1.1 Transdermal Drug Delivery System

1.1.1 General

Transdermal drug delivery systems are defined as self-contained, discrete dosage forms which, when applied to the intact skin, deliver the drug, through the skin, at a controlled rate to the systemic circulation.

At present, the most common form of delivery of drugs is the oral route. While this has the notable advantage of easy administration, it also has significant drawbacks namely poor bioavailability due to hepatic first pass metabolism and the tendency to produce rapid blood level spikes (both high and low), leading to a need for high and/or frequent dosing, which can be both cost prohibitive and inconvenient. To overcome these difficulties there is a need for the development of new drug delivery system; which will improve the therapeutic efficacy and safety of drugs by more precise (i.e. site specific), spatial and temporal placement within the body thereby reducing both the size and number of doses. New drug delivery system are also essential for the delivery of novel, genetically engineered pharmaceuticals (i.e. peptides, proteins) to their site of action, without incurring significant immunogenicity or biological inactivation. Apart from these advantages the pharmaceutical companies recognize the possibility of repattening successful drugs by applying the concepts and techniques of controlled drug delivery system coupled with the increased expense in bringing new drug moiety to the market\(^1\). One of the methods most often utilized has been transdermal delivery - meaning transport of therapeutic substances through the skin for systemic effect. Closely related is percutaneous delivery, which is transport into target tissues, with an attempt to avoid systemic effects. \(^2\)

1.1.2 Anatomy of Skin \(^3,4,5\)

For Understanding the concept of transdermal drug delivery systems, it is important to review the structural and biochemical features of human skin and those characteristics which contribute to the barrier function and the rate of drug access into the body via skin.
Anatomically, the skin has many histological layers but in general, it is described in terms of three major tissue layers: the epidermis, the dermis and the hypodermis. The epidermis results from an active epithelial basal cell population and is approximately 150 μm thick. It is the outermost layer of the skin and the process of differentiation results in migration of cells from the basal layer towards the skin surface. The end result of this process is the formation of a thin, stratified and extremely resilient layer at the skin surface. Below this layer are the other layers of the epidermis-the stratum lucidum, stratum granulosum, stratum spinosum and stratum germinativum. Together, these other layers constitute the viable epidermis. The stratum corneum or the horny layer is the rate-limiting barrier that restricts the inward and outward movement of chemical substances.

The skin is one of the most extensive organs of the human body covering an area of about 2 m² in an average human adult. This multilayered organ receives approximately one-third of all blood circulating through the body. It has varied functions and properties. With a thickness of only a millimeter, the skin separates the underlying blood circulation network from the outside environment, serves as a barrier against physical, chemical and microbial attacks, acts as a thermostat in maintaining body temperature, protects against harmful ultraviolet rays of the sun and plays a role in the regulation of blood pressure.

Human skin has a multifunctional role, primary among which is its role as a barrier against both the egress of endogenous substances such as water and the ingress of xenobiotic material (chemicals and drugs). This barrier function of the skin is reflected by its multilayered structure.

The skin is traditionally divided into three regions:

1. The stratum corneum
2. The viable epidermis
3. The dermis
The top or uppermost layer of the skin known as the stratum corneum (SC) represents the end product of the differentiation process initially started in the basal layer of the epidermis with the formation of keratinocytes by mitotic division. The SC, therefore, is composed of dead cells (corneocytes) interdispersed within a lipid rich matrix. It is the “brick and mortar” architecture and lipophilic nature of the SC, which primarily accounts for the barrier properties of the skin.

The viable epidermis lies below the stratum corneum and consists of stratified keratinizing epithelial cells whose final function is to produce the stratum corneum. This layer does not contain blood vessels relying on nourishment by cell fluid from the dermis layer.

The deepest layer of the skin is dermis, which consists of dense irregularly arranged connective tissue and is nourished directly by blood vessels. Combined, these layers form the skin, which is connected to the subcutaneous tissue by bundles of collagen fiber. Skin also contains endocrine glands, sub-cutaneous glands, apocrine glands and hair follicles. Interestingly, in spite of being a composite structure of so many
components, studies have revealed that it is the ultra-thin, outermost layer stratum corneum, which offers the highest resistance towards drug permeation.

1.1.3 Routes of penetration\textsuperscript{6-10}

Under normal circumstances, the predominant route is through the intercellular spaces. The diffusional pathlength is therefore much longer than the simple thickness of the stratum corneum (~20 mm). Importantly, the intercellular spaces contain structured lipids and a diffusing molecule has to cross a variety of lipophilic and hydrophilic domains before it reaches the junction between the stratum corneum and the viable epidermis. The transepidermal route across the continuous stratum corneum comprises transport via intracellular and intercellular spaces. The polar molecules mainly diffuse through the polar pathway consisting of “boundwater” within the hydrated stratum corneum, whereas the nonpolar molecules dissolve and diffuse through the nonaqueous lipid matrix of the stratum corneum. The transappendageal route transports substances via the sweat glands and the hair follicles with their associated sebaceous glands, but it is considered to be of minor importance because of relatively smaller area (less than 0.1% of total surface).
1.1.4 Benefits of TDDS $^{1,3,4,11,12}$

1. The avoidance of first pass metabolism and other variables associated with the GI tract, such as pH, gastric emptying time.

2. Sustained and controlled delivery for a prolonged period of time.

3. Reduction in side effects associated with systemic toxicity, i.e. minimization of peaks and troughs in blood-drug concentration.

4. No gastrointestinal degradation (pH, enzymatic activity, drug interaction with food, drink and other orally administered drugs).

5. Reduces the chance of over and under dosing through the prolonged preprogrammed delivery of drug at the required therapeutic rate.

6. Ability to modify the properties of the biological barrier to absorption (Penetration enhancement).

7. Relatively large area ($1-2 \, \text{m}^2$) of application in comparison with the buccal or nasal cavity.

8. Improved patient acceptance and compliance.

9. Direct access to target or diseased site, e.g. treatment of skin disorders such as psoriasis, eczema and fungal infections.

10. Ease of dose termination in the event of any adverse reactions, either systemic or local effect.

11. Convenient and painless administration.

12. Ease of use may reduce overall healthcare treatment costs.

13. Provides an alternative in circumstances where oral dosing is not possible (in unconscious or nauseated patients).

1.1.5 Limitations of TDDS $^{1,3,4,11,12}$

1. A molecular weight less than 500 Da is essential to ensure ease of diffusion across the SC since solute diffusivity is inversely related to its size.

2. Sufficient aqueous and lipid solubility, a log $P$ (octanol/water) between 1 and 3 is required for the permeate to successfully traverse the SC and its underlying aqueous layers for systemic delivery to occur.
3. Intra- and inter-variability associated with the permeability of intact and diseased human skin. This implies that there will be fast, slow and normal skin absorption profiles, resulting in varying biological responses. The barrier nature of intact SC ensures that this route is applicable only for very potent drugs that require only minute concentrations (e.g. 10–30 ng/ml for nicotine) in the blood for a therapeutic effect.

4. Pre-systemic metabolism; the presence of enzymes, such as peptidases, esterases, in the skin might metabolize the drug into a form that is therapeutically inactive, thereby reducing the efficacy of the drug.

5. Skin irritation and sensitization, referred to as the “Achilles heel” of dermal and transdermal delivery. The skin as an immunological barrier may be provoked by exposure to certain stimuli; this may include drugs, excipients or components of delivery devices, resulting in erythema, oedema, etc.

1.1.6 Factors affecting drug permeation \(^5,13,14\)

The principle transport mechanism across mammalian skin is by passive diffusion, primarily through the transepidermal route at steady state or through trans-appendageal route at initial non-steady state. The factors controlling transdermal permeability can be broadly placed in the following case.

1.1.6.1 Physico-chemical properties of the penetrant molecules

1.1.6.1.1 Partition co-efficient

Drugs possessing both lipid and water solubility are favorably absorbed through the skin. Transdermal permeability co-efficient shows a linear dependency on partition co-efficient. A lipid/water partition co-efficient of one or greater is generally required.

1.1.6.1.2 pH conditions

The pH value of very high or very low can be destructive to the skin. With moderate pH values, the flux of ionisable drugs can be affected by changes in pH that alter the ratio of charged and uncharged species and their transdermal permeability.
1.1.6.1.3 Penetrant concentration

Increasing concentration of dissolved drug causes a proportional increase in flux. At higher concentrations, excess solid drug functions as a reservoir for prolonged period of time.

1.1.6.2 Physicochemical properties of drug molecule

1.1.6.2.1 Release characteristics

Solubility of the drug in the vehicle determines the release rate. The mechanism of drug release depends on the following factors

- Whether the drug molecules are dissolved or suspended in the delivery system
- The interfacial partition co-efficient of the drug from the delivery system to skin.
- pH of the vehicle.

1.1.6.2.2 Enhancement of transdermal permeation

Majority of drugs will not penetrate the skin at the rates sufficiently high for therapeutic efficacy; the permeation can be improved by the addition of permeation enhancer like dimethyl sulfoxide, dimethyl formamide, ethanol, propylene glycol etc into the system.

1.1.6.3 Physiological and pathological conditions of skin

1.1.6.3.1 Reservoir effect of horny layer

The horny layer is deeper layer, can sometimes act as depot and modify the transdermal permeation of drugs. The reservoir effect is due to irreversible binding of a part of the applied drug with the skin.

1.1.6.3.2 Lipid film

The lipid film on the skin surface acts as a protective layer to prevent the removal of moisture from the skin and helps in maintaining the barrier function of stratum corneum.

1.1.6.3.3 Skin hydration

Hydration of stratum corneum can enhance permeability. Skin hydration can be achieved simply by covering or occluding the skin with plastic sheeting, leading to accumulation of sweat. Increased hydration appears to open up the dense, closely packed cells of the skin and increase its porosity.
1.1.6.3.4 Skin temperature
Raising the skin temperature results in an increase in the rate of skin permeation, this may be due to availability of energy required for diffusivity.

1.1.6.3.5 Regional variation
Difference in nature and thickness of the barrier of skin causes variation in permeability.

1.1.6.3.6 Pathological injuries to the skin
Injuries that disrupt the continuity of the stratum corneum, increase permeability due to increased vasodilatation caused by removal of the barrier layer.

1.1.6.3.7 Cutaneous self-metabolism
Catabolic enzymes present in the epidermis may render the drug inactive by metabolism and thus reduce the topical bioavailability of the drug.

1.1.7 Components of transdermal drug delivery systems\(^1,12,15,16\)

1.1.7.1 Polymer Matrix
The Polymer controls the release of the drug from the device. Possible useful polymers for transdermal devices are

**Natural Polymers:** e.g. Cellulose derivatives, gelatin, shellac, waxes, proteins, gums and their derivatives, natural rubber, starch etc.

**Synthetic Elastomers:** e.g. Polybutadiene, hydrid rubber, polysiloxane, silicone rubber, nitrile, acrylonitrile, butyl rubber, styrenebutadiene rubber, neoprene etc.

**Synthetic Polymers:** e.g. Polyvinyl alcohol, polyvinyl chloride, polyethylene, polypropylene, polycrylate, polyamide, polyurea, polyvinylpyrrolidone, polymethylmethacrylate, etc.

1.1.7.2 Drug
For successfully developing a transdermal drug delivery system, the drug should be chosen with great care. The desirable properties of a drug for transdermal delivery are as mentioned below

- Must be non-ionic.
- The drug should have a molecular weight less than approximately 500 daltons.
• The drug should have affinity for both – lipophilic and hydrophilic phases. Extreme partitioning characteristics are not conducive to successful drug delivery via the skin.

• The drug should have low melting point (< 200°C)

• Dose should be less than 50 mg per day, and ideally less than 10 mg per day.

1.1.7.3 Permeation Enhancers

These are compounds which promote skin permeability by altering the skin as a barrier to the flux of a desired penetrant. These may conveniently be classified under the following main headings

1.1.7.3.1 Solvents

These compounds increase penetration possibly by swallowing the polar pathway and/or by fluidizing lipids. Examples include water alcohols – methanol and ethanol; alkyl methyl sulfoxides – dimethyl sulfoxide, alkyl homologs of methyl sulfoxide dimethyl acetamide and dimethyl formamide; pyrrolidones – 2 pyrrolidone, N-methyl, 2-pyrrolidone; laurocapram (Azone), miscellaneous solvents – propylene glycol, glycerol, silicone fluids, isopropyl palmitate.

1.1.7.3.2 Surfactants

These compounds are proposed to enhance polar pathway transport, especially of hydrophilic drugs. The ability of a surfactant to alter penetration is a function of the polar head group and the hydrocarbon chain length.

• Anionic Surfactants: e.g. Dioctyl sulphosuccinate, Sodium lauryl sulphate, Decodecymethyl sulphoxide etc.

• Nonionic Surfactants: e.g. Pluronic F127, Pluronic F68, etc.

• Bile Salts: e.g. Sodium taurocholate, Sodium deoxycholate, Sodium tauroglycocholate.

• Binary system: These systems apparently open up the heterogeneous multilaminate pathway as well as the continuous pathways. e.g. Propylene glycol-oleic acid and 1, 4-butanediol-linoleic acid.
1.1.7.3.3 Miscellaneous chemicals

These include urea, a hydrating and keratolytic agent; N, N-dimethyl-m-toluamide; calcium thioglycolate; anticholinergic agents. Some potential permeation enhancers have recently been described but the available data on their effectiveness is sparse. These include eucalyptol, di-o-methyl-ß-cyclodextrin and soyabean casein.

1.1.7.4 Other Excipients

1.1.7.4.1 Adhesives

The fastening of all transdermal devices to the skin has so far been done by using a pressure sensitive adhesive which can be positioned on the face of the device or in the back of the device and extending peripherally. Both adhesive systems should fulfill the following criteria

- Should adhere to the skin aggressively, should be easily removed.
- Should not leave an unwashable residue on the skin.
- Should not irritate or sensitize the skin.
- The face adhesive system should also fulfill the following criteria.
  - Physical and chemical compatibility with the drug, excipients and enhancers of the device of which it is a part.
  - Permeation of drug should not be affected.
  - The delivery of simple or blended permeation enhancers should not be affected.

1.1.7.4.2 Backing membrane

Backing membranes are flexible and they provide a good bond to the drug reservoir, prevent drug from leaving the dosage form through the top and accept printing. It is impermeable substance that protects the product during use on the skin e.g. metallic plastic laminate, plastic backing with absorbent pad and occlusive base plate (aluminium foil), adhesive foam pad (flexible polyurethane) with occlusive base plate (aluminium foil disc) etc.
1.1.8 Techniques of permeation enhancement

1.1.8.1 Mechanical methods

1.1.8.1.1 Iontophoresis \(^{17-20}\)

This method involves the application of a low level electric current either directly to the skin or indirectly via the dosage form in order to enhance permeation of a topically applied therapeutic agent. Increased drug permeation as a result of this methodology can be attributed to either one or a combination of the following mechanisms: Electro-repulsion (for charged solutes), electro-osmosis (for uncharged solutes) and electro-perturbation (for both charged and uncharged).

1.1.8.1.2 Electroporation \(^{19-21}\)

This method involves the application of high voltage pulses to the skin that has been suggested to induce the formation of transient pores. High voltages (100 V) and short treatment durations (milliseconds) are most frequently employed. The technology has been successfully used to enhance the skin permeability of molecules with differing lipophilicity and size (i.e. small molecules, proteins, peptides and oligonucleotides) including biopharmaceuticals with molecular weights greater that 7kDa.

1.1.8.1.3 Microneedle-based Devices \(^{22}\)

The very first microneedle systems, described in 1976, consisted of a drug reservoir and a plurality of projections (microneedles 50 to 100 \(\mu\)m long) extending from the reservoir, which penetrated the stratum corneum and epidermis to deliver the drug.

1.1.8.1.4 Skin Abrasion \(^{23}\)

The abrasion technique involves the direct removal or disruption of the upper layers of the skin to facilitate the permeation of topically applied medicaments. Some of these devices are based on techniques employed by dermatologists for superficial skin resurfacing (e.g. microdermabrasion) which are used in the treatment of acne, scars, hyperpigmentation and other skin blemishes.
1.1.8.1.5 Needle-less Injection

Transdermal delivery is achieved by firing the liquid or solid particles at supersonic speeds through the outer layers of the skin using a suitable energy source. The mechanism involves forcing compressed gas (helium) through the nozzle, with the resultant drug particles entrained within the jet flow reportedly traveling at sufficient velocity for skin penetration.

1.1.8.1.6 Ultrasound (Sonophoresis or Phonophoresis)

This technique involves the use of ultrasonic energy to enhance the transdermal delivery of solutes either simultaneously or via pre-treatment. It uses low frequency ultrasound (55 kHz) for an average duration of 15 seconds to enhance skin permeability.

1.1.8.1.7 Laser Radiation

This method involves direct and controlled exposure of a laser to the skin that results in the ablation of the stratum corneum without significantly damaging the underlying epidermis. Removal of the stratum corneum using this method has been shown to enhance the delivery of lipophilic and hydrophilic drugs.

1.1.8.2 Carriers/ Vehicles

1.1.8.2.1 Micro or nanocapsules

These are composed of multiple concentric bilayers of surfactant; separated by a polar liquid medium, generally water in which the hydrophilic additives can be incorporated. Their lipid core allows encapsulation of lipid additives and their multi-lamellar (lipid/water) structure creates good skin affinity leading to cutaneous penetration and good hydration.

1.1.8.2.2 Nanoemulsions/ Sub-micron emulsions (SMEs)/ Mini-emulsions

These are oil-in-water emulsions with an average droplet size ranging from 100 to 500 nm. They have very good stability and they do not undergo phase separation during storage. They have a liquid lipophilic core and are appropriate for lipophilic compound transportation. Many studies showed reduced transepidermal water loss,
which means support to the barrier function of the skin. Nanoemulsion viscosity is very low, which is interesting because they can be produced as sprays.

1.1.8.2.3 Solid lipid nanoparticles (SLNs) ²⁸

These droplets are made by solid lipids. Their sizes range from 50 to 1000 nm. They can also be stabilized by surfactants or polymers. There are mainly three structures: Homogeneous matrix, drug-enriched shell and drug-enriched core. They can protect active components against chemical degradation and modulate compound release. SLNs also present occlusive properties because of the formation of a film on the skin. This film formed by lipid fusion is supposed to be a pore-less film with improved skin hydration and protection properties.

1.1.8.2.4 Multiple emulsions ²⁹

These W/O/W emulsions consist of the dispersion of a W/O emulsion in an aqueous phase under several conditions. One can incorporate different water-soluble ingredients (even if they are incompatible) and also oil soluble additives. Like SLNs, these substances will be protected and sustain release of drug by controlling droplet breakdown. These systems can have high oily phase contents (65%, Trixera, Bain emollient, Avène) and thus present good hydration. Their efficacy has been demonstrated in dermatology to treat stretch marks (Triffadiane, CS Dermatologie).

1.1.8.2.5 Microemulsions ³⁰

These formulations have been shown to be superior for cutaneous delivery compared to other conventional vehicles. These systems are identified as transparent mixtures of water, oil and surfactants. They are thermodynamically stable and optically isotropic. Microemulsions are spontaneously produced in a narrow range of oil-water-surfactant composition, represented on pseudo-ternary diagram phases. They are dynamic systems with continuously fluctuating interfaces. Their good dermal and transdermal delivery properties could be attributed to their excellent solubilising properties. Their high solubilising properties improve biodispensibility and thus reduce the efficient dose thereby increasing tolerability. Furthermore, their restructuring effect on skin and hair (due to their high lipid content) make microemulsion formulations adapt to altered skin and hair conditions.
1.1.8.2.6 Vesicular carriers

1.1.8.2.6.1 Liposomes $^{31-33}$: These are colloidal particles formed as concentric biomolecular layers that are capable of encapsulating drugs. Their delivery mechanism is reported to be associated with accumulation of the liposomes and associated drug in the stratum corneum and upper skin layers, with minimal drug penetrating to the deeper tissues and systemic circulation. It is interesting that the most effective liposomes are reported to be those composed of lipids similar to stratum corneum lipids, which are most likely to enter stratum corneum lipid lamellae and fuse with endogenous lipids.

1.1.8.2.6.2 Niosomes $^{34-36}$: These are vesicles composed of nonionic surfactants that have been evaluated as carriers for a number of drug and cosmetic applications. This carrier has more permeability than liposomes for transdermal drug delivery.

1.1.8.2.6.3 Transfersomes $^{37,38}$: These are vesicles composed of phospholipids as their main ingredient with 10-25% surfactant (such as sodium cholate) and 3-10% ethanol. The surfactant molecules act as “edge activators”, conferring ultradeformability on the transfersomes, which reportedly allows them to squeeze through channels in the stratum corneum that are less than one-tenth the diameter of the transfersome.

1.1.8.2.6.4 Ethosomes $^{39,40}$: These are liposomes with high alcohol content capable of enhancing penetration to deep tissues and the systemic circulation. It is proposed that alcohol fluidizes the ethosomal lipids and stratum corneum bilayer lipids, thus allowing the soft, malleable ethosomes to penetrate.

1.1.8.3 Miscellaneous techniques $^{41}$

1.1.8.3.1 Prodrugs and Ion-Pairs

The prodrug approach has been investigated to enhance dermal and transdermal delivery of drugs with unfavorable partition coefficients. The prodrug design strategy generally involves addition of a pro-moiety to increase partition coefficient and solubility to increase the transport of the drug in the stratum corneum. Upon reaching the viable epidermis, esterases release the active drug by hydrolysis thereby optimizing concentration in the epidermis. Charged drug molecules do not
readily partition into or permeate through human skin. Formation of lipophilic ion pairs has been investigated to increase stratum corneum penetration of charged species. This strategy involves adding an oppositely charged species to the charged drug, forming an ion-pair in which the charges are neutralized so that the complex can partition into and permeate through the stratum corneum. The ion-pair then dissociates in the aqueous viable epidermis releasing the parent charged drug that can diffuse within the epidermal and dermal tissues.

1.1.8.3.2 Vehicle

Saturated and Supersaturated Solutions: The maximum skin penetration rate is obtained when a drug is at its highest thermodynamic activity as is the case in a supersaturated solution. Supersaturated solutions can occur due to evaporation of solvent or by mixing of co-solvents.

1.1.8.3.3 Eutectic Systems

The melting point of a drug influences solubility and hence skin penetration. According to solution theory, lower the melting point, greater the solubility of a material in a given solvent, including skin lipids. The melting point of a drug delivery system can be lowered by formation of a eutectic mixture, which is a binary system. At a constant ratio, the components inhibit the crystallization process of each other, such that the melting point of the two components in the mixture is less than that of each component alone.

1.1.8.3.4 Complexes

Complexation of drugs with cyclodextrins has been used to enhance aqueous solubility and drug stability. Cyclodextrins of pharmaceutical relevance contain 6, 7 or 8 dextrose molecules bound in a 1,4- configuration to form rings of various diameters. The ring has a hydrophilic exterior and lipophilic core in which appropriately sized organic molecules can form non-covalent inclusion complexes resulting in increased aqueous solubility and chemical stability. Cyclodextrins are large molecules, with molecular weights greater than 1000 Da, therefore it would be expected that they would not readily permeate the skin. Complexation with
cyclodextrins has been variously reported to both increase and decrease skin penetration.

**1.2 Ethosomes**

**1.2.1 Introduction**

The ethosomes are soft, malleable vesicular carrier comprising of hydro alcoholic or hydro/alcoholic/glycolic phospholipid in which the concentration of alcohols or their combination is relatively high. These “soft vesicles” represents novel vesicular carrier for enhanced delivery to/through the skin. The size of ethosomes vesicles can be modulated from tens of nanometers to microns.

Ethosomes provide a number of important benefits including improving the drug's efficacy, enhancing patient compliance and reducing the total cost of treatment. The Ethosomes were found to be suitable for various applications within the pharmaceutical, biotechnological, veterinary, cosmetic, and nutraceutical markets.

Ethosomes have been found to be much more efficient in delivering drug to the skin, than liposomes or hydro-alcoholic solution. Ethosomes are the non invasive drug delivery carriers that enable drugs to reach the deep skin layers finally delivering to the systemic circulation. For optimal skin delivery, drug should be efficiently entrapped within ethosomal vesicles. Ethosomal drug delivery system is a new state of the art technique and easier to prepare in addition to safety and efficacy. Ethosomes have become an area of research interest, because of their enhanced skin permeation, improved drug delivery, increased drug entrapment efficiency etc. Ethosomes have been used to deliver many drug molecules like acyclovir, bacitracin,
testosterone, insulin etc. Ethosomal drug delivery system has thus become an active area of research and development for novel therapies.

**Proposed Mechanism of Skin Permeation of Ethosomes**

The figure 1.2.2 shows the schematic representation of mechanism of skin permeation of ethosomes.

- The stratum corneum (SC) lipid multilayers at physiological temperature are densely packed and highly conformationally ordered.

- Ethosomal formulations contain ethanol in their composition that interacts with lipid molecules in the polar headgroup regions, resulting in an increased fluidity of the SC lipids.

- The high alcohol content is also expected to partial extract the SC lipids. These processes are responsible for increasing inter and intracellular permeability of ethosomes.

- In addition, ethanol imparts flexibility to the ethosomal membrane that shall facilitate their skin permeation. The interdigitated, malleable ethosome vesicles...
can forge paths in the disordered SC and finally release drug in the deep layers of skin.

- The transdermal absorption of drugs could then result from fusion of ethosomes with skin lipids. This is expected to result in drug release at various points along the penetration pathway.

**1.2.2 Advantages**

1. Ethosomes offer enhanced permeation of drug through skin for transdermal and dermal delivery.

2. Since the structure of the ethosome offers place to accommodate hydrophilic, lipophilic as well as amphiphilic drug moieties, they can be used for a variety of drugs.

3. Ethosomes are platform for the delivery of large and diverse group of drugs (peptides, protein molecules)

4. Ethosome composition is safe and the components are approved for pharmaceutical and cosmetic use.

5. The vesicles can act as a depot to release the drug slowly and offer a controlled release.

6. They offer increased efficacy of drug.

7. They are compatible with biomembrane since it is made up of phospholipids.

8. They can increase the oral bioavailability of drugs.

9. The technology has no large-scale drug development risk since the toxicological profiles of the ethosomal components are well documented in the scientific literature.

10. The ethosomal drug is administrated in semisolid form (gel or cream), producing high patient compliance. In contrast, iontophoresis and phonophoresis are relatively complicated to use which will affect patient compliance.
11. They offer high market attractiveness for products with proprietary technology. They are relatively simple to manufacture with no complicated technical investments required.

12. The ethosomal system is passive, non-invasive and is available for immediate commercialization.

1.2.3 Disadvantages ⁴⁵⁻⁴⁷

1. Leakage and fusion of encapsulated drug/molecules.
2. Sometimes phospholipids undergo oxidation and hydrolysis like reaction.
3. It has short half-life.
4. Scale up is difficult and production cost is high.

1.2.4 Structure and composition ⁴⁵,⁴⁸

The main components of ethosomes are phospholipids.

1.2.4.1 Phospholipids

Phospholipids are the major structural components of biological membranes such as the cell membrane. Two major types of phospholipids (along with their hydrolysis products) are phosphoglycerides and sphingolipids. The most common phospholipid used is phosphatidylcholine (PC).

![Figure 1.5 Structure of phospholipid molecule](image-url)
Phosphatidylcholine is an amphipathic molecule in which exist a

- hydrophilic polar head group phosphocholine
- glycerol bridge
- pair of hydrophobic acyl hydrocarbon chains.

Molecules of PC are not soluble in water. As shown in figure 1.2.3, in aqueous media PC molecules align themselves closely in planar bilayer sheets in order to minimize the unfavorable action between the bulk aqueous phase and the long hydrocarbon fatty chain. (i.e. they orient themselves so that the fatty acid chains face each other, and the polar heads face the aqueous phase- this reduces the instability which exists when the molecules exist alone). Such unfavorable interactions are completely eliminated when the sheets fold on themselves to form closed sealed vesicles.

![Figure 1.6 Behavior of PC in aqueous media](image)

PC molecules contrast markedly with other amphipathic molecules such as detergents and lysolecithin – in that they form bilayer sheets not micellar structures. This is thought to be because the double fatty acid chain gives the molecule an overall tubular shape, more suitable for aggregation in planar sheets compared with detergents with a polar head and single chain whose conical shape fits nicely into spherical micellar structure.
At various temperatures, lecithin membranes can exist in different phases (phases are states such as solid gel state or fluid liquid state). The transition from one phase to another can be detected by physical techniques as the temperature is increased. The most widely used method for determining the phase transition temperature ($T_c$) is micro-calorimetry. In general increasing the chain length and increasing the saturation of the chains increases the phase transition temperature and also the stability of the molecule. At elevated temperature lipid membrane passes from tightly ordered gel state (stable) to a liquid crystal phase (metastable or unstable) where freedom of movement of the individual molecule is higher due to the fatty acid chain adopting a new conformation other than the all trans straight chain configuration, such as a gauche conformation state (a phenomenon known as chain tilt).

Phosphatidylcholine can be derived from natural and synthetic sources

**Naturally occurring phospholipids are**

- Phosphatidylcholine (one chain is oleoyl and the other is palmitoyl)
- PE (Phosphatidylethanolamine)
- PS (Phosphatidylserine)
Synthetic phospholipids are

- DOPC (Dioleoyl-phosphatidylcholine)
- DSPC (Distearoyl-phosphatidylcholine)
- DOPE (Dioleoyl-phosphatidylethanolamine)
- DSPE (Distearoyl-phosphatidylethanolamine)

1.2.5 Characterization of ethosome

The behavior of ethosomes in both physical and biological systems is governed by the factors such as physical size, membrane permeability, percent entrapped solutes, chemical composition and quantity and purity of the starting material.

1.2.5.1 Physical properties

1.2.5.1.1 Particle size and particle size distribution\(^{49,50}\)

Most precise method to determine size of the ethosome is by electron microscopy, since it allows to view each individual ethosome and to obtain exact information about the profile of ethosome population over the whole range of sizes. Unfortunately it is very time consuming and requires equipments that may not always be immediately available to hand. In contrast, laser light scattering (quasi-elastic laser light scattering) method (Malvern Zetasizer) is very simple and rapid to perform but having disadvantage of measuring an average property of the bulk of the ethosomes. All the methods require very costly equipments. If only approximate idea of size range is required then gel exclusion chromatographic techniques are recommended, since only expense incurred is that of buffers and gel materials.

Light microscopy has been utilized to examine the gross size distribution of large vesicles produced from single chain amphiphiles. If the bilayers are having fluorescent hydrophilic probes, the ethosomes can be examined under a fluorescent microscope. The resolution of the light microscope limits this technique for obtaining the complete size distribution of the preparation but using negative stain electron microscopy, one can obtain an estimate of the lower end of the size distribution. A technical difficulty in obtaining good negative stains of ethosomes is the spreading of
the vesicles on the carbon-coated grid. Treating the grid with 0.1mg/mL solution of bacitracin or coating the support films with silica by the evaporation of silicon monoxide, usually permits a satisfactory spreading of ethosomes for negative staining. Glow discharging of the grids immediately prior to use is of considerable help to the spreading of ethosomes on the grid surface. For the large vesicles (5μm), negative stain electron microscopy is not suitable for determination of the size distribution because vesicle distortion during preparation of the specimen makes it difficult to obtain an estimate of the diameter of the original particle.

In laser light diffraction technique, there is diffraction of light in which monochromatic light bends around particles. When a ray of light is incident on a particle it gets diffracted at an angle. This diffraction causes the light to bend and change its path. Proteins and nanoparticles in suspension undergo Brownian motion that is related to their hydrodynamic radius as expressed by the Stokes-Einstein equation. As biomolecules or a distribution of biomolecules diffuse around the laser-beam coherence area, light scattered from them overlaps and interferes with the transmission of the laser light. A high-sensitivity detector can then record the time-varying signal caused by scattered light and compare it to the constant signal emitted when no molecules are present. This process is known as dynamic light scattering (DLS), or quasi-elastic light scattering and photon correlation spectroscopy, and is analogous to the Doppler shift of sound frequencies emitted from a moving source. Small particles or biomolecules diffuse quickly, causing rapid fluctuations of the scattered light. Larger particles, such as protein aggregates and nanoparticles, diffuse slowly, resulting in less frequent fluctuations in the intensity of the scattered light. Since small particles diffuse more rapidly than large particles, the rate of fluctuation of scattered light intensity varies accordingly. Thus by measuring the rate of fluctuation of the scattered light, the translational diffusion coefficient (D) can be measured. Using this technique one can measure particles in the range of about 3nm to about 3μm.

By measuring the translational diffusion coefficient D, we can then find out the mean hydrodynamic radius Rh of the particle using the Stokes-Einstein Equation as follows

\[ D = \frac{kT}{6\pi\eta Rh} \]

where
D = Translational Diffusion Coefficient
K = Boltzmann’s Constant
T = Absolute Temperature
H = Solvent Viscosity
Rh = mean hydrodynamic radius

The hydrodynamic radius is the radius of a hypothetical hard sphere that diffuses with the same speed as the particle under examination. However, not all macromolecular particles (e.g. proteins or DNA) are perfect hard spheres and hence the hydrodynamic radius calculated from the diffusional properties of the particle is indicative of the apparent size of the dynamic hydrated/solvated particle which would be perfectly hard spheres.

Gel Permeation Exclusion chromatography on large pure gels was introduced to separate SUVs from radial MLVs. However, large vesicles of 1-3 μm diameter usually fail to enter the gel and are retained on the top of the column. A thin layer chromatography system using agarose beads has been introduced as a convenient, fast technique for obtaining a rough estimation of the size distribution of an ethosome preparation. However, it was not reported if this procedure was sensitive to a physical blockage of the pores of the agarose gel as is the more conventional column chromatography.

1.2.5.1.2 Surface charge

A free flow electrophoretic method is used to determine the surface charge of MLVs. A technique has been developed that separates extruded vesicles on the basis of their surface charge by electrophoresis on a cellulose acetate plate in a sodium borate buffer pH 8.8. The lipid samples (5 nmoles) are applied to the plate and electrophoresis is carried out at 4ºC on a flat bed apparatus for 30 min at 18 V/cm. The plate is dried and the phospholipids are visualized by the molybdenum blue reagent. Ethosomes up to 0.2 μm in diameter can migrate on this support and with this technique as little as 2 mole % of charged lipids can be detected in an ethosome.
bilayer. This sensitive assay should prove valuable for examining the charge heterogeneity in ethosome preparation.

1.2.5.1.3 Percent Capture (entrainment)

It is essential to measure the quantity of material entrapped inside ethosomes before the study of behavior of this entrapped material in physical and biological systems, since the effects observed experimentally will usually be dose related. After removal of unincorporated material by the separation techniques, one may assume that the quantity of material remaining is 100% entrapped, but the preparation may change upon storage. For long term stability test and for developing new ethosome formulation or method of preparation, a technique is needed for separating free from entrapped material. In general two methods used are Mini column centrifugation and Protamine aggregation.

In Mini column centrifugation method the hydrated gel (sephadex G-50) is filled in a barrel of 1mL syringe without plunger which is plugged with a whatman GF/B filter pad. This barrel is rested in a centrifuge tube. This tube is spun at 2000 rpm for 3 min to remove excess saline solution from the gel. After centrifugation the gel column should be dried and have come away from the side of the barrel. Then, eluted saline is removed from the collection tube. Ethosome suspension (0.2mL undiluted) is applied dropwise to the top of the gel bed, and the column is spun at 2000 rpm for 3 min. to expel the void volume containing the ethosomes into the centrifuge tube. The elute is then removed and set aside for assay.

The protamine aggregation method may be used for ethosomes of any composition (both + and – vely charged materials). However, a preliminary test should be carried out before to check that the solute material entrapped does not itself precipitate in presence of protamine after release from ethosome. In this method, ethosome suspension (20mg/ml in normal saline) is placed in conical glass centrifuge tube, 0.1mL of protamine solution (10mg/ml) is added with mixing, and allowed to stand for 3 min. 30 mL of saline is added and then the tube is spun for 20min at 2000g at room temperature. The supernatant is removed and assayed for free, untrapped compound by standard methods. The suspended pellet is
resuspended in 0.6mL of 10% triton X-100 and the material completely dissolved. The volume is made up to the desired value and then assayed for entrapped material by standard methods.

1.2.5.1.4 Entrapped Volume

The entrapped volume of a population of ethosomes (in μL/mg phospholipid) can often be deduced from measurements of the total quantity of solute entrapped inside ethosomes assuring that the concentration of solute in the aqueous medium inside ethosomes is the same as that in the solution used to start with, and assuming that no solute has leaked out of the ethosomes after separation from unentrapped material. However, in many cases such assumption is invalid. e.g., in two phase methods of preparation, water can be lost from the internal compartment during the drying down step to remove organic solvent. On other occasions, water may enter or be expelled from the ethosome as a result of unanticipated osmotic differences. The best way to measure external volume is to measure the quantity of water directly, and this may be done very conveniently by replacing the external medium with a spectroscopically inert fluid, and then measuring the water signal, for e.g. by NMR. In this method, ethosomes prepared in aqueous solution consisting of ordinary water are spun at high centrifugal force (200,000g for 6 hours) to give a tight pellet, from which the supernatant is decanted off to remove every drop of excess fluid (including some ethosome, if necessary). The pellet is then resuspended in deuterium oxide (D₂O). The permeability of the membrane to water is such that D₂O and H₂O equilibrate very rapidly throughout the whole of the volume of the medium. A small aliquot is removed for quantification of phospholipid and the remainder is used to obtain an NMR scan of H₂O, the peak height of which can be related to concentration by comparison with standards containing known amount of H₂O and D₂O.

1.2.5.1.5 Lamellarity

The average number of bilayers present in a ethosome can be found by freeze electron microscopy and by 31P-NMR. In the latter technique, the signals are recorded before and after the addition of broadening agent such as manganese ions.
which interact with the outer leaflet of the outermost bilayers. Thus, a 50% reduction in NMR signal means that the ethosome preparation is unilamellar and a 25% reduction in the intensity of the original NMR signal means that there are 2 bilayers in the ethosome. Nowadays, freeze fracturing electron microscopy has become a very popular method to study structural details of aqueous lipid dispersions.

1.2.5.1.6 Phase behaviour of ethosome

An important feature of lipid membrane is the existence of a temperature dependant, reversible phase transition, where the hydrocarbon chains of the phospholipid undergo a transformation from an ordered (gel) state to a more disordered fluid (liquid crystalline) state. These changes have been documented by freeze fracture electron microscopy, but most easily demonstrated by differential scanning calorimetry. The physical state of the bilayers profoundly affects the permeability, leakage rates and overall stability of the liposomes. The phase transition temperature (Tc) is a function of phospholipid content of the bilayers. The Tc can give good clues regarding ethosomal stability, permeability and whether drug is entrapped in the bilayers or the aqueous compartment.

1.2.5.1.7 Drug release

The mechanism of drug release from the ethosomes can be assessed by the use of a well calibrated in-vitro diffusion cell. The ethosome based formulations can be assisted by employing in-vitro assays to predict pharmacokinetics and bioavailability of the drug before employing costly and time-consuming in-vivo studies. The dilution-induced drug release in buffer and plasma was employed as predictor for pharmacokinetic performance of ethosomal formulations and another assay which determined intracellular drug release induced by ethosomes degradation in the presence of mouse-liver lysosome lysate was used to assess the bioavailability of the drug.
1.2.5.2 Chemical properties

1.2.5.2.1 Quantitative determination of phospholipid

It is difficult to measure directly the phospholipid concentration, since dried lipids can often contain considerable quantities of residual solvent. Consequently the method most widely used for determination of phospholipid is an indirect one in which the phosphate content of the sample is first measured. The phospholipids are measured either using Bartlett assay or Stewart Assay.

In the Bartlett assay the phospholipid phosphorous in the sample is first hydrolyzed to inorganic phosphate. This is converted to phospho-molybdic acid by the addition of ammonium molybdate and phospho-molybdic acid is quantitatively reduced to a blue colored compound by amino-naphthyl-sulfonic acid. The intensity of the blue color is measured spectrophotometrically and is compared with the curve of standards to give phosphorous and hence phospholipid content. Bartlett assay is very sensitive but is not reasonably reproducible. The problem is that the test is easily upset by trace contamination with inorganic phosphate. Therefore, precaution is to be taken using a set of borosilicate glass tubes which are washed well and not used for any other purpose. The sensitivity of the Bartlett assay to inorganic phosphate creates problem with measurement of phospholipid ethosomes suspended in physiological buffers, which usually contain phosphate ions. This can be overcome by employing a more specific method which is unaffected by inorganic phosphate. In Stewart assay, the phospholipid forms a complex with ammonium ferrothiocyanate in organic solution. The advantage of this method is that the presence of inorganic phosphate does not interfere with the assay. This method is not applicable to samples where mixture of unknown phospholipids may be present. In this method, the standard curve is first prepared by adding ammonium ferrothiocyanate (0.1M) solution with different known concentrations of phospholipids in chloroform. Similarly, the samples are treated and optical density of these solutions is measured at 485nm and the absorbance of samples compared with the standard curve of phospholipids to get the concentration. TLC method may also be employed for determining the purity and the concentration of lipids. If the compound is pure it should run as a single spot in all elution solvents. Phospholipids
which have undergone extensive degradation can be observed as a long smear with a tail trailing to the origin, compared with the pure material which runs as a one clearly defined spot.

### 1.2.5.2.2 Phospholipid hydrolysis

The major product of lecithin hydrolysis is lysolecithin where one fatty acid chain is lost by de-esterification. Ideally, estimation of phospholipid hydrolysis by quantitation of lysolecithin could be carried out by HPLC where the column outflow can be monitored continuously by UV absorbance to obtain a quantitative record of the eluted components. Unfortunately, many natural phospholipids have fatty acids which are unsaturated and therefore, absorb to different extent in the 1- and 2-position. It is difficult to relate peak height accurately to absolute quantities of lysophosphatidyl choline (LPC), since one does not know the absorbance of the fatty acid species that have been retained on the glycerol bridge. Consequently, methods are preferred which permit detection of LPC via the phosphate group, after first separating the hydrolysis product (LPC) from the parent PC by TLC. The spots can either be stained with iodine, then scraped off and the phosphate measured directly, or they can be measured by scanning densitometry. Hydrolysis products of other phospholipids can be estimated in the same way.

### 1.2.5.2.3 Phospholipid Oxidation

Oxidation of the fatty acids of phospholipids in the absence of specific oxidants occurs via a free radical chain mechanism. The initiation step is abstraction of a hydrogen atom from the lipid chain that can occur most commonly as a result of exposure to electro-magnetic radiation or trace amount of contamination with the transition metal ions. Polychain-saturated lipids are particularly prone to oxidative degradation. A number of techniques are available for determining the oxidation of phospholipids at different stages i.e., UV absorbance method, TBA method (for endoperoxides), iodometric method (for hydroperoxides) and GLC method.
Table 1.1 Examples of ethosomes as a drug carrier

<table>
<thead>
<tr>
<th>Sr.no.</th>
<th>Drug</th>
<th>Purpose of Ethosomal delivery</th>
<th>Application</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Azelaic acid</td>
<td>Improves the sustained release</td>
<td>Treatment of acne</td>
</tr>
<tr>
<td>2</td>
<td>Diclofenac</td>
<td>Selective targeting of cells</td>
<td>NSAIDS</td>
</tr>
<tr>
<td>3</td>
<td>Testosterone</td>
<td>low oral bioavailability dose dependent side effects</td>
<td>Steroidal hormone</td>
</tr>
<tr>
<td>4</td>
<td>Trihexyphenidyl hydrochloride</td>
<td>4.5-times higher than that from liposome</td>
<td>Treatment of Parkinson’s disease</td>
</tr>
<tr>
<td>5</td>
<td>Zidovudine and lamivudine</td>
<td>Better cellular uptake</td>
<td>Anti-HIV</td>
</tr>
<tr>
<td>6</td>
<td>Bacitracin</td>
<td>Better cellular uptake</td>
<td>Antibacterial</td>
</tr>
<tr>
<td>7</td>
<td>Erythromycin</td>
<td>Better cellular uptake</td>
<td>Antibacterial</td>
</tr>
<tr>
<td>8</td>
<td>DNA</td>
<td>Expression into skin cells</td>
<td>Treatment of genetic disorders</td>
</tr>
<tr>
<td>9</td>
<td>Cannabidiol</td>
<td>low bioavailability</td>
<td>Treatment of rheumatoid arthritis</td>
</tr>
<tr>
<td>10</td>
<td>Acyclovir</td>
<td>Poor skin permeation</td>
<td>Treatment of Herpes labialis</td>
</tr>
<tr>
<td>11</td>
<td>Insulin</td>
<td>GIT degradation</td>
<td>Treatment of diabetes</td>
</tr>
<tr>
<td>12</td>
<td>Cyclosporin</td>
<td>GIT degradation</td>
<td>Treatment of inflammatory skin disease</td>
</tr>
<tr>
<td>13</td>
<td>Ammonium glycyrrhizinate</td>
<td>Poor skin permeation</td>
<td>Treatment of inflammatory based skin diseases</td>
</tr>
<tr>
<td>14</td>
<td>Fluconazole</td>
<td>Poor skin permeation</td>
<td>Treatment of candidiasis</td>
</tr>
<tr>
<td>15</td>
<td>Methotrexate</td>
<td>Poor skin permeation</td>
<td>Treatment of psoriasis</td>
</tr>
<tr>
<td>16</td>
<td>Salbutamol</td>
<td>Enhanced drug delivery through skin</td>
<td>Anti-asthmatic</td>
</tr>
<tr>
<td>17</td>
<td>Minoxidil</td>
<td>Piloceaceous targeting</td>
<td>Treatment of baldness</td>
</tr>
</tbody>
</table>
1.3 Gel

1.3.1 Introduction

In general, hydrogels can be prepared from either synthetic polymers or natural polymers. The synthetic polymers are hydrophobic in nature and chemically stronger compared to natural polymers. Their mechanical strength results in slow degradation rate, but on the other hand mechanical strength provides the durability as well. These two opposite properties should be balanced through optimal design.

Polymeric gels are the liquid-solid systems. It means that they have a solid matrix that swells in water and forms a three dimensional network. These polymers do not dissolve in the liquid. Creating this three dimensional network is a result of crosslinking that is again a result of chemical bindings. There are a numerous monomers that have been used to prepare hydrogels. To improve the mechanical properties of hydrogels several manufacturing methods were proposed, among which three resulted in significant improvements in the mechanical properties. Namely,

- Double network hydrogels
- Hydrogels containing sliding cross-linking agents
- Nanocomposite hydrogels

Double network hydrogels: Two hydrogels are combined together. One of them is a highly cross-linked polyelectrolyte and the other one is a loosely cross-linked or
maybe uncross-linked natural hydrogel. This combination will result in an effective relaxation of locally applied stress and dissipation of crack energy.

**Hydrogels containing sliding cross-linking agents:** Two cyclodextrin molecules get cross-linked. These molecules will create double rings that can move slightly along the PEG chains. This will result in an excellent mechanical property for hydrogel. It will provide a hydrogel with a high degree of swelling and a high stretching ratio without fracture.

**Nanocomposite hydrogels:** Polymer N-isopropylacrylamide (NIPAAm) that is clay-contained is combined with hectorite \([\text{Mg}_{5.34}\text{Li}_{0.66}\text{Si}_{8}\text{O}_{20}(\text{OH})_{4}]\text{Na}_{0.66}\) as a multifunctional cross-linker. The mechanical property of the hydrogel was enhanced and the tensile module and strength were proportional with clay content.

**1.3.2 Classification of hydrogels**

**1.3.2.1 Chemically cross-linked hydrogels**

Radical polymerization is usually applied to make these polymers. When these types of hydrogels come in contact with \(\text{H}_2\text{O}\) molecules, they begin to swell up and spread their network.

**1.3.2.2 Physically cross-linked hydrogels**

Physically cross-linked hydrogels do not need introduction of an external cross-linking agent. Cross-linking agents are usually nondegradable and can be toxic and a removal of their residuals may be needed before they can be used in biomedical or pharmaceutical purpose. The physically crosslinked hydrogels are usually biodegradable. Their amorphous hydrophilic phase is held together by highly ordered aggregated chain segments held together by secondary molecular forces such as hydrogen bonding, Van der Waals forces or hydrophobic interaction.

**1.3.3 Preparation of hydrogels**

**1.3.3.1 Use of crosslinkers**

Copolymerization of monomers using multifunctional co-monomer, which acts as cross linking agent, chemical initiator initiates the polymerization reaction which can be carried out in bulk, solution or suspension.
Cross linking of linear polymers by irradiation or by chemical compounds. Monomers used here contain an ionizable group that can be ionized or can undergo a substitution reaction after the polymerization is completed. Thus, the hydrogels synthesized may contain weakly acidic groups like carboxylic acids or weakly basic groups like substituted amines or a strong acidic and basic group like sulfonic acid and quaternary ammonium compounds. Cross linkers incorporated are glutaraldehyde, calcium chloride and oxidized konjac glucomannan (DAK). They impart sufficient mechanical strength to the polymers and thus prevent burst release of the medicaments.

1.3.3.2 Isostatic ultra high pressure (IUHP)

Suspensions of natural biopolymers (eg.-starch) are subjected to ultra high pressure of 300-700 MPa for 5 or 20 minutes in a chamber which brings about changes in the morphology of the polymer (i.e. gelatinization of starch molecules occur). Temperature in the chamber varies from 40 to 52ºC.

1.3.3.3 Use of nucleophilic substitution reaction

A pH and temperature sensitive hydrogel viz. hydrogel of N-2-dimethylamino ethylmethacrylamide (DMAEMA) has been prepared using nucleophilic substitution reaction between methacroyl chloride and 2-dimethylamino ethylamine.

1.3.3.4 Use of gelling agent

Gelling agents like glycophosphate1-2propanediol, glycerol, trehalose, mannitol etc have been used in the preparation of hydrogels. However, presence of negative charged moieties and turbidity are the problems associated with the method.

1.3.3.5 Use of irradiation and freeze thawing

Irradiation method is suitable as well as convenient but the processing is costly along with the poor mechanical strength of the product. Freeze thawing method imparts sufficient mechanical strength and stability to the hydrogels except that they are opaque in appearance with little swelling capacity. However, hydrogels prepared from microwave irradiation are more porous than conventional methods.
1.3.3.6 Synthesis of hydrogel in industry

Formulation of monomer along with initiators and additives lead to polymerization which forms the gel. The gel is dried, sieved and mixed with other additives and post treatment is done if needed. The final formulation is packed and dispatched.

1.3.4 Design criteria for hydrogels

<table>
<thead>
<tr>
<th>Design Criteria</th>
<th>Drug Variables</th>
</tr>
</thead>
<tbody>
<tr>
<td>Polymer transport properties</td>
<td>Molecular weight of polymer</td>
</tr>
<tr>
<td>Molecular diffusion</td>
<td>Molecular weight and size of protein</td>
</tr>
<tr>
<td></td>
<td>Cross linking density</td>
</tr>
<tr>
<td></td>
<td>Hydrogel degradation rate</td>
</tr>
<tr>
<td>Physical properties</td>
<td>Polymer/ cross-linker/ initiator concentrations</td>
</tr>
<tr>
<td>Gelling mechanism/conditions</td>
<td>Temperature, pH, ionic strength</td>
</tr>
<tr>
<td>Structural properties</td>
<td>Molecular properties of polymer</td>
</tr>
<tr>
<td>Biodegradability</td>
<td>Mechanical strength</td>
</tr>
<tr>
<td>Biological properties</td>
<td>Cytotoxicity of the hydrogel</td>
</tr>
<tr>
<td>Biocompatibility</td>
<td>Capsule formation</td>
</tr>
</tbody>
</table>

1.3.5 Application of hydrogels

**Wound Healing** – Modified polysaccharide found in cartilage is used in formation of hydrogels to treat cartilage defects. For example, the hydrogel of gelatin and polyvinyl alcohol (PVA) together with blood coagulants are formulated.

**Soft Contact Lenses** (silicon hydrogels and polyacrylamides) – The first commercially available silicon hydrogels adopted two different approaches.

First approach was a logical extension of its development of silicon monomers with enhanced compatibility in hydrogel forming monomers. The second was the development of siloxy monomers containing hydrophilic polyethylene oxide segments and oxygen permeable polysiloxane units.
**Industrial Applicability** - Hydrogels are used as absorbents for industrial effluents like methylene blue dye. Another example is adsorption of dioxins by hydrogel beads.

**Tissue Engineering** – Micronized hydrogels are used to deliver macromolecules (phagosomes) into cytoplasm of antigen-presenting cells. This property is also utilized in cartilage repairing. Natural hydrogel materials used for tissue engineering include agarose, methylcellulose and other naturally derived products.

**Drug Delivery in GI Tract** – Hydrogel deliver drugs to specific sites in the GIT. Drugs loaded with colon specific hydrogels show tissue specificity and change in the pH or enzymatic actions cause liberation of drugs. They are designed to be highly swollen or degraded in the presence of micro flora.

**Rectal Delivery** – Hydrogels showing bioadhesive properties are used for rectal drug delivery. Miyazaki et al. explored the xyloglucan gel with a thermal gelling property as matrices for drug delivery.

**Ocular Delivery** – Chitoni et al. reported silicon rubber hydrogel composite ophthalmic inserts. Cohen et al. developed *in-situ* forming gelling system of alginate with high gluconic acid contents for the ophthalmic delivery of pilocarpine.

**Transdermal Delivery** – Swollen hydrogels can be used as controlled release devices in the field of wound dressing. Hydrogel based formulations are being explored for transdermal iontophoresis to obtain enhanced permeation of products viz. hormones and nicotine.

**Subcutaneous Delivery** – Hydrogel formulations for subcutaneous delivery of anticancer drugs are being prepared viz. crosslinked PHEMA (Polyhydroxyethylmethacrylate) was applied to cytarabine (Ara-c). Implantable hydrogels are now leading towards the development of biodegradable systems which don’t require surgical removal once the drug has been administered.

**Novel Hydrogel For Controlled Drug Delivery** – HYPAN is the novel hydrogel having properties useful controlled drug delivery. Physical network of crystalline clusters which fully replace the covalent network typical of other hydrogels.
Hydrogel For Gene Delivery – Modification of hydrogel composition leads to effective targeting and delivery of nucleic acids to specific cells for gene therapy. Hydrogel versatility has potential application in the treatment of many genetic and/or acquired diseases and conditions.

Cosmetology – Hydrogels when implanted into breast accentuate them for aesthetic reasons. These implants have silicon elastomer shell and are filled with hydroxyl propyl cellulose polysaccharide gel.

Topical Drug Delivery – Instead of conventional creams, hydrogel formulation are employed to deliver active components like Desonide, a synthetic corticosteroid used as an anti-inflammmatory for better patient compliance.

Protein Drug Delivery – Interleukins conventionally administered as injection are now given as hydrogels which show better compliance and form in-situ polymeric network and release proteins slowly.

1.4 Drug Profile

1.4.1 Felodipine \(^{69-72}\)

Felodipine is a dihydropyridine calcium-channel blocker, is used alone or with an angiotensin converting enzyme inhibitor, to treat hypertension, chronic stable angina pectoris, and Prinzmetal’s variant angina. Felodipine is official in IP (2010), BP (2010) and USP 32 NF 27.

\[\text{IUPAC: Ethyl methyl (4RS)-4-(2,3-dichlorophenyl)-2,6-dimethyl-1,4 dihydropyridine-3,5-dicarboxylate}\]

Structure:
### Table 1.3 Physical properties of Felodipine

<table>
<thead>
<tr>
<th>Property</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Molecular Formula</td>
<td>C_{18}H_{19}Cl_{2}NO_{4}</td>
</tr>
<tr>
<td>Molecular Weight</td>
<td>384.3</td>
</tr>
<tr>
<td>Color and Appearance</td>
<td>White or light yellow, crystalline powder</td>
</tr>
<tr>
<td>Melting point</td>
<td>145 °C</td>
</tr>
<tr>
<td>Solubility</td>
<td>20 mg/l in water, 120 mg/ml in methanol, 30 mg/ml in absolute alcohol (Ethanol)</td>
</tr>
<tr>
<td>Log P</td>
<td>3.8</td>
</tr>
</tbody>
</table>

#### 1.4.1.1 Pharmacodynamics

Felodipine belongs to the dihydropyridine (DHP) class of calcium channel blockers (CCBs), the most widely used class of CCBs. There are at least five different types of calcium channels in Homo sapiens: L-, N-, P/Q-, R- and T-type. It was widely accepted that CCBs target L-type calcium channels, the major channel in muscle cells that mediates contraction; however, some studies have shown that Felodipine also binds to and inhibits T-type calcium channels. T-type calcium channels are most commonly found on neurons, cells with pacemaker activity and on osteocytes. The pharmacologic significance of T-type calcium channel blockade is unknown.

Felodipine also binds to calmodulin and inhibits calmodulin-dependent calcium release from the sarcoplasmic reticulum. The effect of this interaction appears to be minor. Another study demonstrated that Felodipine attenuates the activity of calmodulin-dependent cyclic nucleotide phosphodiesterase (CaMPDE) by binding to the PDE-1B1 and PDE-1A2 enzyme subunits. CaMPDE is one of the key enzymes involved in cyclic nucleotides and calcium second messenger systems. Felodipine also acts as an antagonist to the mineralcorticoid receptor by competing with aldosterone for binding and blocking aldosterone-induced coactivator recruitment of the mineralcorticoid receptor. Felodipine is able to bind to skeletal and cardiac muscle isoforms of troponin C, one of the key regulatory proteins in muscle contraction. Though Felodipine exhibits binding to many endogenous molecules, its vasodilatory effects are still thought to be brought about primarily through inhibition of voltage-gated L-type calcium channels. Similar to other DHP CCBs, Felodipine
binds directly to inactive calcium channels stabilizing their inactive conformation. Since arterial smooth muscle depolarizations are longer in duration than cardiac muscle depolarizations, inactive channels are more prevalent in smooth muscle cells. Alternative splicing of the alpha-1 subunit of the channel gives Felodipine additional arterial selectivity. At therapeutic sub-toxic concentrations, Felodipine has little effect on cardiac myocytes and conduction cells.

1.4.1.2 Pharmcokinetics

**Absorption:** Is completely absorbed from the gastrointestinal tract; however, extensive first pass metabolism through the portal circulation results in a low systemic availability of 15%. Bioavailability is unaffected by food.

**Distribution:** Volume of Distribution 10 L/kg Protein binding 99%, primarily to the albumin fraction.

**Metabolism:** Hepatic metabolism primarily via cytochrome P450 3A4. Six metabolites with no appreciable vasodilatory effects have been identified.

**Route of elimination:** Although higher concentrations of the metabolites are present in the plasma due to decreased urinary excretion, these are inactive. Animal studies have demonstrated that Felodipine crosses the blood-brain barrier and the placenta.

**Clearance:** 0.8 L/min

**Toxicity:** Symptoms of overdose include excessive peripheral vasodilation with marked hypotension and possibly bradycardia. Oral rat LD₅₀ is 1050 mg/kg.

### Table 1.4 Brief Pharmacokinetics of Felodipine

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Plasma protein binding</td>
<td>99%</td>
</tr>
<tr>
<td>T max</td>
<td>2 ½ hr</td>
</tr>
<tr>
<td>C max (with 5 mg oral dose) (&amp; directly proportional to dose)</td>
<td>2 µg/l</td>
</tr>
<tr>
<td>Plasma half life</td>
<td>10-14 hrs</td>
</tr>
<tr>
<td>Volume of distribution</td>
<td>10 l/kg</td>
</tr>
<tr>
<td>Plasma clearance</td>
<td>48 l/hr</td>
</tr>
<tr>
<td>Oral bioavailability</td>
<td>15%</td>
</tr>
</tbody>
</table>
1.4.2 Ropinirole HCl\textsuperscript{73-80}

**Name:** Ropinirole HCl

Ropinirole is an orally administered non-ergoline dopamine agonist.

**Synonym:**

IUPAC: 4-[2-(dipropylamino)ethyl]-1,3-dihydro-2H-indol-2-one hydrochloride

**Molecular Structure:**

![Molecular Structure of Ropinirole HCl](image)

**Table 1.5 Physical properties of Ropinirole HCl**

<table>
<thead>
<tr>
<th>Molecular Formula</th>
<th>C\textsubscript{16}H\textsubscript{24}N\textsubscript{2}O•HCl</th>
</tr>
</thead>
<tbody>
<tr>
<td>Molecular Weight</td>
<td>296.84 (260.38 as free base)</td>
</tr>
<tr>
<td>Color and Appearance</td>
<td>white to yellow solid powder</td>
</tr>
<tr>
<td>Melting point</td>
<td>243° to 250°C</td>
</tr>
<tr>
<td>Solubility</td>
<td>133 mg/ml in water.</td>
</tr>
<tr>
<td></td>
<td>62.5 mg/ml in absolute alcohol (Ethanol)</td>
</tr>
<tr>
<td>Log P</td>
<td>2.3 (base), - 0.55 (salt)</td>
</tr>
</tbody>
</table>

**1.4.2.1 Pharmacodynamics**

Ropinirole HCl is a non-ergoline dopamine agonist with high relative *in-vitro* specificity and full intrinsic activity at the D\textsubscript{2} and D\textsubscript{3} dopamine receptor subtypes, binding with higher affinity to D\textsubscript{3} than to D\textsubscript{2} or D\textsubscript{4} receptor subtypes. Ropinirole HCl has moderate *in-vitro* affinity for opioid receptors. Ropinirole HCl and its metabolites have negligible in vitro affinity for dopamine D\textsubscript{1}, 5-HT\textsubscript{1}, 5-HT\textsubscript{2}, benzodiazepine, GABA, muscarinic, alpha\textsubscript{1}-, alpha\textsubscript{2}-, and beta-adrenoreceptors.
The precise mechanism of action of Ropinirole HCl for the treatment for Parkinson’s disease is unknown, although it is believed to be due to stimulation of postsynaptic dopamine D2-type receptors within the caudate-putamen in the brain. This conclusion is supported by studies that show that Ropinirole HCl improves motor function in various animal models of Parkinson’s disease. In particular, Ropinirole HCl attenuates the motor deficits induced by lesioning the ascending nigrostriatal dopaminergic pathway with the neurotoxin 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) in primates. The relevance of D3 receptor binding in Parkinson’s disease is unknown.

**1.4.2.2 Pharmacokinetics**

**Absorption:** Oral absorption of Ropinirole HCl is rapid and essentially complete with first pass metabolism by the liver. Bioavailability of Ropinirole HCl may be up to approximately 46% and average peak concentrations of the drug are achieved at a median time of 1.5 hours post dose. Wide inter-individual variability in the pharmacokinetic parameters has been seen and the increase in systemic exposure (Cmax and AUC) to the drug is proportional to the increase in dose, over the therapeutic dose range. Administration of Ropinirole HCl with food delayed the rate of absorption (prolonged median Tmax by 2.6 hours and 25% decrease in Cmax); however, there was no marked change in overall systemic availability of the drug.

**Metabolism:** Ropinirole HCl is mainly metabolized by the liver, and it has been shown that the enzyme predominantly responsible for its clearance is CYP1A2, an enzyme known to be induced by smoking and omeprazole, and inhibited by, for example, fluvoxamine, mexiletine, and the older fluoroquinolones such as ciprofloxacin and norfloxacin.

**Distribution:** Consistent with its high lipophilicity, Ropinirole HCl exhibits a large volume of distribution (approx. 7 L/kg) and is cleared from the systemic circulation with an average elimination half life of about 6 hours. Plasma protein binding of the drug is low (10-40%).

**Excretion:** Ropinirole HCl is primarily cleared by CYP1A2 metabolism and its metabolites are mainly excreted in urine. The major metabolite (N-despropyl
derivative) is at least 100 times less potent than Ropinirole in animal models of dopaminergic function. The active metabolite represents a much smaller proportion of circulating dose-derived material and would not notably contribute to the pharmacological activity of Ropinirole. No change in the oral clearance of Ropinirole HCl is observed following single and repeated oral administration. In patients with mild to moderate renal impairment, no change in the clearance of Ropinirole HCl was observed by population kinetics.

1.4.2.3 Indications

Ropinirole HCl is indicated for the treatment of the signs and symptoms of idiopathic Parkinson’s disease and moderate-to-severe primary Restless Legs Syndrome (RLS), including the reduction of associated periodic limb movement and episodes of nocturnal arousal.

1.4.2.5 Adverse Reactions:

The most common adverse reactions during early Parkinson’s disease are nausea, dizziness, somnolence, headache, vomiting, syncope, fatigue, dyspepsia, viral infection, constipation, pain, increased sweating, asthenia, dependent/leg edema, orthostatic symptoms, abdominal pain, pharyngitis, confusion, hallucinations, urinary tract infections, and abnormal vision. The other adverse reactions observed during advanced Parkinson’s disease are dyskinesias, aggravated Parkinsonism, insomnia, injury, falls, upper respiratory infection, increased drug level, arthralgia, tremor, anxiety, dry mouth, hypokinesia and paresthesia.

1.4.2.6 Dosage and Administration

1.4.2.6.1 Dosing for Parkinson's disease

The recommended starting dose for Parkinson’s disease is 0.25 mg 3 times daily to a total dose of 24 mg/day.

1.4.2.6.2 Dosing for Restless Legs Syndrome (RLS)

The recommended adult starting dosage for RLS is 0.25 mg once daily upto 4 mg once a day.
Table 1.6 Brief Pharmacokinetics of Ropinirole HCl

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Plasma protein binding</td>
<td>40-50%</td>
</tr>
<tr>
<td>T max</td>
<td>1 ½ hr</td>
</tr>
<tr>
<td>C max (with 1 mg oral dose) (&amp; directly proportional to dose)</td>
<td>1 µg/l</td>
</tr>
<tr>
<td>Plasma half life</td>
<td>6 hr</td>
</tr>
<tr>
<td>Volume of distribution</td>
<td>7 l/kg</td>
</tr>
<tr>
<td>Plasma clearance</td>
<td>47 l/hr</td>
</tr>
<tr>
<td>Oral bioavailability</td>
<td>40-50%</td>
</tr>
</tbody>
</table>

1.5 Excipients Profile

1.5.1 Lecithin

1.5.1.1 Nonproprietary Names

USPNF: Lecithin

1.5.1.2 Synonyms

Phospholutein, Vitellin, Yelkin TTs, Alcolec S, AF 1, Acti-Flow, Azolectin, Kelecin, Granulestin; Soybean Lecithin; Soy Lecithin; Soya Lecithin; L-a Phosphatidylcholine; Phosphatidylcholine.

1.5.1.3 Functional Category

Emollient; Emulsifying agent; Solubilizing agent.

1.5.1.4 Applications in Pharmaceutical Formulation or Technology

Lecithins are used in a wide variety of pharmaceutical applications. They are also used in cosmetics and food products. Lecithins are mainly used in pharmaceutical products as dispersing, emulsifying, and stabilizing agents and are included in intramuscular and intravenous injections, parenteral nutrition formulations, and topical products such as creams and ointments.
Lecithins are also used in suppository bases to reduce the brittleness of suppositories, and have been investigated for their absorption-enhancing properties in an intranasal insulin formulation. Lecithins are also commonly used as a component of enteral and parenteral nutrition formulations.

There is evidence that phosphatidylcholine (a major component of lecithin) is important as a nutritional supplement to fetal and infant development. Furthermore, choline is a required component of FDA-approved infant formulas. Other studies have indicated that lecithin can protect against alcohol i.e. cirrhosis of the liver, lower serum cholesterol levels, and improve mental and physical performance.

Ethosomes in which lecithin is included as a component of the bilayer have been used to encapsulate drug substances; their potential as novel delivery systems has been investigated. This application generally requires purified lecithins combined in specific proportions.

1.5.1.5 Chemical Composition and Identification

<table>
<thead>
<tr>
<th>Name</th>
<th>CAS #</th>
<th>% by weight</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lecithin</td>
<td>8002-43-5</td>
<td>100</td>
</tr>
</tbody>
</table>

1.5.1.6 Physical and Chemical Properties

<table>
<thead>
<tr>
<th>Physical state and Appearance</th>
<th>Solid (Granular solid)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Odor</td>
<td>Neutral</td>
</tr>
<tr>
<td>Color</td>
<td>Golden to light tan. The color is nearly white when freshly made, but rapidly becomes yellowish to brownish in iar.</td>
</tr>
<tr>
<td>pH (1% solution/water)</td>
<td>6.8</td>
</tr>
<tr>
<td>Specific Gravity</td>
<td>1.0305 at 24 ºC(Water = 1)</td>
</tr>
<tr>
<td>Volatility</td>
<td>1% (v/v).</td>
</tr>
<tr>
<td>Solubility</td>
<td>Soluble in diethyl ether. Insoluble in cold water, acetone. Soluble in</td>
</tr>
</tbody>
</table>
Table 1.8 Stability and Reactivity Data of Lecithin

<table>
<thead>
<tr>
<th>Stability</th>
<th>The product is stable</th>
</tr>
</thead>
<tbody>
<tr>
<td>Conditions of Instability</td>
<td>Excess heat, dust generation</td>
</tr>
<tr>
<td>Incompatibility with various substances</td>
<td>Reactive with oxidizing agents</td>
</tr>
<tr>
<td>Corrosivity</td>
<td>Non-corrosive in presence of glass</td>
</tr>
</tbody>
</table>

1.5.1.8 Handling and Storage

1.5.1.8.1 Precautions: Keep away from heat. Keep away from sources of ignition. Empty containers pose a fire risk, evaporate the residue under a fume hood. Ground all equipment containing material. Do not breathe dust. Keep away from incompatibles such as oxidizing agents.

1.5.1.8.2 Storage: Keep container tightly closed. Keep container in a cool, well-ventilated area. Do not store above 25°C (77°F). Preferably Refrigerate (2-6°C).

1.5.1.8.3 Packaging: Standard packaging 5 kg in double PE-bag and additional Al-bag

1.5.2 Alcohol

1.5.2.1 Nonproprietary Names

BP: Ethanol (96%), JP: Ethanol, PhEur: Ethanol (96%)

USP: Alcohol

1.5.2.2 Synonyms

Ethanolum (96 per centum), ethyl alcohol, ethyl hydroxide, grain alcohol, methyl carbinol
1.5.2.3 Chemical Name
Ethanol

1.5.2.4 CAS Registry Number
64-17-5

1.5.2.5 Empirical Formula
C₂H₆O

1.5.2.6 Molecular Weight
46.07

1.5.2.7 Structural Formula

1.5.2.8 Functional Category
Antimicrobial, preservative, disinfectant, skin penetrant, solvent

1.5.2.9 Applications in Pharmaceutical Formulation or Technology
Ethanol and aqueous ethanol solutions of various concentrations are widely used in pharmaceutical formulations and cosmetics. Although ethanol is primarily used as a solvent, it is also employed as a disinfectant and in solutions as an antimicrobial preservative. Topical ethanol solutions are used in the development of transdermal drug delivery systems as penetration enhancers. Ethanol has also been used in the development of transdermal preparations as a co-surfactant.

1.5.2.10 Typical Properties

1. **Antimicrobial activity:** Ethanol is bactericidal in aqueous mixtures at concentrations between 60% and 95% v/v; the optimum concentration is generally considered to be 70% v/v. Antimicrobial activity is enhanced in the presence of edetic acid or edetate salts.

2. **Boiling point:** 78.15°C

3. **Flammability:** Readily flammable, burning with a blue, smokeless flame.
4. **Flash point:** 14°C (closed cup)

5. **Solubility:** Miscible with chloroform, ether, glycerin, and water (with rise of temperature and contraction of volume).

6. **Specific gravity:** 0.8119–0.8139 at 20°C

   The above typical properties are for alcohol (ethanol 95% or 96% v/v).

### 1.5.2.11 Stability and Storage Conditions

Aqueous ethanol solutions may be sterilized by autoclaving or by filtration and should be stored in airtight containers, in a cool place. Incompatibilities in acidic conditions, ethanol solutions may react vigorously with oxidizing materials. Mixtures with alkali may darken in color owing to a reaction with residual amounts of aldehyde. Organic salts or acacia may be precipitated from aqueous solutions or dispersions. Ethanol solutions are also incompatible with aluminum containers and may interact with some drugs.

### 1.5.2.12 Related Substances

Dehydrated alcohol, denatured alcohol, dilute alcohol, isopropyl alcohol