CHAPTER - 1

Chapter - 1: Introduction

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1. INTRODUCTION

1.1. Introduction

Route of administration is also one of the important influential factors, to be considered, to produce desired therapeutic effect and patient compliance. Different routes are available to administer the drugs which includes IM, IV, SC, Nasal, Oral, transdermal, topical, rectal, etc.

![Figure 1.1: Different routes of drug administration](image)

Though different routes are available, the Oral route is an ancient, highly successful, comfortable and economical route for administration of drugs. Its success rate is limited by different reasons includes first pas metabolism, gastric irritation, nausea, vomiting, unpleasant taste and odour of some drugs, etc. Third highly successful route of administration is transdermal route from ancient
days. Even in current days, the analysis of the global market scenario of the drug delivery market revealing the same\textsuperscript{1}.

![Market segmentation the global drug delivery market in 2007](image)

**Figure 1.2: Market segmentation the global drug delivery market in 2007**

Transdermal drug delivery systems were defined as self-contained, discrete dosage forms which when applied to the intact skin, deliver the drug (s) through the skin at controlled rate to the systemic circulation\textsuperscript{2}.

There is much difference between the transdermal and topical drug delivery systems. In transdermal drug delivery systems the drug reaches to the deeper layers of the skin and from there into the systemic circulation and produces the systemic effect. The concentration of the drug in the plasma is significant.

Eg. NitroDur transdermal patch, Fentanyl transdermal patch.
Topical drug delivery can be defined as the application of a drug containing formulation to the skin to directly treat cutaneous disorders with the intent of containing the pharmacological or other effect of the drug to the surface of skin or within\(^3\).

In topical drug delivery systems, the drug will not reach the deeper layers of the skin but remains upper layers of the skin and produces the localized effect. Concentration of the drug in plasma is insignificant.

Eg. Lidocain patch.

**Figure 1.3: Topical and transdermal drug delivery systems**

The process of administration of drugs to the skin is using over the millennia. They used to administer the drugs to produce localized effect and the medication is used to administer in the form of ointments, gels, creams, pates and poultices, etc. these dosage forms are incapable to produce systemic effect. Administration of drugs
through skin to produce systemic effect is a relatively new phenomenon and is introduced to overcome the drawbacks associated with oral administration and IV routes\textsuperscript{4}.

The first adhesive transdermal delivery system (TDDS) patch was approved by the Food and Drug Administration in 1979 (scopolamine patch for motion sickness). Nitroglycerine patches were approved in 1981. This method of delivery became widely recognized when nicotine patches for smoking cessation were introduced in 1991. Transdermal drug delivery systems growth between the 2003-2007 was more than tripled. Today, there are 19 transdermal delivery systems available in the market and the drug is administering by different techniques such as usage of the permeation enhancers, iontophoresis, etc\textsuperscript{5}.

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{image.png}
\caption{Number of Transdermal drugs approved in each year.}
\end{figure}
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<tr>
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1.2. Advantages of Transdermal drug delivery system

- Avoidance of first pass metabolism of drugs.
- Transdermal medication delivers a steady infusion of a drug over a prolonged period of time. Adverse effects or therapeutic failures frequently associated with intermittent dosing can also be avoided.
- The simplified medication regimen leads to improved patient compliance and reduced the side effects, inter and intra-patient variability.
- No interference with gastric and intestinal fluids.
- Maintains stable or constant and controlled blood levels for longer period of time.
- Comparable characteristics with interavenous infusion.
- It increases the therapeutic value of many drugs via avoiding specific problems associated with the drug like GI irritation, lower absorption, decomposition due to ‘hepatic first pass’ effect.
- This route is suitable for the administration of drugs having very short half life, narrow therapeutic window and poor oral availability.
- Improved patient compliance and comfort via non-invasive, painless and simple application.
- Flexibility of terminating the drug administration by simply removing the patch from the skin.
- Self administration is possible in these systems.
1.3. Disadvantages of Transdermal drug delivery system

- The possibility of local irritation may develop at the site of application. Many problems like Erythema, itching, and local edema can be caused by the drug, the adhesive, or other excipients in the patch formulation.
- Drugs having large molecular size make absorption difficult. So drug molecule should ideally be below 800-1000 daltons.
- Many drugs with a hydrophilic structure having a low penetration through the skin and slowly to be of therapeutic benefit. Drugs with a lipophilic character, however, are better suited for transdermal delivery.
- The barrier function of the skin changes from one site to another on the same person, from person to person and with age.
- Transdermal drug delivery system cannot achieve high drug levels in blood/plasma.

1.4. Physiology of the skin

The skin, also considered as the cutaneous membrane which covers the external surface of the body. It is the largest organ of the body with respect to surface area and weight. In healthy adults, the skin covers approximately an area of 2m$^2$ (22ft$^2$) and weight 4.5-5 kg (10-11 lb), about 7% of total body weight. It ranges in thickness from 0.5 mm on the eyelids to 4.0 mm on the heels. The average thickness of the skin is 1-2mm thick. Anatomically, the skin has many histological layers but in general, it is described in terms of three
major tissue layers. Figure 1.5 shows the structure of the skin. The three layers of the skin are as follows.

1. The epidermis
2. The dermis and
3. The hypodermis.

The superficial thin portion is said to be epidermis and is composed of epithelial tissues. Below to the epidermis, there will be thicker portion which is called as dermis. Below to the dermis, there will be a subcutaneous layer and is not part of the skin. The subcutaneous layer is also called as hypodermis which contains adipose and areolar tissues.

Deep to the dermis, but not part of the skin, is the subcutaneous layer. Also called the hypodermis, this layer consists of areolar and adipose tissues, Fibers which came from the dermis fix the skin to underlying bones, the connective tissue around muscles and fascia. The hypodermis acts as fat depositing centre and also contains large number of blood vessels that supply the skin. The hypodermis also contains pacinian corpuscles, nerves ending, which are highly sensitive to pressure.

1.4.1. Epidermis

The epidermis consists of keratinized stratified squamous epithelium. It contains four types of cells which includes

1. Keratinocytes
2. Melanocytes
3. Langerhans cells and
4. Merkel cells.
Among all epidermal cells keratinocytes occupy 90% and they are arranged in 4-5 layers. As they produce protein called keratin, they are named as keratinocytes. Keratin is a hard, fibrous protein which helps in protection of skin and underlying tissues from heat, abrasions, chemicals, microbes, etc. The keratinocytes also produce lamellar granules which liberates a water repellent sealant. The sealant decreases the water entry and loss and also prevents the entry of foreign materials.

Approximately 8% of the total epidermal cells are melanocytes. They develop from the ectoderm of a developing embryo and these melanocytes produce the pigment called melanin. The melanocytes possess long, slender projections which extend between the keratinocytes and transfer melanin granules. Melanin appeared as brown-black or yellow-red in colour which is responsible for the skin colour. This absorbs the damaging ultraviolet light. The melanin granules form a protective layer over the nucleus and protect the DNA from UV light. Though the melanin granules protect the keratinocytes effectively, the melanocytes are highly susceptible to damage caused by the UV light.

Langerhans cells also said to be epidermal dendritic cells which were arise from red bone marrow and migrate to the epidermis. These cells constitute a tiny proportion of the total epidermal cells. They take part in immune responses raised against microbes that invade the skin and are easily damaged by the UV light. The function of these
cells in the immune response is to aid other cells of the immune system to recognize an invading microbe and obliterate it.

Merkel cells are the very least proportion of the total epidermal cells and are located in the deepest layer of the epidermis, where they contact the flattened process of a sensory neuron called a merkel (tactile) disc. Merkel cells and their allied merkel discs identify touch sensations.

Numerous discrete layers of keratinocytes in different stages of development form the epidermis. Generally the stratum corneum consists of four different layers namely stratum basale, stratum spinosum, stratum granulosum and a thin stratum corneum. But, the areas of the skin which expose to friction such as palms, finger tips, soles, etc will consists of five layers. i.e. stratum basale, stratum spinosum, stratum granulosum, stratum lucidem, and a thick stratum corneum.

1.4.1.1. Stratum basale

Stratum basale is the deepest layer of the epidermis and is composed of a single row of columnar or cuboidal keratinocytes. Few cells in this layer are stem cells which undergo cell division constantly to produce new keratinocytes. The nuclei of the keratinocytes present in the stratum basale are large in size and their cytoplasm contains numerous ribosomes, a small Golgi complex, a few mitochondria, and few rough endo-plasmic reticulums. The cytoskeleton present within the keratinocytes of the stratum basale includes scattered intermediate filaments which are called as keratin intermediate filaments.
(tonofilaments). These keratin intermediate filaments form the hard protein keratin and its more superficial epidermal layers. Keratin intermediate filaments attached to desmosomes which bind the cells of the stratum basale to each other & to the cell of the adjacent stratum spinosum and to hemidesmosomes, which binds the keratinocytes to the basement membrane positioned between the epidermis and the dermis. Melanocytes and merkel cells with their associated merkel discs are scattered among the keratinocytes of the stratum basal layer. The stratum basale is also known as the stratum germinativum to indicate its role in forming new cells. Keratin protects the deeper layers from injuries.

1.4.1.2. Stratum spinosum

Stratum spinosum present on the surface of the stratum basale and consists of abundant keratinocytes arranged in 8-10 layers. The cells which are present in the superficial layers are little flattened. The keratinocytes present in the stratum spinosum will have the same organelles as cells present in the stratum basale and some retained their ability to divide. The keratinocytes of these layers produce bundles of keratin in intermediate filaments. Though they are rounded and larger in living tissue, cells of the stratum spinosum shrink and pull apart when prepared for microscopic examination so that they appear to be covered with thorn like spins. Bundles of keratin intermediate filaments insert in to the desmosomes at each projection, which tightly joins the cells to each other. This pact provides both
flexibility and strength to the skin. Projections of melanocytes and Langerhans cells are also present in the stratum spinosum.

1.4.1.3. **Stratum granulosum**

Stratum granulosum is made up of flat keratinocytes and are arranged in 3-5 layers which will undergo apoptosis. The nuclei and other organelles of these keratinocytes begin to degenerate as they move away from their source nutrition. Even though the keratin intermediate filaments are no longer being produced by these cells, they will become more apparent because the organelles in the cells are regress. These cells contain darkly stained granules of a protein called keratohyalin. These granules filled with cystine and histidine rich proteins which binds the keratin filaments together. This is important job the keratohyalin granules.

A typical quality of these cells present in this layer is the presence of darkly stained granules of a protein called keratohyalin and is assembles the keratin intermediate filaments into keratin. During transition between stratum granulosum and stratum corneum, these cells secrete lamellar bodies, containing proteins and lipids, into extracellular spaces. This phenomenon is leads to formation of hydrophobic lipid envelop which is responsible for barrier properties of the skin.

1.4.1.4. **Stratum Lucidum**

This layer is present in the areas where thick skin will present such as soles, palms, fingertips, etc. the stratum lucidum consists of 4-6 layers of clear flat dead keratinocytes. These cells contain large
quantities of keratin. This may be the responsible for additional toughness in this region than other parts of the skin.

1.4.1.5. Stratum Corneum

This layer contains on average of 25-30 layers of flattened dead keratinocytes. The cells are extremely thin, flat hexagonal in shape and are free from nucleus. These are end product of the differentiation of keratinocytes. The cells are arranged in the form of brick-and-mortar structure. Adjacent cell layers are also form strong connections with one another. The plasma membranes of neighboring cells are arranged in complex, wavy folds that fit together like pieces of a jigsaw puzzle to hold the layers together. In this stratum corneum of the epidermis, the cells are continuously shed and replaced by new cells from the deeper layers. Its multiple layers protects from microbial invasions and injuries. Constant exposure of the skin to friction stimulates amplified cell production which results in the formation of a callus i.e. an abnormal thickening of the stratum corneum.

1.4.2. Dermis

Below to the epidermis the dermis will present which is second deeper layer of the skin. The dermis consists of dense irregular connective tissue containing elastic fibers and collagen. The fibers are arranged as woven network which will give great tensile strength. It has ability to stretch and recoil and is thicker than the epidermis. The thickness of the dermis will vary from one region to another in same subject and subject to subject. Leather used for manufacturing of shoes, belts, basketballs, baseball gloves, etc is the treated, dried
dermis of animals. The predominant cells present in the dermis are fibroblasts. The other cells which are resent in the dermis are macrophages and adipocytes. Adipocytes will present in the near to the subcutaneous layer. Nerves, blood vessels, hair follicles and glands are rooted in the dermis. It is an essential layer for the survival of the epidermis. The dermis is divided as two regions viz. thick deeper reticular region and thin superficial papillary region.

The papillary region occupy 1/5th thickness of the total thickness of the dermis and is composed of fine elastic fibers and thin collagen. Its surface area in increased by dermal papillae which appears as small, nipple shaped structures. These papillae project into the undersurface of the epidermis. All epidermal papillae consist of capillary loops and few also contain tactile receptors called corpuscles of touch or meissner corpuscles. Few other dermal papillae contain free nerve endings, dendrites which will not have any clear structural specialization. Different nerve endings initiate the signals of different sensations like warmth, pain, itching, tickling, coolness, etc.

The reticular region, attached to the subcutaneous layer, contains bundles of scattered fibroblasts, thick collagen fibers and various wandering cells. Some adipose cells along with some coarse elastic fibers will present in the deepest part of the layer. In the reticular region, the collagen fibers are arranged like net and the arrangement is more regular than those in the papillary region. The more regular arrangement of the large collagen fibers aids the skin
resist stretching. Nerves, blood vessels, hair follicles, sweat glands and sebaceous (oil) glands, occupy the spaces between fibers.

The combination of elastic and collagen fibers in the reticular region provides different properties like strength, extensibility, elasticity, etc to the skin. The surfaces of the fingers, palms, toes and soles have a series of ridges and grooves. They appear either as a pattern of loops or as straight lines and whorls, as on the tips of the digits. These epidermal ridges are formed during the third month of fetal development as downward projections of the epidermis into the dermis between the dermal papillae of the papillary region.

The epidermal ridges build a strong bond between the dermis and epidermis in a region of high mechanical stress. The epidermis ridges also boost the surface area of the epidermis and thus increase the grip of the hand or foot by increasing friction. The epidermal ridges also greatly increase surface area, which increase the number of meissner corpuscles and thus increases tactile sensitivity.
1.5. Pathways of drug absorption through the skin

Several investigations were carried out to determine the penetration pathways of the topically applied active principles into/through the skin to produce both localized and/or systemic effects. The investigations proposed different pathways through which drugs can be absorbed into and/or across the skin depending on the physicochemical properties of the drug. Both hydrophilic and lipophilic drugs are absorbed from different routes of the skin. The barrier properties of the stratum corneum will not permeate drugs through the skin but due to the presence of various absorption routes
facilitates the permeation of drugs and allows the drugs to reach the systemic circulation. The different absorption routes proposed by the researchers are as follows:

1. Transfollicular route
2. Transcellular route
3. Intercellular route

1.5.1. Transfollicular route

This route is the shortest pathway for drugs to reach the systemic circulation that provides a large area for diffusion of drugs. Various oil glands, hair follicles, sweat glands and pores openings present on the outer surface of the skin via their ducts. These ducts acts continuous channels for transportation of the drugs across the stratum corneum but extent of drug transportation across the skin will be influenced by various factors like secretions from the glands, amount of secretion and content etc. However transappendageal route occupies only 0.1% of total skin surface and therefore contributes minimum.

1.5.2. Transcellular route

In transcellular route drug transportation will takes place from the corneocytes which consists of highly hydrated keratin. This corneocytes will create hydrophilic pathways for transportaion of drugs. Around the corneocytes there will be lipids which connect these cells. Therefore drugs require partitioning and diffusion steps for permeation into the systemic circulation. This is the widely using route for transportation of various drugs. In this route the drugs will
pass through the matrix (cytoplasm) of the cells and is suitable route for hydrophilic drugs. The highly hydrated keratin provide aqueous pathway to the hydrophilic drugs. A number of partitioning and diffusion steps are require to pass the drug through the cell matrix.

1.5.3. **Intercellular route**

In intercellular route, the drugs diffuse through the lipid matrix present between the cells. The barrier property of this route is due to the tortuous structure formed by corneocytes and the drug has to pass through the alternating lipid and aqueous domain by partitioning into the lipid bilayer and diffusing to the inner side. It has been found that water has to travel 50 times more by this route so; it is suitable mainly for uncharged lipophilic drugs.

![Figure 1.6: Pathways of drug absorption through the skin](image)

**Figure 1.6: Pathways of drug absorption through the skin**
1.6. General method of drug absorption from skin to produce systemic effect

To produce desired therapeutic effect, the drug concentration in the plasma should lie in the therapeutic window, i.e. between minimum effective concentration (MEC) and maximum safe concentration (MSC). All drugs of desired concentration will not reach the therapeutic window when applied on the skin by means of conventional dosage forms such as ointments, creams, gels, etc because of various reasons like barrier properties of the skin, high molecular weight, high melting point, less lipophilicity of the drug etc. To overcome these difficulties and to achieve of desired concentration in the plasma different researchers introduced different technologies. The different technologies introduced by the researchers for administration of drugs across the skin to produce systemic effect are as follows.

- Chemical potential adjustment
- Ion pairs and complex coacervates
- Eutectic systems
- Hydration
- Chemical penetration enhancers
- Electrophoresis
- Iontophoresis
- Ultrasound (Phonophoresis, Sonophoresis)
- Laser radiation and photochemical waves
- Radio frequency
Magnetophoresis
Temperature ("thermophoresis")
Microneedle based devices
Skin puncture and perforation
Needleless injection
Suction ablation
Application of Pressure
Skin stretching
Skin abrasion
Pharmaceutical carrier systems, etc

1.6.1. Chemical potential adjustment

The highest rate of penetration of drug across the skin is observed when drug is at its peak thermodynamic activity which is similar to that of super saturated. Diffusion of paraben across the silicone membrane from saturated solutions in 11dissimilar solvents was determined. As there is variation in solubility of parabens in different solvents, the concentration is varied two folds. But, the flux of paraben from all solvents was same, since thermodynamic activity left constant due to the maintenance of saturated conditions entire experiment. Supersaturated solutions can crop up by evaporation of solvent or by mixing of cosolvents. Evaporation of solvent from skin surface is the most common mechanism to achieve supersaturation in many topically applied preparations. Apart from this, the water present in the skin was taken into the vehicle and works as antisolvent, the permeant thermodynamic activity would increase.⁸⁻¹¹.
1.6.2. **Ion pairs and complex coacervates**

Charged drugs substances do not readily permeate or partition into/through the skin. A new strategy i.e. formation of lipophilic ionpairs has been studied to enhance the permeation of charged drug molecules across the skin. This technique consists of mixing of an oppositely charged species to the charged drug molecule resulting in the formation of a complex in which the charges will be neutralized. The resultant new complex can permeate or partition into/through the skin. The complex has been dissociated in aqueous viable epidermis and releases the parent charged drug molecule which can diffuse within the skin tissues.\textsuperscript{12}

1.6.3. **Eutectic systems**

Melting point of drugs is an important parameter which will influence on their solubility which intern influence on the penetration into the skin. Regular solution theory states that drugs having lower melting point will exhibit higher solubility in given solvents and even in skin lipids also. Drug delivery systems melting point can be decreased by forming eutectic mixture. Eutectic mixtures are prepared by mixing two or more components at certain fixed concentrations. The components inhibit the crystalline process of each other such that the melting point of all components in the mixture is less than that of each component alone. A variety of eutectic mixtures containing penetration enhancers have been reported and the penetration acts as second component.
Eg: Ibuprofen with terpenes\textsuperscript{13}, and methyl nicotinate\textsuperscript{14}, propranolol with fatty acids\textsuperscript{15} and lignocaine with menthol\textsuperscript{16}.

1.6.4. Hydration

Water is the most widely used and accepted hydrating agent. Hydration is the safest method to enhance the penetration of hydrophilic\textsuperscript{17} and lipophilic\textsuperscript{18} drugs across the skin. Around 15-20\% of the water of dry weight is present in stratum corneum and whose concentration may change based on the humidity present in external environment. Additional water present within the stratum corneum could change permeant solubility which modifies partitioning from the vehicle into the membrane. Apart from this hydration of skin causes swelling of the stratum corneum which opens the microscopic structure and causes increase in permeation of drugs.

Scheuplein and Blank proved that the diffusion coefficients of alcohols in hydrated skin were ten times than that of dry skin\textsuperscript{19}. Hydration of skin can be increased by different means such as occlusion with plastic films; paraffins, oils, wax. The components of ointments and water-in-oil emulsions prevent the loss of transepidermal water and oil-in-water emulsions that donate water. Among all techniques plastic occlusive films or oily vehicle have most intense effect on hydration and penetration rate\textsuperscript{20}.

Eg: use of lignocaine and prilocane in occlusive condition for enhanced anaesthesia.
1.6.5. Chemical penetration enhancers (CPEs)

The use of CPEs over the other techniques has certain advantages, including design flexibility of the patch and ease of patch application over a large area (>10 cm²)²¹. An ideal penetration enhancer should reversibly reduce the barrier resistance of the SC without damaging the skin cells. According to Finnin et al.²²: ideal penetration enhancers should possess the following properties:

- Pharmacologically inert
- Nontoxic, nonirritating, and non-allergenic
- Rapid onset of action; predictable and suitable duration of action for the drug used
- Reversible effect of the CPE on the barrier property of SC
- Chemically and physically compatible with the delivery system
- Readily incorporated into the delivery system
- Inexpensive and cosmetically acceptable

Because the skin provides such a formidable barrier to the delivery of most drugs, a broad range of different chemical additives have been tested to enhance transdermal penetration during the last two decades. Much of the cited literature is found in patents²³ as well as pharmaceutical science literature²⁴. Even though many chemical entities have been identified, only a few were introduced in the market due to several limitations, which include their economic feasibility and the toxic effects on skin, which make them undesirable for developing transdermal patches. Penetration enhancers may act by means one and/or more of following three mechanisms²⁵:
Mechanism - 1: An interaction with intercellular protein.

Mechanism - 2: Disruption of the highly ordered structure of stratum corneum lipid.

Mechanism - 3: An improved partition of the drug, solvent or coenhancer into the stratum corneum.

1.6.5.1. Sulphoxides and similar chemicals

Dimethyl sulphoxides (DMSO) is one of the most primitive and an extensively studied penetration enhancer. It is a strong aprotic solvent and is considered as universal solvent. It appeared as colourless and odourless in nature. It is used as solvent in many areas in pharmaceutical sciences, to treat systemic inflammation by applying topically, penetration enhancer, etc. Though it has wide applications, it is associated with few drawbacks. The extent of permeation is concentration dependent and in general cosolvents containing greater than 60% Dimethyl sulfoxide are required to produce desired enhancement effect. Nevertheless, it can cause erythema. Denature of few skin proteins leads to erythema, contact urtcaria, scaling, burning, etc. As DMSO causing adverse effects, researchers introduced a similar kind of permeation enhancers with fewer side effects i.e. Dimethylacetamide (DMAC) and dimethylformamide (DMF). These are similar kind of aprotic solvents as that of DMSO. Conversely, Southwell and Barry, showed a 12-times increase in the flux of caffeine permeation across a DMF-treated human skin and they also concluded that there is an irreversible membrane damage by DMF. DMF caused an irreversible damage to
the human skin membranes but at the same time it had shown greater bioavailability of betamethasone-17-benzoate. This was measured by vasoconstrictor assay\textsuperscript{28, 29} in invivo study. DMSO may also extract lipids, making the horny layer more permeable by forming aqueous channels.

It has been postulated that DMSO denatures the intercellular structural proteins of the stratum corneum, or promotes lipid fluidity by disruption of the ordered structure of the lipid chains. In addition, DMSO may alter the physical structure of the skin by elution of lipid, lipoprotein and nucleoprotein structures of the stratum corneum. Decylmethylsulfoxide (DCMS) is thought to promote permeation enhancement as a result of protein-DCMS interaction creating aqueous channels, in addition to lipid interactions.

1.6.5.2. Alkanes

Long chain alkanes (C7-C16) have been shown to enhance skin permeability by non-destructive alteration of the stratum corneum barrier\textsuperscript{30}. These findings were confirmed in studies in which nonane was investigated as an enhancer\textsuperscript{31}, although there must be some destructive solubilisation and biochemical extraction caused by these lipophilic solvents.

1.6.5.3. Azone

Azone, 1-dodecylazacycloheptan-2-one or laurocapran, was the first molecule exclusively designed as a skin penetration enhancer. It is a highly lipophilic, colourless, odourless liquid with a melting point of \(-7^\circ\text{C}\) and log P of 6.2. It possesses a smooth, oily texture and is
soluble in and compatible with most of the organic solvents including propylene glycol and alcohol. It enhances the permeation of wide variety of drugs such as antiviral agents, steroids, antibiotics, etc across the skin. Generally, it is used as permeation enhancer at a concentrations between 0.1-5% and very specifically between concentrations 1-3%\textsuperscript{32}. Azone provokes dynamic structural disorder of the intercellular lamellar lipid structure throughout the \textit{stratum corneum} and the creation of fluid domains involving the intercellular lipids, which was suggested by 2H NMR assay. Another mechanism was also proposed based on the alteration of the lateral bonding within \textit{stratum corneum} lipid lamellae\textsuperscript{33}. Azone/PG increase penetration through the stratum corneum by affecting both the hydrophilic and lipophilic routes of penetration. Azone increases the fluidity of the lipid layer, while PG increases the water content of the proteinaceous region and helps azone partition into the aqueous region. A combination of these two helps the penetration of hydrophilic drugs greatly\textsuperscript{34}.

1.6.5.4. \textbf{Pyrrolidones}

Pyrrolidones have been used as permeation enhancers for both lipophilic (hydrocortisone and progesterone) and hydrophilic (e.g. 5-flurouracil and mannitol) molecules. N-methyl-2-pyrolidone was employed as permeation enhancer in the manufacturing of a matrix-type transdermal patch for administration of captopril and got limited success\textsuperscript{35}. These pyrrolidones get partitioned well within the tissues of human stratum corneum and they may act by changing the nature of
the solvent of the membrane. These have been used to develop reservoirs within the skin and these reservoirs will acts as potential for sustained release of permeant from the skin over extended period of times.\textsuperscript{35}

1.6.5.5. Urea

It enhances the permeation of drugs across the skin by hydrating the stratum corneum and also by forming the hydrophilic diffusion channels within the barrier, stratum corneum. Urea is less potential for enhancing the permeation of drugs through the stratum corneum. Hence researchers are trying to develop analogues high permeation enhancing capability. Wong et al developed cyclic ureas and studied their permeation capability of indomethacin across the shed snake skin and hairless mouse skin and compared with that of azones. Finally, they concluded that cyclic ureas as potent as that of azones. compared the permeation enhancing effect across the shed snake skin with azones using indomethacin as model. Finally, they concluded that the cyclic ureas as potent as that of azone. These are biodegradable and are non-toxic in nature which consists of a polar parent moiety and a chain alkyl ester group. Due to the presence of these groups, the mechanism of permeation may be a consequence of both hydrophilic activity and lipid disruption mechanism\textsuperscript{37}.

1.6.5.6. Fatty acids and Esters

By means of different fatty acids and their esters, the percutaneous absorption of many drugs has been increased. The widely used fatty acid is oleic acid. In general, unsaturated fatty acids
are more capable than their saturated products in enhancing the percutaneous absorption of drugs. It is of interest to note that many penetration enhancers such as azone contain saturated or unsaturated hydrocarbon chains and some structure–activity relationships have been drawn from the extensive studies of Aungst who employed a range of fatty acids, acids, alcohols, sulphoxides, surfactants and amides as enhancers for naloxone. Shin and his research team studied about different penetration enhancers like fatty acids (capric acid, myristic acid and lauric acid), glycols (tetraethylene glycol and diethylene glycol) and nonic surfactant (polyoxyethylene-2-stearly ether, polyoxyethylene-2-oleyl ether) on the release of triprolidone. Lauric acid in Propylene glycol enhanced the permeation of highly lipophilic antiestrogen. Oleic acid used as permeation enhancer in permeation of salicylic acid and 5-flurouracil across the human skin, *In vitro* and was found enhanced transdermal flux 28, 56-folds respectively.

1.6.5.7. Alcohols, fatty alcohols and glycols

Alcohols may influence transdermal penetration by a number of mechanisms. The alkyl chain length of the alkanols (fatty alcohols) is an important parameter in the promotion of permeation enhancement. Augmentation appears to increase as the number of carbon units increases, up to a limiting value. In addition, lower molecular weight alkanols are thought to act as solvents, enhancing the solubility of drugs in the matrix of the stratum corneum. Disruption of the stratum corneum integrity through extraction of biochemicals by the
more hydrophobic alcohols almost certainly also contributes to enhanced mass transfer through this tissue$^{44}$. The most commonly used alcohol as transdermal permeation enhancer is ethanol. By extracting the large quantities of lipids of stratum corneum, ethanol acts as permeation enhancer. Ethanol also enhances the number of keratin free sulphydryl groups in the stratum corneum proteins. Generally, pretreatment of the skin with ethanol results in enhanced transdermal permeation of hydrophilic compounds where as hydrophobic drugs permeation decreased$^{45}$. The molecular complexity of different glycol molecules is a determinant of their efficacy as permeation enhancers. Solubility of the drug in the delivery vehicle is markedly influenced by the number of ethylene oxide functional groups on the enhancer molecule; this solubility modification may either enhance or retard transdermal flux depending on the specific drug and delivery environment. The activity of propylene glycol (PG) is thought to result from solvation of a keratin within the stratum corneum; the occupation of proteinaceous hydrogen bonding sites reducing drug-tissue binding and thus promoting permeation$^{46}$. Propylene glycol (PG) is extensively used as a vehicle for penetration enhancer and exhibited synergistic action when used along with oleic acid.

**1.6.5.8. Surfactants**

Many surfactants are capable of interacting with the *stratum corneum* to increase the absorption of drugs and other active compounds from products applied to the skin. Skin penetration
measurements are valuable in quantifying these effects and observing the influence of surfactant chemistry and concentration. A surfactant interacts with skin by depositing onto the *stratum corneum*, thereby disorganizing its structure. Then surfactant can solubilise or remove lipids or water-soluble constituents in or on the surface of the *stratum corneum*. Finally it can be transported into and through the *stratum corneum*. This last effect is related to the surfactant and *stratum corneum* protein interaction and epidermal keratin denaturation. In general, anionic surfactants are more effective than cationic and nonionic surfactants in enhancing skin penetration of target molecules. Some anionic surfactants interact strongly with both keratin and lipids, whereas the cationic surfactants interact with the keratin fibrils of the cornified cells and result in a disrupted cell-lipid matrix. Nonionic surfactants enhance absorption by inducing fluidization of the *stratum corneum* lipids. Scheuplein and Ross reported that the capacity of the *stratum corneum* to retain significant quantities of membrane-bound water is reduced in the presence of sodium dodecanoate and sodium dodecyl sulfate. This effect is readily reversible upon removal of the agents. These investigations proposed that anionic surfactants alter the permeability of the skin by acting on the helical filaments of the *stratum corneum*, thereby resulting in the uncoiling and extension of _-keratin filaments to produce _- keratin. Then they cause an expansion of the membrane, which increases permeability. However, more recent findings suggest that impairment of the skin’s barrier properties is unlikely to result
from changes in protein conformation alone. Based on differential scanning calorimetry results, sodium lauryl sulfate (SLS) disrupted both the lipid and the protein components. The amount of surfactant that penetrates the skin after the disruption of the skin barrier depends on the monomer activity and the critical micelle concentration (CMC). Above the CMC, the added surfactant exists as micelles in the solution and micelles are too large to penetrate the skin. The extent of barrier disruption and penetration enhancement of a surfactant is also strongly dependent on surfactant structure, especially alkyl chain length. In general, studies have shown that surfactants having 12 carbons in their alkyl chain cause more disruption to the skin barrier and allow drugs to penetrate more readily than those that have more or less than 12 carbons. The explanation for this optimum of 12 carbons is not known so far.

### 1.6.5.9. Terpenes, Essential oil and terpenoids

Terpenes, found in essential oils, are compounds containing oxygen, hydrogen and carbon atoms. Several terpenes have been used as medicines, fragrance, flavoring agents, etc. The essential oils of ylang-ylang, chenopodium and eucalyptus have been used and proved as effective penetration enhancers for in vivo transdermal administration of 5-fluorouracil. Cornwell et al \(^{50}\) examined influence of 12 sesquiterpenes on 5-fluorouracil permeation across the human skin. The absorption of 5-fluorouracil was enhanced across the skin when it is pretreated with sesquiterpene oil or solid saturated in dimethyl isosorbide. In vitro permeation of morphine hydrochloride,
imipramine hydrochloride and hydrocortisone across the hairless rat skin was studied using L-menthol as permeation enhancer\textsuperscript{51} and also proved enhanced permeation of the same. The possible mechanism for enhanced permeation of these agents across the skin is modifying the nature of the solvent of the stratum corneum. Terpenes possibly will also alter the diffusivity of the drug across the semipermeable membrane.

1.6.5.10. Cyclodextrins

Cyclodextrins are biocompatible substances that can form inclusion complexes with lipophilic drugs with a resultant increase in their solubility, particularly in aqueous solutions. However, cyclodextrins alone were determined to be less effective as penetration enhancers than when combined with fatty acids and propylene glycol\textsuperscript{52}

Figure 1.7: Chemical structure of typical chemical penetration enhancers
1.6.6. Electroporation

The use of electroporation, as a method of enhancing diffusion across biological barriers, dates back as far as 100 years\(^5\). Electroporation involves the application of high voltage pulses to induce skin perturbation. High voltages (≥100 V) and short treatment durations (milliseconds) are most frequently employed. Other electrical parameters that affect delivery include pulse properties such as waveform, rate and number. The increase in skin permeability is suggested to be caused by the generation of transient pores during electroporation. 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 a molecular weight greater than 7kDa, the current limit for iontophoresis. Genetronics Inc (San Diego, California) have developed a prototype electroporation transdermal device, which has been tested with various compounds with a view to achieving gene delivery, improving drug delivery and aiding the application of cosmetics. Other transdermal devices based on electroporation have been proposed by various groups however, more clinical information on the safety and efficacy of the technique is required to assess the future commercial prospects.
Figure 1.8: Basic design of electroporation drug delivery device

1.6.7. Iontophoresis

This method involves enhancing the permeation of a topically applied therapeutic agent by the application of a low level electric current either directly to the skin or indirectly via the dosage form. Increase in drug permeation as a result of this methodology can be attributed to either one or a combination of the following mechanisms; electrorepulsion (for charged solutes), electro osmosis (for uncharged solutes) and electropertubation (for both charged and uncharged). Parameters that affect design of an iontophoretic skin delivery system include; electrode type, current intensity, pH of the system, competitive ion effect and permeant type. The launch of commercialised systems of this technology has either occurred or is currently under investigation by various companies. Extensive literature exists on the many types of drugs investigated using
iontophoretic delivery and the reader is referred to the following extensive reviews. The Phoresor™ device (Iomed Inc.), was the first iontophoretic system to be approved by the FDA in the late 70’s as a physical medicine therapeutic device. In order to enhance patient compliance the use of patient-friendly, portable and efficient iontophoretic systems have been under intense development over the years. Such improved systems include the Vyteris and E-TRANS iontophoretic devices. Previous work has also reported that the combined use of iontophoresis and electroporation is much more effective than either technique used alone in the delivery of molecules across the skin. The limitations of ionotophoretic systems include the regulatory limits on the amount of current that can be used in humans (currently set at 0.5 mA cm$^{-2}$) and the irreversible damage such currents could do to the barrier properties of the skin. In addition, iontophoresis has failed to significantly improve the transdermal delivery of macromolecules of $>7000$ Da$^{55}$. 
Figure 1.9: Basic principle of iontophoresis. A current passed between the active electrode and the indifferent electrode repelling drug away from the active electrode and into the skin.

1.6.8. Ultrasound (sonophoresis and phonophoresis)

Ultrasound involves the use of ultrasonic energy to enhance the transdermal delivery of solutes either simultaneously or via pre-treatment and is frequently referred to as sonophoresis or phonophoresis. The proposed mechanism behind the increase in skin permeability is attributed to the formation of gaseous cavities within the intercellular lipids on exposure to ultrasound resulting in disruption of the SC. Ultrasound parameters such as treatment duration, intensity and frequency are all known to affect percutaneous absorption, with the latter being the most important. Although frequencies between 20 kHz-16 MHz have been reported to enhance skin permeation, frequencies at the lower end of this range (< 100 kHz) are believed to have a more significant effect on transdermal drug delivery with the delivery of macromolecules of molecular weight up to
48 kDa being reported. The SonoPrep ® device (Sontra Medical Corporation) uses low frequency ultrasound (55 kHz) for an average duration of 15 s to enhance skin permeability. This battery operated hand held device consists of a control unit, ultrasonic horn with control panel a disposable coupling medium cartridge, and a return electrode. The ability of the SonoPrep® device to reduce the time of onset of action associated with the dermal delivery of local anaesthetic from EMLA cream was recently reported. In the study by Kost et al.(37), skin treatment by ultrasound for an average time of 9 s resulted in the attainment of dermal anaesthesia within 5 min, which was comparable to the 60 min required in for non-treated skin. The use of other small, lightweight novel ultrasound transducers to enhance the In vitro skin transport of insulin has also been reported by a range of workers57.
Figure 1.10: Basic principle of phonophoresis. Ultrasound pulses are passed through the probe into the skin fluidizing the lipid bilayer by the formation of bubbles caused by cavitation.

1.6.9. Laser radiation and photomechanical waves

Lasers have been used in the clinical therapies for decades, therefore their effects on biological membranes are well documented. Lasers are frequently used for the treatment of dermatological conditions such as acne and to confer ‘facial rejuvenation’ where the laser radiation destroys the target cells over a short frame of time (~300 ns). Such direct and controlled exposure of the skin to laser radiation results in ablation of the SC without significant damage to the underlying epidermis. Removal of the SC via this method has been shown to enhance the delivery of lipophilic and hydrophilic drugs. The extent of barrier disruption by laser radiation is known to be controlled by parameters such wavelength, pulse length, pulse energy,
pulse number and pulse repetition rate. A hand-held portable laser device has been developed by Norwood Abbey Ltd (Victoria, Australia). In a study involving human volunteers, the Norwood Abbey laser device was found to reduce the onset of action of lidocaine to 3-5 min, whilst 60 min was required to attain a similar effect in the control group. The Norwood Abbey system has been approved by the US and Australian regulatory bodies for the administration of a topically applied anaesthetic. Pressure waves (PW), which can be generated by intense laser radiation, without incurring direct ablative effects on the skin have also been recently found to increase the permeability of the skin. It is thought that PW form a continuous or hydrophilic pathway across the skin due to expansion of the lacunae domains in the SC. Important parameters affecting delivery such as peak pressure, rise time and duration has been demonstrated. The use of PW may also serve as a means of avoiding problems associated with direct laser radiation. Permeants that have been successfully delivered in vivo include insulin\textsuperscript{58}, 40 kDa dextran and 20 nm latex particles. A design concept for a transdermal drug delivery patch based on the use of PW has been proposed by Doukas & Kollias.

1.6.10. Radio-frequency

Radio-frequency involves the exposure of skin to high frequency alternating current (~ 100 kHz) resulting in the formation of heat-induced microchannels in the membrane similar to when laser radiation is employed. The rate of drug delivery is controlled by the number and depth of the microchannels formed by the device, which
is dependent on the properties of the microelectrodes used in the device. The Viaderm device (Transpharma Ltd) is a hand held electronic device consisting of a microprojection array (100 microelectrodes/cm²) and a drug patch. The microneedle array is attached to the electronic device and placed in contact with the skin to facilitate the formation of the microchannels. Treatment duration takes less than a second, with a feedback mechanism incorporated within the electronic control providing a signal when the microchannels have been created, so as to ensure reproducibility of action. The drug patch is then placed on the treated area. Experiments in rats have shown the device to enhance the delivery of granisetron HCL, with blood plasma levels recorded after 12 h rising to 30 times higher levels than that recorded for untreated skin after 24 h. A similar enhancement in diclofenac skin permeation was also observed in the same study. The device is reported not to cause any damage to skin with the radio-frequency-induced microchannels remaining open for less than 24 h.

**1.6.11. Magnetophoresis**

This method involves the application of a magnetic field which acts as an external driving force to enhance the diffusion of a diamagnetic solute across the skin. Skin exposure to a magnetic field might also induce structural alterations that could contribute to an increase in permeability. *In vitro* studies by Murthy showed a magnetically induced enhancement in benzoic acid flux, which was observed to increase with the strength of the applied magnetic field.
Other *In vitro* studies using a magnet attached to transdermal patches containing terbutaline sulphate (TS), demonstrated an enhancement in permeant flux which was comparable to that attained when 4% isopropyl myristate was used as a chemical enhancer. In the same paper the effect of magnetophoresis on the permeation of TS was investigated *in vivo* using guinea pigs. The preconvulsive time (PCT) of guinea pigs for those subjected to magnetophoretic treatment was found to last for 36 h which was similar to that observed after application of a patch containing 4% IPM. This was in contrast to the response elicited by the control (patch without enhancer), when the increase in PCT was observed for only 12 h. In human subjects, the level of TS in the blood was higher but, not significantly different to that observed with the patch containing 4% IPM. The fact that this technique can only be used with diamagnetic materials will serve as a limiting factor in its applicability and probably explains the relative lack of interest in the method.

1.6.12. **Microneedle based devices**

One of the first patents ever filed for a drug delivery device for the percutaneous administration of drugs was based on this method\(^6\). The device as described in the patent consists of a drug reservoir and a plurality of projections extending from the reservoir. These microneedles of length 50-110 µm will penetrate the SC and epidermis to deliver the drug from the reservoir. The reservoir may contain drug, solution of drug, gel or solid particulates and the various embodiments of the invention include the use of a membrane
to separate the drug from the skin and control release of the drug from its reservoir. As a result of the current advancement in microfabrication technology in the past ten years, cost effective means of developing devices in this area are now becoming increasingly common. A recent commercialisation of microneedle technology is the Macroflux® microprojection array developed by ALZA Corporation. The macroflux® patch can either be used in combination with a drug reservoir or by dry coating the drug on the microprojection array; the latter being better for intracutaneous immunization. The lengths of the microneedles have been estimated to be around 50-200 µm and therefore are not believed to reach the nerve endings in the dermo-epidermal junction. The microprojections/ microneedles (either solid or hollow) create channels in the skin, hence allowing the unhindered movement of any topically applied drug. Clinical evaluations report minimal associated discomfort and skin irritation and erythema ratings associated with such systems are reportedly low. This technology serves as an important and exciting advance in transdermal technology due to the ability of the technique to deliver medicaments with extremes of physicochemical properties (including vaccines, small molecular weight drugs and large hydrophilic biopharmaceuticals. Dual purpose hollow microneedle systems for transdermal delivery and extraction which can be coupled with electrotransport methods are also described by Allen et al.$^{62}$ These mechanical microdevices which interface with electronics in order to
achieve a programmed or controlled drug release are referred to as microelectromechanical systems (MEMS) devices.

Figure 1.11: Basic design of microneedle delivery devices. Needles of approximately with or without centre hollow channels are placed onto the skin surface so that they penetrate the stratum corneum and epidermis without reaching the nerve endings present in the upper dermis.

1.6.13. Skin puncture and perforation

These devices are similar to the microneedle devices produced by microfabrication technology. They include the use of needle-like structures or blades, which disrupt the skin barrier by creating holes and cuts as a result of a defined movement when in contact with the skin. Godshall and Anderson, described a method and apparatus for disruption of the epidermis in a reproducible manner. The apparatus consists of a plurality of microprotrusions of a length insufficient for penetration beyond the epidermis. The microprotrusions cut into the outer layers of the skin by movement of the device in a direction
parallel to the skin surface. After disruption of the skin, passive (solution, patch, gel, ointment etc) or active (iontophoresis, electroporation etc) delivery methods can then be utilised. Descriptions of other devices based on a similar mode of action have been described by Godshall\textsuperscript{63}, Kamen\textsuperscript{64}, Jang\textsuperscript{65} and Lin \textit{et al}\textsuperscript{66}.

1.6.14. Needleless injection

Needleless injection is reported to involve a pain free method of administering drugs to the skin. This method therefore avoids the issues of safety, pain and fear associated with the use of hypodermic needles. 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. Over the years there have been numerous examples of both liquid (Ped-O-Jet\textsuperscript{®}, Iject\textsuperscript{®} Biojector2000\textsuperscript{®}, Medi-jector\textsuperscript{®} and Intraject\textsuperscript{®}) and powder (PMEDTM device formerly known as powderject\textsuperscript{®} injector) systems. The latter device has been reported to deliver successfully testosterone, lidocaine hydrochloride and macromolecules such as calcitonin and insulin\textsuperscript{66,67}. Problems facing needless injection systems include the high developmental cost of both the device and dosage form and the inability, unlike some of the other techniques described previously, to programme or control drug delivery in order to compensate for inter-subject differences in skin permeability. In addition, the long-term effect of bombarding the skin with drug particles at high speed is not known thus, such systems may not be suitable for the regular administration of drugs. It
may however be very useful in the administration of medicaments which do not require frequent dosing e.g. vaccines.

1.6.15. **Suction ablation**

Formation of a suction blister, involves the application of a vacuum or negative pressure to remove the epidermis, whilst leaving the basal membrane intact. The cellpatch® (Epiport Pain Relief, Sweden) is a commercially available product based on this mechanism. It comprises a suction cup, epidermatome (to form a blister) and device (which contains morphine solution) to be attached to the skin this method which avoids dermal invasivity thereby avoiding pain and bleeding is also referred to as skin erosion. Such devices have also been shown to induce hyperaemia in the underlying dermis in *in vivo* studies which was detected via laser Doppler flowmetry and confirmed via microscopy, and is thought to further contribute to the enhancement of dextran and morphine seen with this method. The disadvantages associated with the suction method include the prolonged length of time required to achieve a blister (2.5 h), although this can be reduced to 15-70 min by warming the skin to 38 °C. In addition, whilst there is no risk of systemic infection compared to the use of intravenous catheters, the potential for epidermal infections associated with the suction method cannot be ignored even though the effects might be less serious.

1.6.16. **Application of Pressure**

The application of modest pressures (i.e. 25 kPa) has been shown to provide a potentially noninvasive and simple method of
enhancing skin permeability of molecules such as caffeine. These workers attributed the increase in transcutaneous flux to either an improved transapendageal route or an increased partition of the compound into the SC when pressure was applied. This method may also work due to the increased solubility of caffeine in the stratum corneum caused by the increase in pressure.

![Figure 1.12: Enhancement of transdermal permeation by pressure wave](image)

1.6.17. Skin stretching

These devices hold the skin under tension in either a unidirectional or multidirectional manner. The authors claim that a tension of about 0.01 to 10 mP results in the reversible formation of micropathways. The efficiency of the stretching process was demonstrated by monitoring the delivery of a decapeptide (1 kDa) across the skin of hairless guinea pigs using a microprotrusion array. The results of the study showed that the bi-directional stretching of
skin after microprotrusion piercing, allowed the pathways to stay open (i.e. delayed closure) hence facilitating drug permeation to a greater extent ($27.9 \pm 3.3 \, \mu g/cm^2 \, h$) than in the control group ($9.8 \pm 0.8 \, \mu g/cm^2 \, h$), where the skin was not placed under tension after microneedle treatment. However, increased skin permeation in the absence of microneedle pre-treatment was found not to occur. Other methods involving the use of skin stretching with subsequent use of delivery devices based on electrotransport, pressure, osmotic and passive mechanisms have also been suggested but the value of skin stretching alone without the benefit of a secondary active delivery device remains to be seen.

1.6.18. **Skin abrasion**

These techniques, many of which are based on techniques employed by dermatologists in the treatment of acne and skin blemishes (e.g. microdermabrasion), involve the direct removal or disruption of the upper layers of the skin to enhance the permeation of topically applied compounds. The delivery potential of skin abrasion techniques are not restricted by the physicochemical properties of the drug and previous work has illustrated that such methods enhance and control the delivery of a hydrophilic permeant, vitamin C vaccines and biopharmaceuticals. One current method is performed using a stream of aluminium oxide crystals and motor driven fraises Sage & Bock also describe a method of pre-treating the skin prior to transdermal drug delivery which consists of a plurality of microabraders of length 50-200 µm. The device is rubbed against the
area of interest, to abrade the site, in order to enhance delivery or extraction. The microabraders/microprotrusions terminate as blunt tips and therefore do not penetrate the SC. The device functions by removing a portion of the SC without substantially piercing the remaining layer. Some of these methods are claimed to offer advantages such as minimal patient discomfort, increased patient compliance, ease of use and less risk of infection compared to their more “invasive” predecessors such as ablation and the use of hypodermic needles/cannulas to deliver medicaments across the skin.

1.6.19. Pharmaceutical carrier systems

Different types of pharmaceutical carriers are present. They are - particulate, polymeric, macromolecular, and cellular carrier. Particulate type carrier also known as a colloidal carrier system, includes lipid particles (low and high density lipoprotein-LDL and HDL, respectively), microspheres, nanoparticles, polymeric micelles and vesicular like liposomes, niosomes pharmacosomes, virosomes, etc. These carriers were introduced for different purposes and all will not have same qualities.
1.7. Vesicular Carrier Systems

Since two decades the interest on the vesicles has been increased tremendously because of the potential advantages of vesicles. Huge research work is going on the vesicles to achieve different objectives. They become vehicle of choice for drug delivery to researchers and are using these vesicles in the areas like immunology, membrane biology, diagnostic techniques, genetic engineering, etc and are using in the transport and targeting of active pharmaceutical ingredients.

The vesicular systems are highly ordered assemblies of one or several concentric lipid bilayers formed, when certain amphiphilic building blocks are confronted with water. Vesicles can be formed from a diverse range of amphiphilic building blocks. The terms such as synthetic bilayers allude to the non-biological origin of such...
vesiculogenes. Biologic origin of these vesicles was first reported in 1965 by Bingham, and was given the name Bingham bodies.

Figure 1.14: Structure of typical vesicle

The different advantages of the vesicular carrier systems are as follows.

1. Lipid vesicles have evolved successfully, as vehicles for controlled delivery.

2. Vesicles can be administered from different routes such as oral, IM, nasal, transdermal, vaginal, rectal, ocular, etc. and possible to produce both the localized and/or systemic effects.

3. Enhances the bioavailability of the drugs by increasing the mean residence time in biological environment and/or by enhancing the solubility.

4. Reduces the adverse effects associated with the high doses of the drugs by increasing the bioavailability of the active principles.
5. Enhances the stability of the drugs by avoiding the exposure of the drugs to the susceptible environments.

6. Drug targeting can be possible by means of these vesicles.

7. Both hydrophilic and lipophilic drugs can incorporate in these vesicles.

As these vesicles have potential applications, the interest on these vesicles have been increased and developed different vesicles for different purposes. The different vesicles include liposomes, niosomes, ethosomes, transferosomes, pharmacosomes, etc.

1.7.1. Liposomes

Liposomes are simple microscopic lipid vesicles ranging from 20 nanometers to several micrometers in size and in these vesicles the lipid bilayer encloses the aqueous environment. Both hydrophilic and lipophilic drugs can be successfully entrapped\textsuperscript{72} in these vesicles. Hydrophilic and lipophilic drugs entrapped in the central aqueous environment and within the lipid bilayers respectively\textsuperscript{6}. Liposomes can be used for delivery of drugs through oral, topical, ocular, vaginal, etc routes.
Phospholipid and cholesterol are the main ingredients for manufacturing of liposomes. Phosphatidylcholine (PC), a natural phospholipid, is most commonly used for preparation of liposomes. One can also use other phospholipids such as phosphatidyl ethanolamine (PE), phosphatidyl serine (PS), phosphatidyl inositol (PI) and phosphatidyl glycerol (PG) for manufacturing of liposomes. Cholesterol is used for different purposes such as fluidity buffer and also provides rigidity to the lipid bilayer.

Figure 1.15: structure of conventional liposome

Figure 1.16: Chemical structure of Phosphatidylcholine (PC)
Liposomes, as particulate carriers, naturally can target cells of the mononuclear phagocytic system (MPS), particularly macrophages to treat a number of diseases such as infectious diseases, cancer and atherosclerosis and inflammatory diseases. To achieve targeting of liposomes to monocytes, macrophages and dendritic cells, the physicochemical properties of liposomes has been modified by addition of surface ligands such as proteins, peptides, antibodies, polysaccharides, glycolipids, glycoproteins and lectins. Doijad Rajindra C et al prepared zidovudine, loaded liposomes for targeting to liver followed by lungs, kidney and spleen against human immunodeficiency virus (HIV)\textsuperscript{73}. Though this liposomes have wide advantages, its success rate decreased due to its bigger size, impermeable to deeper layers of the skin, fusion, leakage, etc.

The proposed mechanisms for drug delivery across the skin through liposomes are intact vesicular skin permeation, the penetration enhancing effect, the adsorption effect and the penetration of liposomes through the transappendageal route.

1.7.2. **Emulsomes**

Emulsomes are lipid vesicles designed for poorly soluble drugs to administer them through the parenteral route\textsuperscript{74}. The internal core of emulsomes is prepared with fats and triglycerides and is stabilized in form of o/w emulsion by adding high concentration of lecithin. These will posses both liposomes and emulsions characteristics. Entrapment efficiency of lipophilic drugs can be enhanced by the process of solidification or semisolidification of internal oily core which
also controls the release of drugs from vesicles. As liposomes, in these vesicles also one can encapsulate hydrophilic drugs in aqueous compartments of surrounding phospholipid layers. The solvent-free and surfactant-free emulsion technologies were developed to increase the encapsulation capacity of water insoluble antifungal and anticancer drugs which showed improved preclinical efficacy for oral route. Vyas et al developed zidovudine emulsomes for sustained and targeted drug delivery to liver for the treatment of life-threatening viral infections like hepatitis, HIV and Epstein-Barr virus infection.

![Figure 1.17: Structure of emulsomes](image)

### 1.7.3. Ethosomes

These lipid vesicles developed to deliver the drugs having poor penetration through the skin. They are soft in nature having size range from tens of nano meters to microns. These vesicles contains more concentration of ethanol (20-50%) which brings soft malleable nature to the vesicles and also solubilizes the inter cellular lipid and
enhances the penetration of the drugs through the skin\textsuperscript{75}. Ethanol induces surface negative net charge to the vesicles which decreases the size of the vesicles. Concentration of ethanol in vesicles is inversely proportional to the size of the vesicles. Ethosomes has been used as carrier for delivery of many drugs through the skin successfully to produce both localized and/or systemic effects. The drugs includes antifungal agents (fluconazole) antiviral agents (lamivudine, acyclovir, stavudine, and zidovudine), NSAIDS (aceclofenac, diclofenac), antibiotics (cannabidol, erythromycin), etc. in all the studies ethosomes has shown enhanced therapeutic effect when compared with conventional methods.

Exact mechanism of drug permeation across the skin through ethosomes is not proposed. It is assumed that a combination of two processes contribute to the enhancing effect. The two processes are solubilisation of stratum corneum lipid and on vesicles fluidity as well as dynamic interaction between ethosomes and the stratum corneum.
1.7.4. Enzymosomes

These are developed to deliver the therapeutic proteins like enzymes and are developed by complexing the enzymes with the lipids. Superoxide Dismutase, a therapeutic agent for oxidative stress related diseases like rheumatoid arthritis and ischaemia/reperfusion situations, loaded enzymosomes have been developed for long circulation time in the blood, in order to accumulate at inflammed target sites, while maintaining enzymatic activity in its intact form.

1.7.5. Sphingosome

These are introduced to overcome the stability problems associated with liposomes and are called as sphingosomes due to the presence of the sphigolipids in place of phospholipids in liposomes. Phospholipids readily undergo chemical degradation such as
oxidation, hydrolysis due to the presence of ester linkage. In sphingolipids either an ether or amide linkage will present which are resistant to chemical degradation and brings stability to the sphingolipids than the phospholipids. Sphingosomes are efficient carriers because of high stability, for targeting of the drug to the site of action and are being biodegradable, innocuous nature and also identical to biological membrane.

Sphingosomes offer selective passive targeting to tumour tissues and flexibly bind with site-specific ligands to achieve active targeting. These are much more stable to acid hydrolysis and have better drug retention characteristics. Sphingosomal products e.g., Marqibo(TM) (vincristine loaded sphingosomes) are loaded with active, cell cycle-specific anticancer agents that are benefited from increased targeting and long duration of drug exposure at the tumor site. Vinorelbine and topotecan are also selected for sphingosomal formulation specifically for their ability to benefit from this novel encapsulation.

![Figure 1.19: structure of sphingolipids](image)
1.7.6. **Transferosomes**

Transferosome was introduced by Gregor Cevc in the year 1991. The word “Transferosome” means “carrying body” and is derived from Latin word “transferre” means “to carry across” and the Greek word “soma” means “body”. The unique nature of these vesicles is that they have deformable nature. Due to its deformable nature these vesicles penetrate through the pores having less size than the vesicles and deliver the drugs across the skin without making puncture of the skin and with less loss.

![Structure of transferosomes](image)

**Figure 1.20: Structure of transferosomes**

These are used as carriers for many drugs such as hormones, proteins, peptides and various drug molecules. These vesicles are also used successfully to deliver the active principles, such as insulin, interferons, which are highly unstable in GIT, through the skin. Large molecular weight substances such as proteins are difficult to deliver through skin due to their large m.wt which can also delivered successfully through the skin by these vesicles. In a
study it was shown that these vesicles delivered diclofenac sodium 10times more than the commercial hydrogel across the skin and shown longer effect\textsuperscript{76}.

Two mechanisms have been proposed to explain the drug permeation across the skin by means of transferosomes. First, vesicles can act as drug carrier systems, where by intact vesicles enter the saturation stratum corneum carrying vesicle bound drug molecules into the skin. Second vesicles can acts as penetration enhancers, whereby vesicle bilayers enter the stratum corneum and subsequently modify the inter cellular lipid lamellae.

![Figure1.21: Mechanism of transferosomes permeation across the skin](image)

**1.7.7. Pharmacosomes**

Pharmacosomes are novel vesicular drug delivery systems having unique advantages over other drug delivery systems\textsuperscript{77}. Pharmacosomes are amphiphilic lipoidal colloidal dispersions of
drugs, covalently bound to lipids with potential to improve bioavailability of poorly water soluble as well as poorly lipophilic drugs. Any drug possessing a free carboxyl group or an active hydrogen atom (-OH, -NH2) can be esterified (with or without a spacer group) to the hydroxyl group of a lipid molecule, thus generating an amphiphilic prodrug. An amphiphilic prodrug is then converted to pharmacosomes upon dilution with water. The prodrug conjoins hydrophilic and lipophilic properties (thereby acquiring amphiphilic characteristics), reduces interfacial tension, and at higher concentration exhibits mesomorphic behavior. Because of decrease in interfacial tension, the contact area increases, therefore bioavailability also increases. As the drug is covalently conjugated with lipids, loss due to leakage of drug does not occur. Hence, provides maximum entrapment efficiency. The three main components for the preparation of pharmacosomes are drug, solvent and lipid. Drug should contain active hydrogen atom (-COOH, OH, NH2), can be esterified with lipid and form amphiphilic complexes, which facilitate membrane transfer. The solvent should have high purity, volatility and intermediate polarity (between the polarity of phospholipid and drug) for the preparation of pharmacosomes. The most commonly used lipid for the preparation of pharmacosomes is phosphatidylcholine. The pharmacosomes can be prepared by hand-shaking and ether-injection methods. These have been prepared for various non-steroidal anti-inflammatory drugs, proteins, cardiovascular and antineoplastic drugs. Drug release from pharmacosomes is by hydrolysis (including
enzymatic) unlike liposomes the release of drug is by diffusion through bilayer, desorption from the surface or degradation of liposomes.

1.7.8. Virosomes

Virosomes are reconstituted viral envelopes that are composed of a lipid bilayer in which inserted viral glycoproteins can be derived from different enveloped viruses. Virosomes are described as liposomes with influenza virus hemagglutinin (HA) and neuraminidase (NA) spikes on their surface. Virosomes closely mimic the intact virus except that they do not contain virus replication machineries. They retain the cell entry and membrane fusion characteristics of the virus derived from. The two pathways by which reconstituted vesicles are able to enter the cells and deliver their contents into the cytoplasm are plasma membrane fusion (Sendai virus) and acid-induced fusion from within endosomes (Influenza virus). As a result, foreign substances encapsulated within the lumen of virosomes are effectively delivered to the cytosol of target cells. Virosomes can be used in vaccination for the efficient induction of antibody responses against the virus they are derived from.  

1.7.9. Non-Ionic Surfactant Vesicles/Niosomes

First, in 1979, Handjanivila et al reported the niosomes will form upon hydration of a mixture of cholesterol and single alkyl chain. These are also said to be non-ionic surfactant vesicles. Since 1979, different non-ionic surfactants such as polyglycerol alkyl ethers, polyoxyethylene alkyl ethers, ester linked surfactants, steroid linked surfactant, brij and a series of spans, tweens, etc have to used to
manufacture the niosomes\textsuperscript{79}. These have microscopic lamellar structure of size range 10-1000nm consisting of spherical, uni or multilamellar and polyhedral vesicles in aqueous media. Cationic, anionic and ampholytic surfactants also used to prepare niosomes but as they causes skin irritation, non ionic surfactants are preferred which causes no irritation. These niosomes have many advantages over lipid vesicles with respect to stability, manufacturing and storage. These will not require special condition for manufacturing and storage because the non ionic surfactants are less prone to oxidation comparatively lipids which are used in preparation of liposomes and other lipid vesicles. These have shown better patient compliance and better therapeutic effect in comparison to oily formulations.

\textbf{Figure 1.22: Non-ionic surfactant vesicle / Niosome}

\textbf{1.7.10. Bilosomes}

Bilosomes are the novel innovative drug delivery carriers consist of deoxycholic acid incorporated into the membrane of niosomes. As conventional vesicles (liposomes and niosomes) can
cause dissolution and undergo enzymatic degradation in gastrointestinal tract but incorporation of bile salts (commonly used penetration enhancers) in niosomal formulation could stabilize the membrane against the detrimental effects of bile acids in GI tract. These bile salt stabilized vesicles are known as bilosomes. These are highly biocompatible and have been found to improve the therapeutic efficacy of drugs due to their stability in gastrointestinal tract. Bilosomes have been found to increase the bioavailability of drugs as they can readily absorbed through small intestine to the portal circulation (hepatocirculation). Through this circulation they approach to liver and release the drug, so found to be an effective tool in drug targeting to liver. Shukla et al showed that HBsAg loaded bilosomes produced both systemic as well as mucosal antibody responses upon oral administration. For extended humoral, cell-mediated and mucosal immune responses, additional coating carrier system provided better protection against disease for longer period of time. Optimum mannan coating was found to stabilize the vesicles in gastrointestinal environment and also act as a targeting ligand for mannose receptors expressed on macrophages and dendritic cells\(^80\).

1.7.11. **Aquasomes**

Aquasomes firstly developed by Kossovsky, are one of the most recently developed delivery system for bioactive molecules\(^81\). Aquasomes are three layered structures (i.e. core, coating and drug) that are self-assembled through non covalent bonds, ionic bonds and vander waals forces. They consist of tin oxide, nanocrystalline carbon
ceramic (diamonds) or brushite (calcium phosphate dihydrate) core coated with oligomeric film to which biochemically active molecules are adsorbed by copolymerization, diffusion or adsorption with or without modification. The solid core provides the structural stability, while the carbohydrate coating protects against dehydration and stabilizes the biochemically active molecules. Aquasomes are spherical 60-300nm size particles called Âbodies of waterÊ. Their water like properties protects and preserves fragile biological molecules. Mechanism of action of aquasomes is controlled by their surface chemistry, which deliver contents through combination of specific targeting, molecular shielding and slow and sustained release process. Due to their size and structural stability, these avoid clearance by reticuloendothelial system and degradation by other environmental changes. Aquasomes can be used as red blood cell substitutes for the release of oxygen by haemoglobin. Aquasomes can be used as vaccines for delivery of viral antigen, for targeted intracellular gene therapy, for delivery of insulin and enzymes like DNAase and pigments/dyes.