhancer added into device formulation is responsible for keeping suitable skin hydration state in order to promote percutaneous drug absorption [10].

Basics on Hydrogel

Hydrogel was first reported by Wichterle and Lím (1960). A hydrogel is a three-dimensional (3D) hydrophilic polymer network linked together by chemical or physical cross-linking of individual polymer chains, and it can swell in water and retain a large amount of water while maintaining its structure (Fig. 1). Because of its significant water content, hydrogels also possess a degree of flexibility quite similar to natural tissues. The hydrophilicity of the hydrogel network is due to the presence of hydrophilic groups such as $-NH_2$, $-COOH$, $-OH$, $-CONH_2$, $-CONH-$, and $-SO_3H$. In response to certain physical and chemical stimuli, hydrogels undergo a significant volume phase transition or gel-sol phase transition. These physical stimuli include temperature, pressure, light intensity, electric and magnetic fields, and solvent composition, whereas the chemical or biochemical stimuli include pH, ions, and specific chemical compositions [4-6, 25].

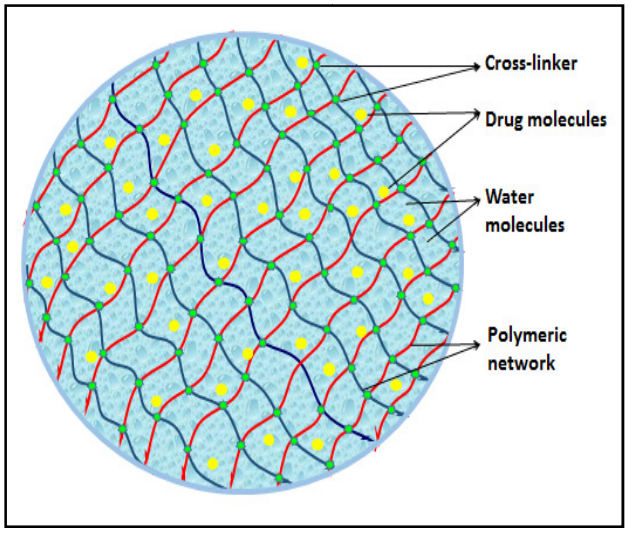

Figure 1: Structure of Hydrogel

Properties of Hydrogel

Hydrogel are hydrophilic polymer matrices, with a capacity to absorb and retain water when placed in an aqueous environment. Due to their significant water content, hydrogels also have a degree of flexibility very similar to natural tissue. The structural matrix arrangement and the ability of hydrogel to swell, under biological conditions, make it an ideal medium as a carrier for use in drug delivery and immobilization of proteins, peptides and other biological compounds.Hydrogels are physically or chemical ly cross-linked three-dimensional structure.

Hydrogel is a network of polymer chains that are water-insoluble, this insoluble cross-linked structure can be effectively used to entrapped and immobilized active agents and bio-molecules, and allows control over its release in well-defined specific manner [26].

Hydrogel are non-Newtonian system having either plastic flow or pseudo plastic flow which makes its suitable for transdermal application. An ideal yield stress value of hydrogel suitable for transdermal application should be 50-80 Pa [27].

Advantages of Hydrogel in TDDS

Hydrogels have a very high capacity to absorb water and similar physicochemical characteristics to that of tissues, which makes it suitable for various biomedical applications. Hydrogels prepared from natural polymers shows similar characteristics to the extracellular matrix and therefore may exhibit desirable properties such as biocompatibility, biodegradability and inherent cell surface interactions [27].

Hydrogel acts as a matrix or delivery system for other carriers like micro and nano particles (ethosomes, Niosome, etc.)Synthetic hydrogels derived from a wide range of monomer have been found to have many applications particularly in drug delivery devices, as carriers for implantable devices, and in tissue-engineering scaffolds [28].

Hydrogel acts as a matrix for controlling drug release from the formulation. Hydrogel is a successful controlled drug delivery to administer protein and peptide based drugs for treatment of a number of diseases. Hydrogel have organized polymeric networks, with well-defined physiochemical properties and reproducibility of drug release profile with ease [29].

Classification of Hydrogel

Hydrogel are classified based on the polymerization, ionic charge, cross linking, physical/ chemical responses, and physical properties [25]. Various classification of hydrogel is shown in Fig. 2.

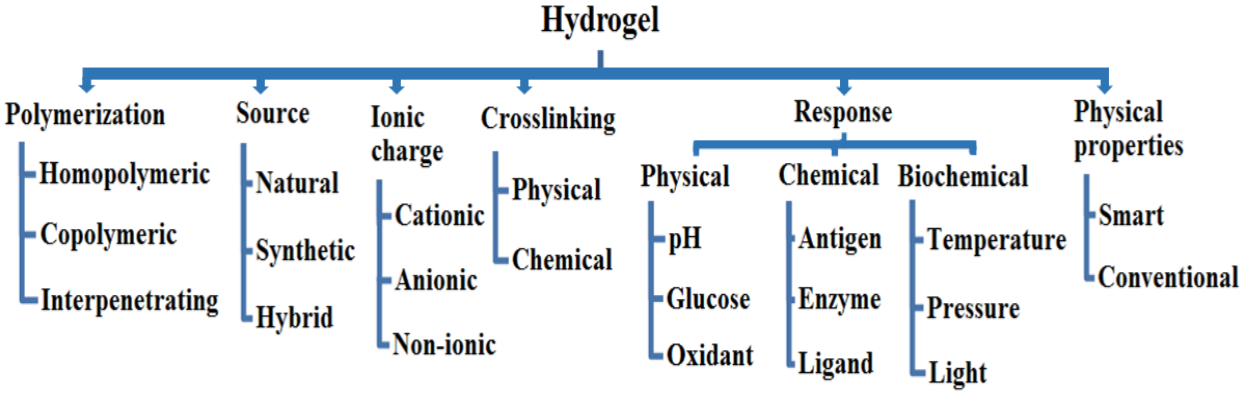

Figure 2: Classification of Hydrogel

Method of Preparation of Hydrogel

The methods of preparation of hydrogel are mainly classified into two types of crosslinking, i.e., physically cross-linked hydrogel and chemically cross-linked hydrogel. The methods are briefly described as follows:

Physically Cross-Linked Gels

Physically cross-linked hydrogels have gained more interested in the current era due to the lack of cross-linkers used for synthesis. The different methods used to synthesize physically cross-linked hydrogels are described as follows:

By Hydrogen Bonds

Polyacrylic acid and polymethacrylic acid undergoes complexation with polyethylene glycol. Such complexes are bonded together by hydrogen bonds between oxygen of polyethylene glycol and polyacrylic carboxylic group, while poly (meth) hydrophobic acrylic acid interactions also play a role. Hydrogen bonding has also been found in poly (methacrylic acid-g-ethylene glycol). Hydrogen bonds are formed only when the carboxylic acid group is protonated, showing highly pH-dependent swelling of these gels [30].

From Amphiphilic Graft and Block Polymers

Amphiphilic block and graft co-polymers can self-assemble in water to create structured constructions such as polymeric hydrogels that aggregate the hydrophobic sections of the polymer. Typically, amphiphilic copolymers create micelles, lamellar stages, etc. In general, cross-linked hydrogels are acquired from multi-block co-polymers or co-polymers [31].

Cross-Linking by Crystallization

Polyvinyl alcohol is a natural hydrophilic polymer. When aqueous polyvinyl alcoholis kept at room temperature and a little mechanical strength is applied, they gradually form a gel. When aqueous solution of polyvinyl alcohol undergoesfreeze-thaw process a strong and highly elastic gel is produced.The properties of

the gel dependon the molecular weight and the concentration of polyvinyl alcoholin water, the temperature and time of freezing andthe number of freezing cycles [32].

By Stereo Complex Formation

PDLA and PLLA (semi-crystalline materials) are the homopolymers of D-lactic acid and L-lactic acid respectively. Either stereoisomer's high molecular weight PLLA or PDLA has 170°C melting point but in combination of PDLA and PLLA, a phase with higher melting temperature (230°C) is formed, which is due to stereo complex formation [33].

Cross-Linking by Ionic Interactions

Ionic crosslinking involves the association of polymer chains by non-covalent interactions. Cross-linking with ionic interactions can takes place at room temperature and physiological pH. An example of ionic interactions cross-linked gel is alginate gels. Calcium ions are used to cross-link alginate and formed a gel. Alginate gels can be used as a matrix for the release of protein and the encapsulation of living cells [34].

Cross-Linking by Protein Interaction

Tirrell and Cappello have pioneered protein engineering as a new development in materials chemistry. The benefit of protein engineering due to the sequence of peptides and therefore their physical and chemical properties can be precisely altered by rational design of the genetic code in synthetic DNA sequences [35-36].

By Antigen–Antibody Interactions

Takashi *et al*. have developed a technique for the preparation of a molecular sensitive hydrogel through the introduction of stimulus-sensitive cross-linking structures. Specific antigen identification of an antibody may provide the basis for the manufacture of detecting equipment with a broad range of uses for identification of antigen and immunoassay. A hydrogel which is antigen-sensitive can be prepared by the application of the antigen-antibody binding at cross-linking points in the hydrogel. Due to the replacement of polymer-bound antigen, the hydrogel lightly swelled in the presence of free antigen, which results in releasing of antibodies, thus results in decreasing in the cross-link density [37].

Chemically Cross-Linked Gel

The chemically cross-linked gel has gained increasing interest in the present time because of the good mechanical strength. The solubility properties of hydrophilic polymers are mainly due to the presence of functional groups like –OH, -COOH, -NH, etc. which are useful for hydrogel formation.Chemical crosslinking is the process in which polymer chains are linked by covalent bonds, creating three-dimensional networks that reduce the structure's mobility and typically strengthen its mechanical and barrier properties and its water resistance [38].

Cross-linking by Complementary Groups Chemical Reaction

Covalent bonding between polymer chains can be form by the reaction of functional groups with complementary reactivity, such as an amine carboxylic acid or an iso-cyanate OH/NH reaction.

Cross-Linking with Aldehydes

Aldehydes use as cross linking agents examples are glutaraldehyde, formaldehyde acetaldehyde, etc. Chitosan-polyvinyl alcohol blend hydrogels can be prepared by using glutaraldehyde as cross linking agents. The hydrogel has higher tensile strength and elongation which indicates that the hydrogel has good deformability and flexibility because of the numerous entanglements and strong physical interactions among the chains of mixed polymers [39].

By Addition Reactions

Hydrogels can be produced from Hydrophilic polymers by addition reactions using bis (or higher) functional cross linking agents which react with functional groups of water-soluble polymers. Dextran-based hydrogel can be prepared using thiol-Michael addition reaction by cross-linking glycidyl methacrylate derivatized dextran (Dex-GMA) and dithiothreitol (DTT) under physiological conditions. By altering or changing the pH of buffer, the mechanical properties, gelation process and degree of swelling of the hydrogel can be easily adjusted. The properties of the hydrogel can be easily adjusted by the changing the concentration of the polymer and the amount of cross-linking [40].

By Condensation Reactions

Hydrogel can be prepared by the condensation reactions between hydroxyl group and amines with carboxylic acids or derivatives. These reactions are often use for polymer synthesis such as polyesters and polyamides, N-(3-dimethylamino propyl)-N-ethyl carbodiimide

(EDC) is the most effective cross-linking agent for crosslinking water-soluble polymers with amide bonds [41].

By High Energy Radiation

High energy radiation such as electron beam and gamma rays can be used for polymerization of unsaturated substances. Hydrogel can be synthesized by irradiation of an aqueous solution of poly (methyl vinyl ether) PMVE with electrons.For irradiation, a diluted PMVE solution which is prepared by diluting the high concentrated PMVE solution with distilled water. Oxygen can interfere with the free radical reaction. Thus, it is to be removed by degassing the homogenized polymer solution by blowing argon solution under stirring before starting the irradiation [42].

Cross-Linking by Free Radical Polymerization

Hydrogels can be obtained by free radical polymerization of low molecular weight monomers in the presence of cross-linkers.Graft P (MAA-g-EG) copolymers can be synthesized by free radical solution polymerization of methacrylic acid, purified by vacuum distillation and poly (ethylene glycol) mono methacrylate. Tetra ethylene glycol di methacrylate (TEGDMA) is added as a crosslinking agent. The reaction mixture is diluted with a 50:50 of ethanol and water mixture and a 50:50 mixture of ammonium per sulfate and sodium metabi-sulfite is used as a redox initiator in the amount of 0.025 wt% of the co monomers [43].

Cross-Linking Using Enzymes

Hydrogels prepared by using enzyme systems like tyrosinases, transferases and lysyl oxidases show interesting characteristics as dynamic scaffolds and as systems for controlled release. Enzyme-mediated cross linking has proven its efficiency and attention has now shifted to the development of enzymatically cross linked hydrogels with higher degrees of complexity, mimicking extracellular matrices. Predominantly, trans-glutaminases and horseradish peroxidases can be highlighted as the best studied enzyme systems involved inhydrogel crosslinking for tissue engineering approaches [44-46].

Basics on Ethosomes

Ethosomes are non-invasive delivery carriers that allow drugs to penetrate deep into the skin layers and/or the systemic circulation.These are soft, malleable vesicles that are tailored to improve the delivery of active agents [47-48]. Ethosomes also enable controlled release of the drug over an extended period of time, and keep the drug shielded from immune response or other removal systems and thus enable the release of drug at just the right amount and maintain constant concentration for longer period [49]. Ethosomes are the slight modification of the liposome primarily intended as a carrier for transdermal drug delivery. Ethosomes (Fig. 3) are lipid vesicles that contain phospholipids, relatively high concentrations of alcohol (ethanol and isopropyl alcohol) and water. The sizes of ethosomes ranges from tens of nanometers (nm) to microns (μ) and ethosomes permeates more easily through the layers of the skin and has significantly higher transdermal flux [50].

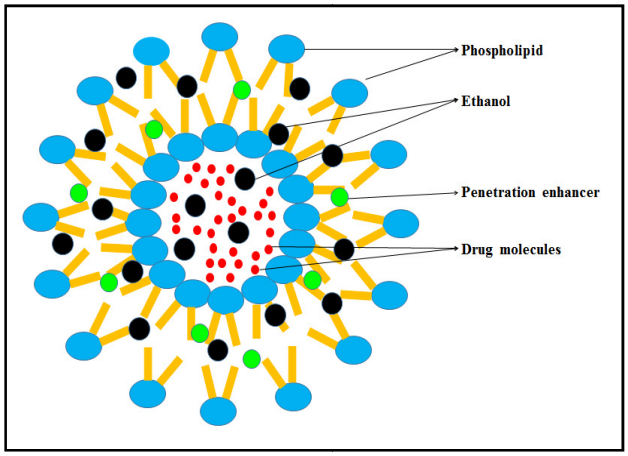

Figure 3: Structure of Ethosome

Advantages of Ethosome in TDDS

Ethosomes since they contain large amount of ethanol, they enhance the delivery of drug through skin and also made possible for transdermal drug delivery of large molecules (peptides, protein molecules) [51-53].

Disadvantages of Ethosomal in TDDS

Ethosomes can cause skin interaction or dermatitis due to interaction between skin tissue with excipients or enhancers used in ethosome. The stability of the ethosomes is of great concern because incase if the shell closure is inaccurate, the ethosomes may coalescence and falls apart on transfer to water and loss of product during transfer from organic to water media [53].

Composition of Ethosomes

Ethosomes are composed of phospholipids, high concentration of ethanol, water and other additives shown in Table 3.

Table 3: Composition of Ethosomes

Types	Uses
Phospholipids e.g. Phospholipon 90 G, Di-palmityl phosphotidyl choline	Phospholipids are one of the prominent vesicles forming unit in ethosomes which are of hydro-alcoholic or hydro/alcoholic/glycolic in character having hydrophilic head and a hydrophobic tail performing central segment in forming bilayer constitution. Increasing phospholipid concentration will increase vesicular size slightly or moderately, but will increase entrapment efficiency significantly but only until a certain concentration, whereby further increase will have no effect on the entrapment efficiency [51].
Ethanol	Ethanol plays the central character in manipulating the physicochemical properties and inimitable identity of the ethosomes. Ethanol is use as a solvent to dissolve the active pharmaceutical ingredients and it also acts as a penetration enhancer which is mainly responsible for the successful transdermal delivery of drug molecules by ethosomes. Incorporation of high concentration of ethanol in ethosomes makes lipid vesicular more malleable. However, it has been reported that higher ethanol concentration can cause leakage of drug from the ethosomes as well as severe skin irritation [8, 23].
Polyglycol (PG) e.g. Propylene glycol, Transcutol RTM	PG is a widely used penetration enhancer. It is used in the preparation of binary ethosomes at a concentration range of 5%–20% and found to influence the ethosomal properties of size, entrapment efficiency, permeation, and stability. Incorporation of PG in ethosomal systems will lead to further reduction in particle size in comparison to systems without PG [49].
Cholesterol	Cholesterol is a rigid steroid molecule, and its incorporation in ethosomal systems enhances the stability and entrapment efficiency of drugs [23].

Methods of Preparation of Ethosomes

Classical Cold Method

This method was first developed by E. Toutoiu in 1996. It is the simplest method for preparing ethosomes. In this method the organic phase and aqueous phase are prepared separately. For the preparation of organic phase, the phospholipids are dissolved in ethanol with addition of penetration enhancer at room temperature. For aqueous phase distilled water, buffer solution or normal saline solution can be used. Using a syringe pump, the aqueous phase is to be added drop wise to the organic phase at a constant rate of 200 µL/ min while stirring at 700-2000 rpm using a magnetic stirrer. The mixing is done for 5–30 min to obtain the required ethosomal suspension. The drug to be incorporated in the ethosomal system will be dissolved in either the aqueous or the organic phase, depending on its physicochemical properties [54]. The above method is shown in Fig. 4.

Ethanol Injection–Sonication Method

This method involves the addition of the organic phase to the aqueous phase by injection process. The organic phase can be prepared by dissolving the phospholipids in ethanol and the aqueous phase involves dissolving the drug in distilled water. The organic phase is added to the aqueous through a syringe (at flow rate of 200 µL/min) which is connected hermetically to prevent ethanol from evaporating. The mixture is then homogenized at 50°c by sonication for 5 minutes by bath or probe sonicator to obtained colloidal solution which is to be filter through disposable filter then the ethosomal formulation is obtain in the filtrate (Fig. 5) [55].

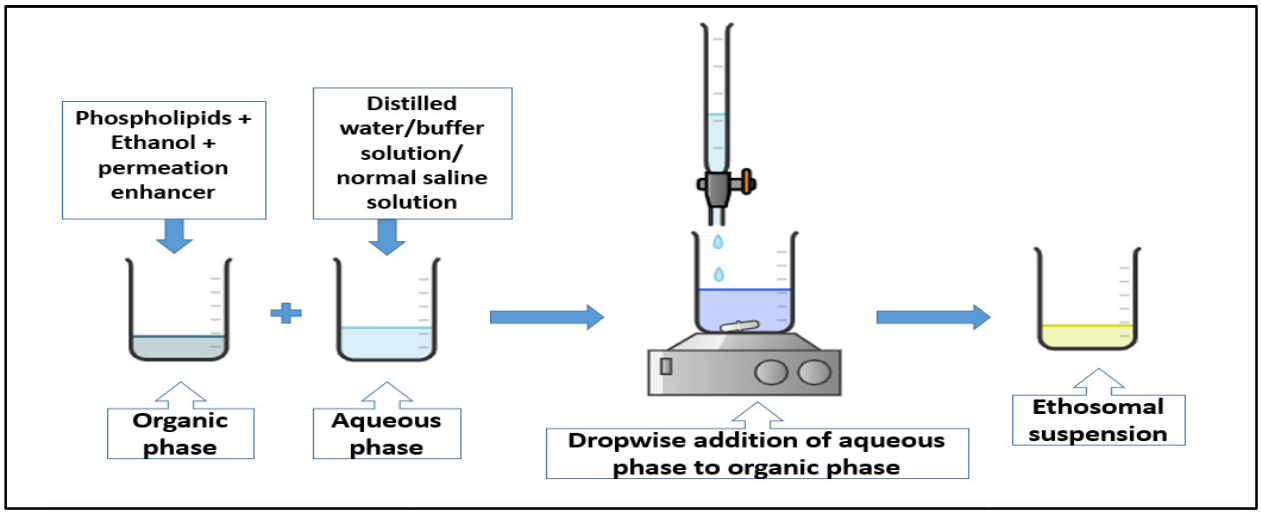

Figure 4: Classical Cold Method

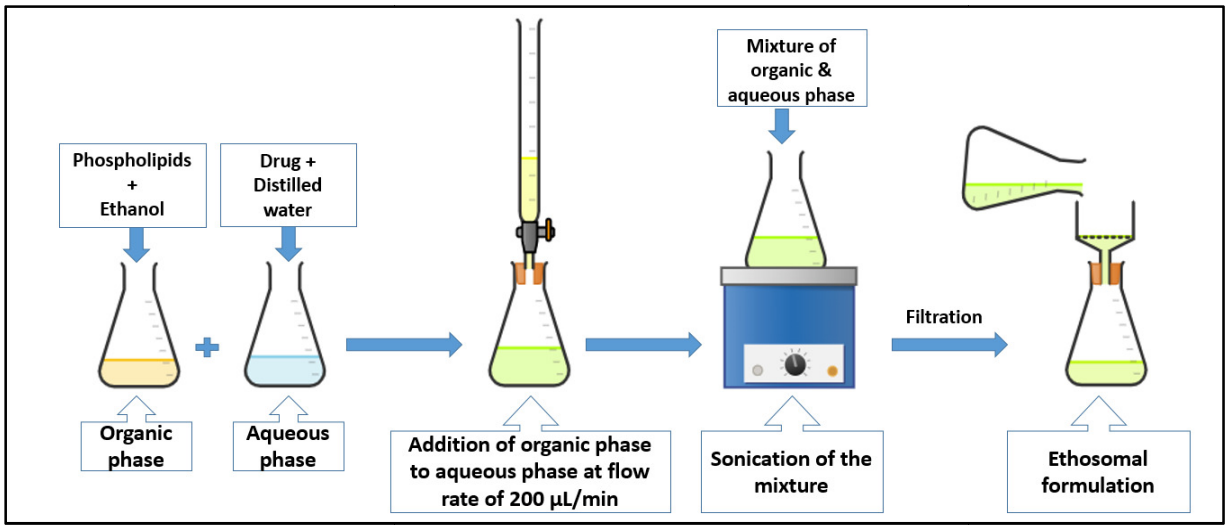

Figure 5: Ethanol Injection-Sonication Method

Hot Method

In this method, in one flask a colloidal solution is prepared by dispersing the phospholipid in water by heating in a water bath at 40°C. In another flaska mixture of ethanol and propylene glycol is prepared by continuous mixing and heating to 40°C. Then the organic phase is added to the aqueous phase under constant stirring.The drug is either dissolve inorganic phase or in aqueous phase depending on its hydrophilic/hydrophobic properties [50]. The whole process is represented in Fig. 6.

Thin-Film Hydration Method

In this method, the organic phase is prepared by dissolving phospholipids in chloroform in a round bottom flask at 90 rpm for 15 min at 45 by rotary evaporation until the organic solvent is removed and a homogeneous thin lipid film is formed on the inner surface of the flaskand dried under vacuum dryer. The deposited lipid film is then hydrated with by addition of solutionof drug and ethanol under vigorous vortexing for 15 min for ethosomal formulations. The formulation is then sonicated by a bath sonicator at 4°C for size reduction and extruded through a polycarbonate membrane filter assembly [8]. The above method is shown in Fig. 7.

Reverse-Phase Evaporation Method

In this method, the phospholipids are mixed in solvent (diethyl ether, chloroform, isopropyl ether, etc.) in a round-bottom flask and the solvent is removed under reduced pressure by a rotary evaporator. The system is then purged with nitrogen and lipids are re-dissolved in the organic phase, in which the reversed phase vesicles will be formed. The resulting two-phase system is sonicated briefly (2-5 min) in a bath-type until the mixture becomes a clear one-phase dispersion.

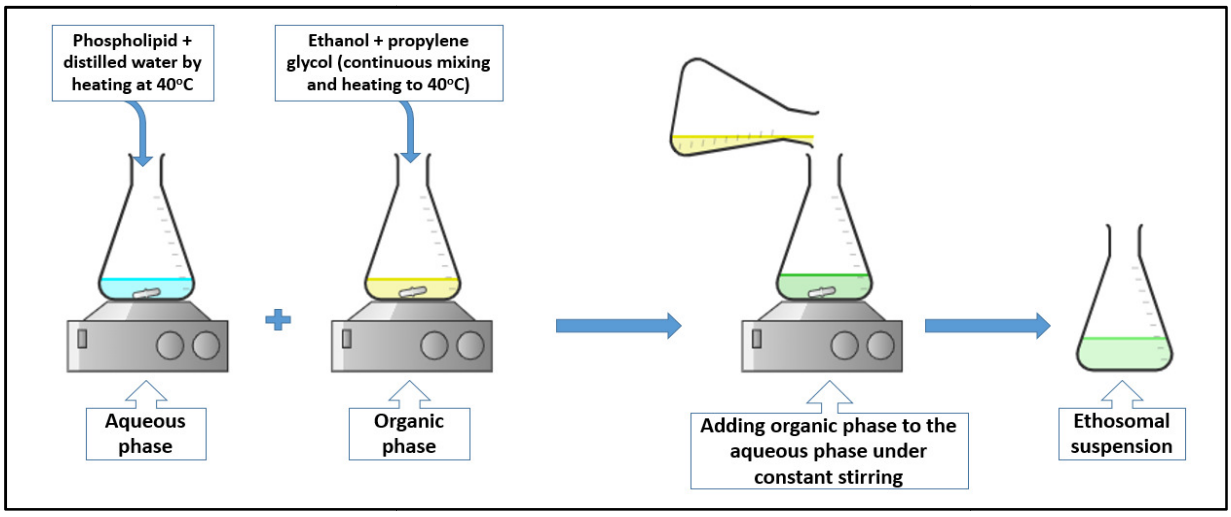

Figure 6: Hot Method

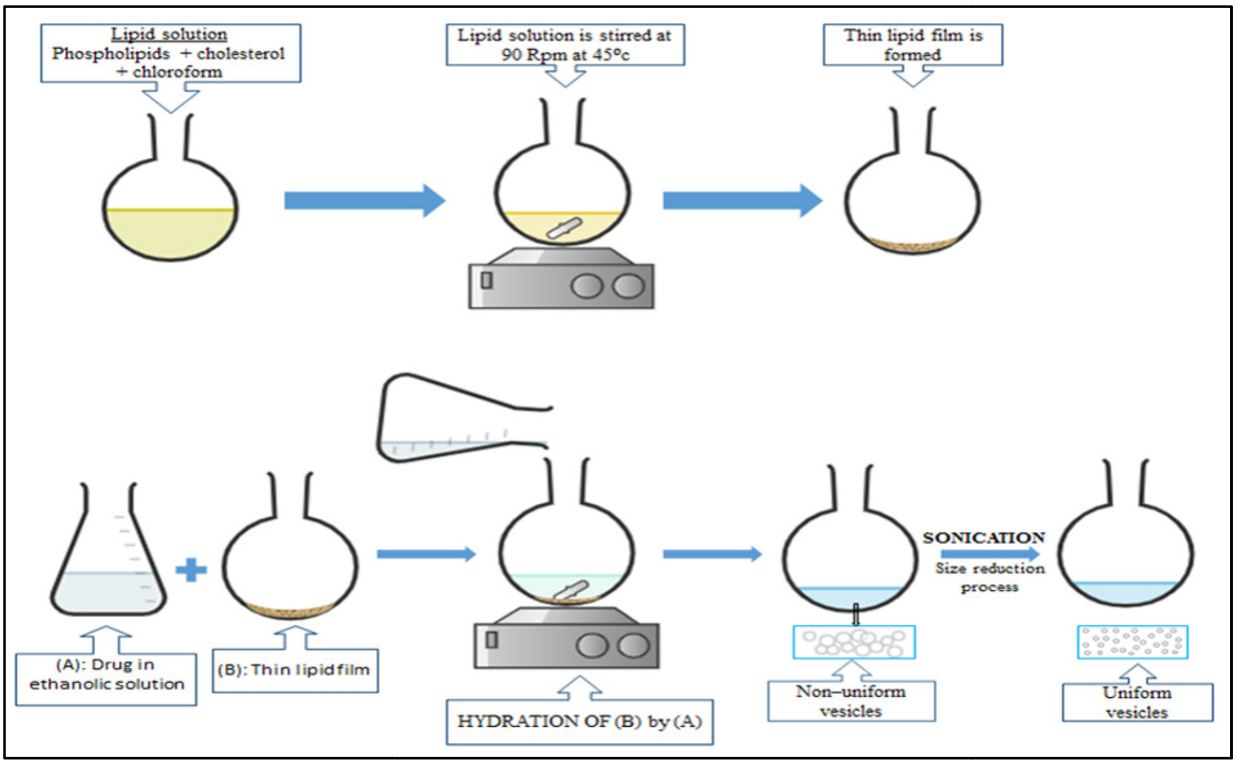

Figure 7: Thin-Film Hydration Method

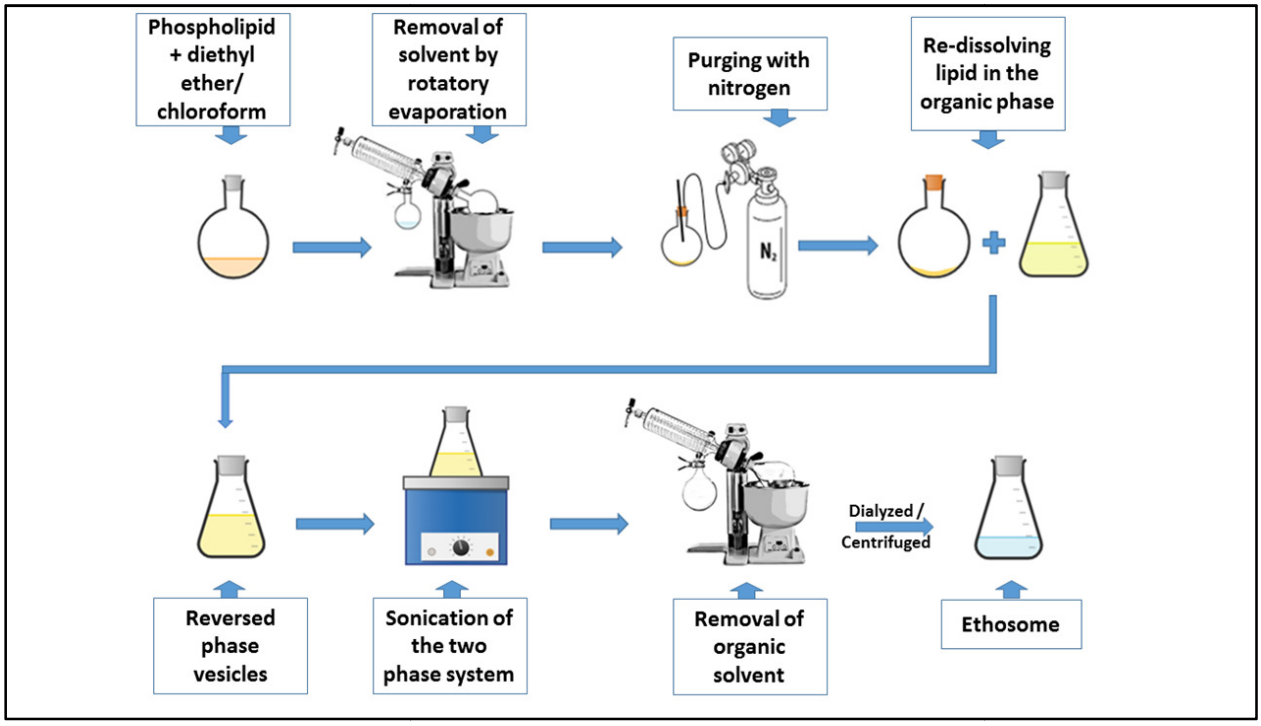

Figure 8: Reverse-Phase Evaporation Method

The mixture is then placed on the rotary evaporator and the organic solventis removed under reduced pressure (water aspirator) at 20-25°C, rotating at approximately 200 rpm. The preparation is then either dialyzed or centrifugedto remove non-encapsulated material and residual organic solvent [56]. This method is represented in Fig. 8.

Trans-Membrane pH-Gradient Method

The concept of this method was first developed for the preparation of liposomes and then it was later also used in the preparation of ethosomes. This method involves two steps (Fig. 9). In the first step empty binary ethosomes is prepared by dissolving phospholipids in ethanol and isopropyl alcohol with drop-wise addition of

citrate buffer under stirring at 700 rpm at 30°C. In the second step, the drug is added and mixed to the drug-free ethosomes suspension under stirring at 700 rpm in a closed container and the pH is adjusted by an alkali (sodium hydroxide), followed by incubation at 40°c for 1 hourto allow the unionized drug to actively pass the bilayer of the ethosomal vesicles and get entrapped. Then, the resultant suspension is to be extruded 5 times through polycarbonate filters to obtain homogeneous drug-loaded ethosomes [57-58].

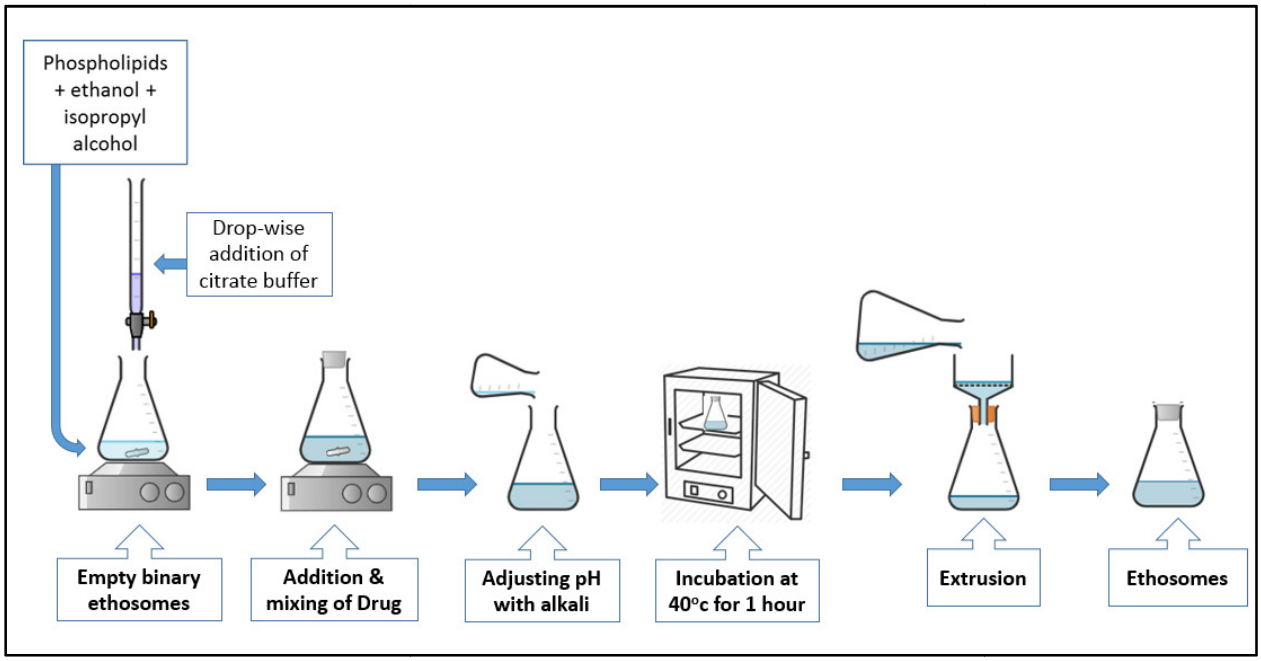

Figure 9: Trans-Membrane Ph-Gradient Method

Physical Characterization of Ethosomes

Some of the factors to be consider for ethosomes are vesicle shape, size, and size distribution, zeta potential and entrapment efficiency.

Vesicle Shape

Visualization of ethosomes can be done using transmission electron microscopy (TEM) and by scanning electron microscopy (SEM). The vesicle shape should be spherical or near-spherical-shaped lipidic vesicular structures.

Vesicle Size and Size Distribution

The size distribution of ethosomal vesicular systems can be determined by DLS (Dynamic Light Scattering) technique. It has already been reported in numerous article that particle size plays an important role in skin penetration of ethosomes. Smaller size vesicles will facilitates ethosomes to pass through small pores of the skin resulting in enhanced skin permeation [59].

Zeta Potential

It is an important parameter that can influence both vesicular stability and vesicle–skin interaction of ethosomes. Zeta potential (ζ) measurements can be determined using a Zetasizer. A high ζ-potential (> -30 mV) is beneficial to vesicles physical stability as it prevents aggregation between vesicles owing to electrostatic repulsion. Also, owing to the negative surface charge of skin, these vesicles can affect skin permeation [8, 59].

Entrapment Efficiency (EE)

The % EE of ethosomes can be measured by the ultracentrifugation technique and dialysis. However, during ultracentrifugation entrappedmight be lost from these elastic vesicles, probably due to the deformation of lipid membranes at such a high speed. These findings illustrate that, in the case of ethosomes, dialysis is a more suitable method than ultracentrifugation. Drug content of the ethosomes can be determined using UV spectrophotometer. This can also be quantified by a modified high performance liquid chromatographic method [60].

Skin Permeation Studies

The ability of the ethosomal preparation to penetrate into the skin layers can be determined by using confocal laser scanning microscopy (CLSM). Different skin models are described in Table 4.

Table 4: Different Skin Models for TDDS

Model	Description
Human skin	
In Vivo	This method involves human trials. This is the least used model for determination of skin penetration due to the high cost of human trials and concerns over applying substances or materials with potentially toxic effects. As well, in vivo responses may be difficult to measure and interpret and subject to significant variability [61].
Ex Vivo	This is an alternative method to invivo human studies. In this method excised human skin is used for permeation studies. Excised human skin is most commonly obtained from plastic surgery or cadavers, and in both cases, appropriate ethical approval is required to use the tissue [61].
Animal Skin	
In Vivo	A wide range of animal models has been used as alternatives to human skin to evaluate percutaneous permeation of substances. Pig, guinea pig, and hairless rat are generally selected for this purpose. Porcine (pig) skin have histologically similar barrier to human skin, but they are difficult to handle whereas rodents have a different barrier properties from humans [61-62].
In Vivo **Chimeric Model**	Chimeric animals are made up of cells from derived from two (or sometimes more) organisms. These "parent" organisms may be of the same or different species. Human/non-human animal chimeras have been used for a number of research purposes, including human disease modeling [60, 63].
Ex Vivo **Skin**	Skin of rodents (mice, rat, and guinea pigs) is the most commonly used in ex-vivo percutaneous permeation studies, due to its availability, their small size, and relatively low cost. There are different hairless strains of each species that are reported to mimic the permeation properties of human skin better than the hairy variety. Among rodents, rat skin is most structurally similar to human skin and it is the most frequently used rodent model [63].

Ethosomal Hydrogel or Ethosome-Loaded Hydrogel

Ethosomal hydrogel or ethosome-loaded hydrogel is an innovative approach for transdermal drug delivery system in which the ethosomal formulation is incorporated in a hydrogel. In this formulation the ethosomes acts as a carriers of the drug molecules and the hydrogel acts as a base for the carriers and as an adhesives for adhesion to the skin [6, 64].

Ethosomes-loaded hydrogel or ethosomal hydrogel combines the advantages or benefits of both the hydrogel and ethosomes in transdermal drug delivery system as ethosomes are known for enhance delivery of drug through skin whereas the hydrogel are known for its bio-compatibility with skin tissues and provide comforts and increase the patient compliance [65-66]. Ethosomal hydrogel can be formulated in such a way that we can preserve the drug and control the release of drug by stimuli responsive conformation or biodegradable bond into the polymer networks [67].

Advantages of Ethosomes-Loaded Hydrogel

Ethosomes-loaded hydrogel or Nano-hydrogel combines the advantages or benefits of both the hydrogel and ethosomes in transdermal drug delivery system. Nano-hydrogel can preserve and control the release of drug by stimuli responsive conformation or biodegradable bond into the polymer networks. The incorporation of vesicular systems into hydrogel bases can improve the stability and deposition of the formulation through the skin. . Hydrogels have been shown to be compatible with ethosomal systems providing the required viscosity and bio-adhesive properties in addition to upgraded dissolution and transport of drugs [68].

Preparation of Ethosomes-Loaded Hydrogel

Ethosomal hydrogel can be prepared by simple mixing of the ethosomes and hydrogel. The optimized formulation of the ethosome is added to the neutralized hydrogel which is prepared earlier by mixing it at 200 rpm for 5 min. The rheological properties of hydrogel before and after addition of ethosome dispersion were evaluated and compared [65, 69].

Mechanisms of Drug Release and Transport from Ethosomal-Hydrogel Formulations

Step 1: Release of Ethosome from Hydrogel

The release of ethosomes from the hydrogel is a rate limiting step which can take place by any of the following mechanism:

1. Diffusion-controlled
2. Swelling-controlled
3. Chemically-controlled

Diffusion-controlled is the most widely applicable mechanism for describing drug release from hydrogels. Diffusion-controlled matrix systems where the drug is uniformly dissolved or dispersed in a polymer matrix

generally exhibit release rates continuously diminishing with time. This is the result of the increasing diffusional resistance and decreasing area at the penetrating diffusion front. Solute behavior in hydrogels has been explained in terms of reduction in hydrogel free volume, enhanced hydrodynamic drag on the solute, increased path length due to obstruction, and a combination of hydrodynamic drag and obstruction effects [70].

Swelling-controlled mechanism is for initially dehydrated hydrogel which generally involves simultaneous water absorption and desorption of drug via a swelling-controlled diffusion mechanism.Thus, as water penetrates the hydrogel matrix containing dispersed drug, the polymer swells and its glass transition temperature is lowered. At the same time, the dissolved drug diffuses through this swollen rubbery region into the external releasing medium. Such diffusion and swelling generally do not follow a Fickian diffusion mechanism. The rate of release of entrapped drug in the hydrogel matrices can be regulated by controlling water swelling and cross linking density [71].

Chemically-controlled release is used to describe molecule release determined by reactions occurring within a delivery matrix. The most common reactions that occur within hydrogel delivery systems are cleavage of polymer chains via hydrolytic or enzymatic degradation or reversible or irreversible reactions occurring between the polymer network and releasable drug. Under certain conditions the surface or bulk erosion of hydrogels will control the rate of drug release. Alternatively, if drug-binding moieties are incorporated in the hydrogels, the binding equilibrium may determine the drug release rate [72].

Step 2: Penetration of Ethosomes in to the Skin

The main factor affecting the drug penetration is the stratum corneum which mainly acts as the "barrier" function of the skin. Ethosomes are known to have good penetration through skin; this is due to its constituent as they contain phospholipids and high concentration of ethanol in their constituent [73].

Ethanol enters the skin and penetrates into intercellular lipids and removes measurable quantities of the lipid barrier material from the stratum corneum and decrease the density of lipid multilayer of cell membrane. This lipid extraction may lower the skin barrier function and render the membrane more permeable, which is the most likely explanation for the effect of ethanol as a skin penetration enhancer. The mechanism of ethanol as a skin permeation enhancer was described to be a so-called 'pull' or 'drag' effect, which means that the permeation of the enhancer subsequently facilitates that of the solute [74].

Ethanol and increases the fluidity of cell membrane lipids and. Increased cell membrane lipid fluidity caused by the ethanol of ethosomes results increased skin permeability. So the ethosomes permeates very easily inside the deep skin layers, where it got fused with skin lipids and releases the drugs into deep layer of skin as shown in Fig. 10 [75].

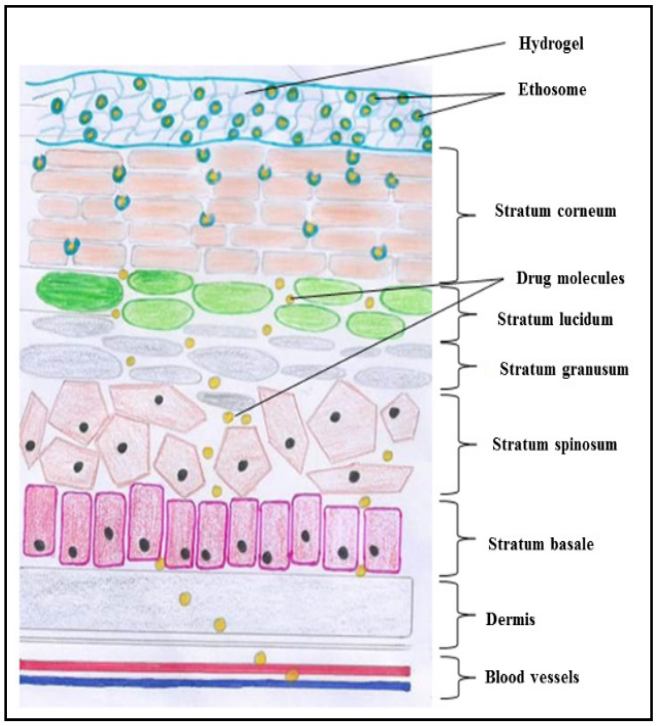

Figure 10: Transdermal delivery of ethosomal-loaded hydrogel

Characterization of Ethosomes-Loaded Hydrogel

Effect of Polymer Grade

Different polymers have different rheological properties which might have different plastic flow and yield stress, elasticity and viscosity and thermal stability, these characteristics might have effects on the viscosity and the elasticity of final formulation which might in turn affects the product quality and the compatibility of the formulation with the skin tissues [61].

Effect of Polymer Concentration

After the selection of polymer for hydrogel, it is important to understand the effect of polymer concentration on rheological properties. The degree of polymer crosslinking and consequently the rheological properties of hydrogel are strongly dependent on the polymer concentration used in the investigation. However, specifically for transdermal application of ethosome vesicles, higher polymer concentration is undesirable. At higher concentration level, there will be more crosslinking of the polymer chain that can hinder the movement of ethosome vesicles from the hydrogel to the skin surface. Therefore, for incorporation of ethosome in hydrogel, the goal was to select the lowest possible concentration level of polymer that shows plastic flow and has sufficient mechanical strength [62].

Effect of Addition of Ethosomal Formulation to Hydrogel

The addition of ethosome dispersion into the hydrogel could affect the flow and viscoelastic behavior of hydrogels. Therefore, flow ramp and frequency sweep tests of formulations can be performed to understand the effect of ethosome incorporation on the hydrogel properties at room temperature.Though hydrogel formulations maintained non-newtonian plastic flow before and even after the addition of ethosome, but at specific concentrations, the addition of ethosome formulation might reduce the yield stress and viscosity values. The decrease in the magnitude of flow (yield stress and viscosity) and viscoelastic parameters after the addition of ethosome could be attributed to the presence of ethanol in ethosome formulation, which increases the polymer chain mobility resulting in the decrease of flow and viscoelastic parameters.The addition of ethosomes might cause a dilution effect which facilitates the decrease in yield stress to the desirable limit (around 50 to 80 Pa). It is also important to acknowledge that even after addition of ethosome; the hydrogel should be able to maintain both plastic flow and viscoelastic behavior with sufficient mechanical strength [6].

Effect of Temperature on Ethosome-Loaded Hydrogel

The ethosomes are composed of temperature sensitive soy phosphatidylcholine and ethanol, the rheological properties of ethosome-loaded Carbopol hydrogels could be drastically affected by change in temperature. Therefore, effect of temperature on ethosome-loaded hydrogels need to be studied from at different temperature ranges from 4°C to 45°C to cover the storage temperature (~ 4°C), room temperature (~ 25°C), and skin temperature (~ 32°C) conditions to which these ethosome-loaded hydrogels might be exposed. Furthermore, flow and viscoelastic properties of ethosome-loaded hydrogels needs to be studied at individual temperature of 4°C, 25°C and 32°C, respectively [6, 75].

CONCLUSION

Transdermal delivery is an effective alternative for conventional and parenteral dosage form and the combination of hydrogel and ethosomes have shown promising result in successful delivery of drugs through transdermal route. This review article provides valuable information regarding hydrogels, ethosomes and their combination for transdermal drug delivery. It has been reported that both hydrogel and ethosomes all of these systems have great potentials to deliver active ingredient via transdermal route of administration. The inclusion of ethosomes into hydrogels could enhance their stability, prolong drug release, enhance transdermal permeability, and increase localization of the drug in the skin. Hydrogel combinations with ethosomes could be of great potential for increasing transdermal drug delivery and clinical research in the future and this technology can be widely utilized for transdermal delivery of drugs for various disease conditions.

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CONFLICTS OF INTEREST

The authors declare no conflicts of interest.

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