1. INTRODUCTION

1.1 Physiology of Human skin

The largest organ of man is skin which provides about 10% of the body mass of an average individual. Skin of average human contains about 200-250 sweat ducts and about 40-70 hair follicles per sq. cm of skin. These skin appendages occupy about 0.1% of total surface of stratum corneum hence the transappendageal route of percutaneous absorption has a small contribution in the total kinetic profile of drug permeation across the skin. Hence, permeation of most neutral molecule can thus be considered as primary diffusion in the interfollicular region through intact stratum corneum. So, for fundamental understanding of drug delivery system through skin, the structure of skin should be understood. (*Gros L, et al; 1980*)

Essential functions of skin include thermoregulation, protection, sensory detection, immune responsiveness, biochemical synthesis and sexual and social communication. Skin is large and easily accessible area for the administration of therapeutic agents. However, it also forms a highly efficient barrier between internal and external parts of the body. Skin is highly resistant to the penetration by any exogenous compound including chemicals and also organisms like bacteria and viruses. Transverse section of human skin is shown in figure 1.1.

Structure of human skin is made up of three layers, viz. epidermis, dermis and hypodermis.
1.1.1. Epidermis

Epidermis is the outermost skin layer. There are number of different strata in which the cells have distinct anatomical features in typical part of epidermis. Skin showing layers of epidermis are shown in figure 1.2. From below, the first stratum is the basal layer or layer of Malpighi. Its cells are mostly polygonal in shape, the deepest tending to a cylindrical columnar form, and the most superficial becoming somewhat flattened. Active mitotic proliferation takes place in the deeper layers, the development of new cells leading to a gradual displacement of the older cells towards the surface. Hence, this stratum is also called stratum germinativum. The epidermis is quiet avascular and between the cells of stratum germinativum there are fine intercellular channels which probably allow the transmission of nutrient fluids derived from capillary blood vessels in the adjacent dermis. These channels are bridged across by delicate protoplasmic threads connecting one cell with another. Epidermis is multi-layered membrane with varied thickness depending upon part of the body. The thickness on eyelids is about 0.06 mm whereas it is about 0.8 mm on palms of the hand and soles of the feet. It does not contain blood vessels. It is built up by several cells which can be further divided into: 1) Stratum germinativum or stratum basale, 2) Stratum spinosum, 3) Stratum granulosum and, 4) Stratum corneum, from inside to outside of the skin. (Chen K., et al 1925, Nelson H.S., 1995)
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Figure 1.1 Transverse section of human skin

1) Stratum germinativum: The stratum germinativum (stratum basale) is the basal layer which contains cells similar to other tissues in the body. It contains keratinocytes, melanocytes, Langerhans’ cells, and Merkel cells (sensory). The main skin cells are keratinocytes, named since they produce keratin, also is a basic component of hair and nails. For production of melanin, melanocytes are responsible. Melanin is a pigment which gives its colour to the skin and protects the body from the UV light. People have same number of melanocytes in an average. The people who normally produce more
melanin are dark-skinned. The production of melanin increases due to exposure to sunlight which may develop a tan.

2) Stratum spinosum: It is on the top of the basale layer. Keratinocytes gets differentiated within this layer and keratin is synthesized which aggregate to form tonofilaments.

3) Stratum granulosum: Keratinocytes continue to differentiate from stratum spinosum to the stratum granulosum and synthesize keratin and start to flatten. It also contains keratohyalin granules composed of profilagrin, loricrin and cystein-rich protein. The
fillagrin subunits of profillagrin play an important role of matrix molecule in aggregation and alignment of keratin filaments.

4) Stratum corneum: Stratum corneum is the outermost part containing a dry keratinized layer of dead cells and represented as a ‘brick and mortar’ model (Elias P M, 1981; Williams M L, et al, 1987). Its thickness varies in different parts of the body between approximately 10 to 50 μm thick. It is thinnest on the lips and thickest on the palms and soles. It acts a principal barrier to permeation of drugs through skin and it also assists in regulation of water loss from the body. Stratum corneum is traversed by the ducts of sweat glands and hair where these are present. (Yiew W. Chien, 1982; Wertz P.W, et al, 1987)

1.1.2 Dermis
Dermis is about 3-5 mm thick, made up of blood vessels, nerve endings, and connective tissue. Collagen and elastin, which combine in fibres, are the compounds in the dermis which provide support and also elastic tissue to give man flexibility in the movement. It contains the sebaceous glands and sweat producing glands. Sebaceous glands surround hair follicles and pores and empty their secretions into them producing sebum which lubricates the skin and hair. Sebaceous glands are abundantly present in the skin of face, upper back, shoulders and chest. Sweat glands (eccrine and apocrine) are helpful in regulating the body temperature by-producing sweat. Eccrine glands can be found almost everywhere in our body. They are mainly located in forehead, palms and soles of the feet. Apocrine glands become active at puberty and are concentrated in the armpits and pubic region. (Singh S, et al 1993; Ritschel W A, et al 1998)

1.1.3 Subcutaneous fat tissue
Subcutaneous fat tissue is like a sheet of fat containing the areolar tissues that attach dermis to underling structures. This composite structure is pierced at various places by two types of potential diffusion shunts. When the drug is topically applied it get distributed after absorption into systemic circulation and followed by transport to target tissues.

The permeation may occur with the aid of diffusion via any of the route mentioned below and as shown in figure 1.3:

1. Transcellular permeation, across the stratum corneum
2. Intracellular permeation, across the stratum corneum
3. Transappadageal permeation, via hair follicles, sebaceous and sweat glands.

Figure 1.3: Permeation pathways of skin.
Transcellular permeation and intracellular permeation shows diffusion through the epidermis and dermis whereas transappadageal permeation permits diffusional leakage into the epidermis and direct permeation into the dermis. The appendages play an important role at short diffusional time and for polar molecules. The drugs which penetrate directly through the intact stratum corneum enter by transcellular or intercellular permeation.

The relative importance of these alternatives is influenced by various physiochemical characteristics of the penetrant such as its partition coefficient, molecular size, pKa, solubility, stability and binding affinity. The properties that also have influence are density of sweat glands and follicles, integrity and thickness of stratum corneum, skin hydration and metabolism and vehicle effects.

1.2 Drug Delivery through Skin

In ancient times lotions and potions have been used on the skin (Y. W. Chien, 1992) and the method of drug delivery through the skin is practiced since 16th century BC. In ancient times, the husk of the castor oil plant was used to be crushed in water and was placed on an aching head and it was cured. (B. C. Finnin, et al 1999). Transdermal drug delivery in the late 1970’s was believed as a methodology that such dosage form can maintain blood drug concentrations controlled by a device. (Rathbone M J et al, 2004).

1.2.1 Factors affecting drug delivery system through skin

(A) Physicochemical properties of permeant


The molecules with an intermediate partition coefficient (log K 1 to 3) and for highly lipophilic molecules (log K>3), cross the stratum corneum by intercellular route. However, for these molecules a further consideration is the ability to partition out of the stratum corneum into the aqueous viable epidermal tissues. For more hydrophilic molecules (log K<1), the transcellular route predominates probably.

In determining the flux of a material through human skin is the molecular size plays an important role. Molecular weight is generally taken as an approximation of molecular size for simplicity. It was observed that an inverse relationship existed between transdermal flux and molecular weight of the molecule.

3. Solubility/melting point (Vinod KR et al, 2010) Number of the organic materials with high melting points have relatively low aqueous solubility at normal temperature and pressure. The lipophilic molecules tend to permeate through the skin faster than more hydrophilic molecules. However, while lipophilicity is a desired property of transdermal candidates, it is also necessary for the molecule to exhibit some aqueous solubility since topical medicaments are generally applied from an aqueous formulation.


According to pH-partition hypothesis, only the unionized forms of the drug can permeate through the lipid barrier in significant amounts.


Assuming membrane related transport, increasing concentration of dissolved drug causes a proportional increase in flux. At concentration higher than the solubility, excess solid drug functions as a reservoir and helps maintain a constant drug constitution for a prolonged period of time.


Penetration of drug depends on diffusion coefficient of drug. At a constant temperature the diffusion coefficient of drug depends on properties of drug, diffusion medium and interaction between them.

7. Other factors (Vinod KR et al, 2010)

Along with the factors mentioned above, there are other molecular properties that can affect drug delivery through the skin. Drug binding is a factor that should be considered while selecting appropriate candidates. Interactions between drug substances and the
tissue can vary from hydrogen bonding to weak Vander Waals forces and the effect of drug binding (if any) on flux across the tissue will vary depending on the permeant, e.g. with a poorly water soluble drug in an aqueous donor solution, significant binding to the stratum corneum may completely retard drug flux. As a result, there will be a delay between applying a drug to the surface of the tissue and its appearance in a receptor solution (in vitro) or the blood (in vivo).

(B) Physicochemical properties of the drug delivery system (Patel D, et al 2011)

1. Release characteristics

Release rate of the drug in the vehicle is a measure of its solubility. Mechanism of drug release depends on the following factors:

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

2. Composition of the drug delivery systems

Composition of the drug delivery systems, e.g. boundary layers, thickness, polymers, vehicles affects the rate of drug release and permeability of the stratum corneum by means of hydration, making with skin lipids, or other sorption promoting effects, e.g. benzocaine permeation decreases with PEG of low molecular weight.

3. Enhancement of transdermal permeation

Majority of drugs will not penetrate skin at rates sufficiently high for therapeutic efficacy. In order to allow clinically useful transdermal permeation of most drugs, the penetration can be improved by the addition of a permeation promoter into the drug delivery systems.
(C) Physiological factors

- Skin barrier properties in the neonate and young infant:

  Skin of newborns is relatively susceptible to irritants. pH and hydration of stratum corneum may enhance the irritant potential to newborn skin. Skin surface pH values in newborns are higher in all body sites than those in adult skin, but stabilize at values similar to adults within the first month.

- Skin barrier properties in aged skin:

  There are changes in the physiology of aged skin (>65 years). Moisture content of human skin decreases with age. There is a flattening of the dermoepidermal junction and consequently, the area available for diffusion into the dermis is diminished.

- Race:

  Racial differences between black and white skins have shown variations in anatomical and physiological functions. In black skin, increased intracellular cohesion, higher lipid content and higher electrical skin resistance levels was observed as compared to whites. Black skin appears to have a decreased susceptibility to cutaneous irritants, but this difference is not detected in stripped skin, suggesting the stratum corneum modulates the different racial response to irritants.

- Body site:

  It is readily apparent that skin structure varies to some degree over the human body. Relative permeability of different skin sites is not simply a function of stratum corneum thickness as different permeants exhibit varied rank orders through different skin sites. It is apparent that genital tissue usually provides the most permeable site for transdermal drug delivery. Skin of head and neck
is comparatively permeable compared to other sites of body such as the arms and legs.

- Skin temperature

The human body maintains a temperature gradient across the skin from around 37º C to around 32º C at the outer surface. Since diffusion through the stratum corneum is a passive process, elevation of the skin temperature can induce structural alterations within the stratum corneum, and these modifications can also increase diffusion through the tissue.

- Blood supply

Changes in peripheral circulation can affect transdermal absorption.

- Skin metabolism

Skin metabolizes steroids, hormones, chemical carcinogens and some drugs. So skin metabolism determines efficacy of drug permeated through the skin.

1.2.2 Gels- an Overview:

Gels are semisolid dosage forms and are transparent to opaque. They contain high ratio of solvent to gelling agent. Gelling agents merge or entangle on dispersing in an appropriate solvent to form a three dimensional colloidal network structure. The fluid flow of solvent molecules by entrapment and immobilization get stopped due to the network. It is supposed that the resistance of gel to deformation and hence the viscoelastic properties of gel is because of the network structure. (Alfred Martin et al 1991; Howard C. Ansel, et al 2000)

Classification of gels:

Gels are classified (Howard C. Ansel, et al 2005) as below:

a) Depending on the type of colloid phase
   i) Organic gels
   ii) Inorganic gels

b) Depending on type of solvent
i) Aqueous gels

ii) Non aqueous gels

1.2.2.1 Basic components of gel:

Gel formulations include the following components (Vyas S. P. et al 2002):

(A) Polymer: It is major component of gel. Various types of polymers have been used to control the drug release. Mechanism and rate of drug release is affected by the physicochemical properties of drug and polymer incorporated. Based on the type of polymer used, different types of excipients may be incorporated to modify some properties like solubility by addition of cosolvents.

Some examples of the polymers used in the formulation of gel:

a. Natural polymers : Alginic acid, agar, gelatin, polysaccharides, tragacanth, etc.

b. Synthetic polymers : PVA, polyethylene, carbomers, poloxamer, polyacrylamide, etc.

c. Semisynthetic polymers: Hydroxyethyl cellulose, hydroxypropyl cellulose, hydroxypropyl methyl cellulose, carboxymethyl cellulose, methylcellulose, etc.

d. Surfactants: Cetostearyl alcohol, Brij – 96, etc.

(B) Drug Substance: For the successful development of a formulation intended to be permeated across the skin, selection of the drug is the most important. Ideal physicochemical properties of a drug for formulation of gel include adequate lipophilicity, molecular weight less than 500 Daltons and pH of aqueous solution of drug between 5 to 9. Whereas, the biological properties include non-irritancy to the skin, stable in GIT.

(C) Penetration Enhancers: These promote the skin permeability by modifying the skin structure as a barrier to the flux of a desired penetrant. Hence these play a critical role in the formulations. In order to diffuse the drug molecules across skin so as to maintain therapeutic levels in blood, the resistance of skin (stratum corneum) to diffusion of drugs
should be reduced. They interact with the applied formulation or with the skin itself and alter the skin’s barrier to penetration.

Ideal characteristics of permeation enhancer to be used are:

- Non-toxic, non-allergenic, non-irritating properties.
- Pharmacological inertness.
- Odourless, colorless and economical and cosmetically acceptable.
- Physically and chemically and compatible with drugs and excipients used.
- After removal of the formulation, the stratum corneum should retain its normal barrier property immediately.

Penetration Enhancers disrupt the structure of stratum corneum and enhance solubility of penetrant thereby increasing the penetration of permeants. Keratin gets swelled and leached out the essential structural material from the stratum corneum because of the accelerants. So the resistance to diffusion is reduced and permeation of drugs through the skin is increased. Some of the commonly used penetration enhancers are essential oils, dimethylsulphoxide, fatty acid and alcohols, surfactants, terpenes and derivatives, etc.

(D) Excipients: Some of the commonly used excipients are given Table 1.1.
1.2.3 Transdermal Films/Patches- an Overview:

Nowadays the transdermal drug delivery is well accepted drug delivery system for delivering drug to systemic circulation. Skin resists the delivery of most of the drugs but a few compounds can cross the barrier (R. H. Guy, 1996). However, it has been proved that the transdermal drug delivery system is a widely accepted drug delivery system. Transdermal drug delivery systems are the dosage forms that deliver a therapeutically effective amount of drug across the skin of patient. These are also referred as patches or films.

With the introduction of newer techniques for enhancement of drug across skin and also with the development of novel methods for reducing skin irritation, the transdermal market has a great scope to widen. Clinical trials on broad variety of drugs are going on which proves that the transdermal drug delivery systems has a great future in near future. Transdermal drug delivery systems are beneficial over other routes of drug delivery in the sense of application. The active drug component permeates by passive diffusion across the skin. (Scheindlin Stanley, 2004)

Traditionally used preparations include creams, gels, ointments, etc. First transdermal patch prepared in the early 1980s was containing Scopolamine for motion sickness. It has shown some benefits and since then the development in transdermal drug was started. (B.
J. Thomas, et al 2004). It was approved by USFDA for the treatment of motion sickness and later on nitroglycerine patch was marketed for the management of angina pectoris (M. R. Prausnitz, et al 2004). Since then, numbers of drugs viz. clonidine, nitroglycerine, fentanyl, oxybutonin, scopolamine, lidocaine and testosterone have been successfully delivered through transdermal route (M. Aqil, et al 2006).


Advantages of transdermal drug delivery:
1. No first-pass hepatic metabolism.
2. Varied conditions of absorption and metabolism as observed with the oral therapy are avoided.
3. Continuity of drug administration in TDDS facilitates use of a drug having short biological half-life.
4. Risk and inconvenience of intravenous therapy is avoided.
5. Reduced gastrointestinal side effects
6. Increased patient compliance in following manner
   • Provisions of simplified therapeutic regimen.
   • Painless delivery of drug.
   • Eliminates swallowing.
   • No chances of forgetting the dose once the device is applied on skin.
   • Easy to carry a patch in wallet or ladies purse.
7. Patches offer less friability problems of wear and tear than the tablets.
8. In a multi drug regimen TDDS avoids drug interaction in GIT.
9. Therapy can be easily stopped just by removing the drug delivery device from the skin surface.
10. Can be administered without any aid, which makes it most suitable formulation; for instance, tablet and capsule need little water. Liquid oral preparation needs teaspoon and parenteral delivery needs specialized person whereas if a patient is told to apply
TDDS patch, he/she can do it any where e.g. in office, in theatre, in club, in house without any aid.

11. Need not be sterile, obviates processing problem.

Disadvantages of transdermal drug delivery system (Charro B. D., et al 2001)

1. It is limited to potent drug molecule typically those requiring a daily dose on the order of 20 mg or less.
2. Even if the drug is sufficiently potent, it must yet satisfy other criteria to be considered a viable candidate for transdermal drug delivery.
3. The pharmacokinetic and pharmacodynamic characteristic of the drug must be such that relatively sustained and slow input provided by transdermal delivery makes sense.
4. Drugs that can be given once a day orally, with reproducible bioavailability and which are well tolerated by patient do not really need a patch formulation.

Considerable efforts are being exercised to overcome the impermeability of intact human skin. Several researchers have described that main fractions of epidermal lipid are ceramides (45-50%), cholesterol (25%) and free fatty acids (10-15%). (Elias, P.M, et al 1981; Lampe, M.A, et al 1983)

Technologies for transdermal drug delivery system (Chien Y W., 2009)

a) Polymer membrane permeation-controlled Systems: The drug reservoir is fixed in between rate controlling polymeric membrane and drug impermeable backing laminate as shown in figure 1.4. Drug gets released through the rate-controlling polymeric membrane. The drug reservoir compartment contains drug solids dispersed homogeneously in a solid polymer matrix. The drug solids are suspended in viscous liquid medium to form a paste like consistency or are dissolved in a solvent to form a clear drug solution. The rate-controlling membrane may be microporous or nonporous polymeric membrane.
b) Polymer matrix diffusion controlled system: In this system, the drug is dispersed homogeneously in hydrophilic or lipophilic polymer matrix to form the drug reservoir. The mixture is then shaped into medicated disks having a defined surface area and controlled thickness. The disks so formed are mounted into an occlusive base-plate in a compartment fabricated from a drug impermeable plastic backing. The medicated discs are surrounded by adhesive rim along the circumference as shown in figure 1.5.
c) Drug reservoir gradient controlled system: The thickness of diffusional path across which drug molecules get diffused was found to be increased with time in this system. The time-dependant increase in diffusional path was compensated by loading the drug in the multilaminate adhesive layer as shown in figure 1.6. The Nitroglycerin releasing patches were prepared by this method.

Figure 1.5: Cross-sectional view of a Polymer matrix diffusion controlled system.

Figure 1.6: Cross-sectional view of a drug reservoir gradient – controlled system
d) Micro reservoir dissolution-controlled System:

![Figure 1.7: Cross-sectional view of a Micro reservoir dissolution-controlled System.](image)

In this system the drug reservoir is prepared by suspending the drug solids in an aqueous solution of water miscible drug solubilizer. The suspension is homogeneously dispersed in a lipophilic polymer by applying high-shear mechanical force and thus to form microscopic drug reservoir. The so formed thermodynamically unstable dispersion then immediately stabilized by cross-linking the polymer chains *in-situ*. Thus medicated polymer disks are formed which has constant surface area and a fixed thickness. The disks are then mounted on adhesive pad.

1.2.3.1 Basic components of Transdermal Films/Patches

Basis components of transdermal devices are as mentioned below:

a) Polymer Matrix

Polymers are the major components of transdermal drug delivery system. The drug release from the device can be controlled by incorporation of polymer matrix. Some of the properties that the polymer should possess are:

i) Drug diffusion should not get influenced by the chemical functionality, molecular weight and glass transition temperature of the polymer.
ii) The polymer must be compatible with the drug.

iii) The polymer should not degrade to produce toxic degradation products.

Commonly used polymers used for preparation of transdermal devices include chitosan, polyvinyl pyrrolidone, ethyl cellulose, hydroxyl propyl methyl cellulose, sodium alginate, etc.

b) Drug

Some of the ideal physicochemical properties of drug for formulation of transdermal drug delivery include its affinity towards lipophilic and hydrophilic phases, low melting point, molecular weight less than 500 daltons, etc. The biological properties include its short half life, its non-irritating properties. The drugs those are unstable in GI tract gets degraded or gets inactivated by hepatic first pass effect are considered to be ideal candidates for transdermal delivery.

c) Permeation enhancers

These compounds encourage permeability of skin by modifying the skin structure as barrier to the entry of drug. Permeation enhancers are hypothesized to affect one or more of these layers to achieve skin penetration enhancement. Permeation enhancer should fulfill the following criteria,
i) It should be pharmacologically inert.

ii) It should be not produce toxic, irritant effects and have a low index of sensitization.

iii) The penetration enhancing action should occur rapidly and should last for a suitable duration.

iv) The enhancer should be chemically and physically compatible with drugs and excipients.

v) It should be odourless, tasteless and colourless.

d) Other Excipients

i) Adhesives- The transdermal devices are tightened to the skin with the help of pressure sensitive adhesive. The adhesive is placed on the face or at the back of the device and extending peripherally. The adhesive systems should be non-irritant and non-sensitizing to the skin and it must not produce imbalance in the normal skin flora during its contact time with the skin. During the dosing interval it should adhere to the skin firmly without disturbing its position at the time of bathing, exercise. It should be easy to remove and unwashable residue should not be left on the skin.

ii) Backing membrane- It is flexible and does not allow the drug to leave from dosage form through the top. It gives a good bond to the drug reservoir and accepts printing. It is impervious substance which protects the product during use on the skin.

Different approaches adopted to enhance skin permeability of drugs by physical and chemical techniques based on use of permeation enhancers are summarized in figure 1.9. A number of factors influence the systemic delivery of drugs from delivery system across skin. (Brandner et al., 2002) A number of steps are involved in the drug release from a formulation applied on the skin and its transport to the systemic circulation. The steps involves (a) drug dissolution within and release from the formulation (b) partitioning into the skin’s outermost layer, the stratum corneum (SC), (c) diffusion across SC, via lipidic intercellular pathway, (d) partitioning from the SC into the aqueous viable epidermis (e) diffusion across the viable epidermis and into the upper dermis (f) uptake into the local capillary network and eventually the systemic circulation. The absorption enhancers may act by one or combination of mechanisms, including influence on the thermodynamic
activity of the drug in solution, alteration of the molecular structure of the cell membrane ranging from temporary membrane pore formation to complete membrane destruction, loosening of the tight junctions (TJs) between epithelial cells, inhibition of the protease activity present in the mucosa, and alteration of the characteristics of the mucus in ways which reduce its diffusion barrier properties.

In mammalian skin with normal barrier function and normal stratum corneum, a colocalization of all known epidermal TJ proteins is found in the stratum granulosum of the interfollicular epidermis and in cell layers facing the hair shaft and the stratum corneum of the hair follicle (Langbein et al., 2002; Pummi et al., 2001) suggesting a continuous TJ system in the skin, which extends from the epidermis into the skin appendages.
Figure 1.9 Summary of the approaches useful for enhancing percutaneous permeation delivery of drugs
1.2.4 Mechanism of Rate-Controlled drug delivery through skin (Misra A N, 2004)

The permeation of drug from the site of application to target tissue through skin layers depends on the physicochemical properties of drug. Rate of permeation dQ/dt across various layers of skin tissue can be expressed mathematically as

\[ \frac{dQ}{dt} = P_s (C_d - C_r) \quad (1.1) \]

Where, \( C_d \) and \( C_r \) respectively represent the concentration of donor and compartment receptor, \( P_s \) is overall permeability coefficient.

\( P_s \) can be defined as follows:

\[ P_s = \frac{K_{sd} D_{ss}}{h_s} \quad (1.2) \]

Where, \( K_{sd} \) = partition coefficient, \( D_{ss} \) = apparent diffusivity, \( h_s \) = thickness of the skin tissue.

\( P_s \) can be considered as a constant, if \( K_{sd}, D_{ss} \) and \( h_s \) terms in the above equation are constant under a given set of conditions. Equation 1.1 suggests that, constant rate of drug permeation can be achieved when the concentration of drug at surface of stratum corneum (\( C_d \)) is maintained greater than the concentration of drug in the receptor side (\( C_r \); \( C_d > C_r \)) under such condition equation 1.1 can be converted to,

\[ \frac{dQ}{dt} = P_s C_d \quad (1.3) \]

Rate of permeation across skin \( \frac{dQ}{dt} \) should not change, if magnitude of \( C_d \) value does not change during the course of skin permeation. By making \( C_d \) greater than \( C_r \) the concentration of drug on the surface of skin \( C_d \), is maintained same or greater than equilibrium (or saturation) solubility of drug in stratum corneum \( C_s^e \); \( C_d \geq C_s^e \). Highest rate of skin permeation \( \left( \frac{dQ}{dt} \right)_m \) was expressed by the following equation 1.4:

\[ \left( \frac{dQ}{dt} \right)_m = P_s C_s^e \quad (1.4) \]

Apparently, the magnitude of \( \left( \frac{dQ}{dt} \right)_m \) is calculated by the permeability coefficient \( P_s \) of skin to drug and the equilibrium solubility of the drug in the stratum corneum \( C_s^e \). Hence, skin permeation appears to be stratum corneum limited.
1.2.5 *In-vitro testing of drug delivery system through the skin*

Proper skin preparation and appropriate cell design gives good *in-vivo* results. Skin preparation includes selection of proper skin. One can choose human skin or can also go for animal or artificial membrane like Nylon, Cellulose acetate etc. Skin separation includes the treatments needed for separation of required part of skin from other unwanted parts. Then separated skin is mounted on the diffusion cell which can be one chambered (one donor compartment) or two chambered (two donor compartments). Various *in-vitro* testing procedures used for assessment of drug permeation/drug diffusion are shown in **figure 1.10**.
**Figure 1.10:** *In-vitro* testing procedure
Selection of membrane for in-vitro study of transdermal drug delivery system
Different skin models used by various researchers are given in figure 1.11. Though there is no rule regarding to selection of the skin model. But generally researchers starts with artificial membrane, then in-vitro animal skin, then in-vitro human skin (cadaver skin), then in-vivo animal skin, then finally in-vivo human skin.
Franz diffusion cell (Jung S, 2003)
It is one chambered (vertical) type cell most widely used for in-vitro testing of TDDS as shown in figure 1.12. Many modifications have been made in the franz diffusion cell design according to the requirement. The skin is mounted on the plate above O ring. 20-70 ml phosphate buffer solution with pH 7.4 is filled in reservoir compartment. Transdermal patch/gel is applied on upper layer of skin. Diffusion medium in reservoir is stirred at particular rpm. Sampling is done at particular interval from reservoir compartment i.e. specified volume of fluid is withdrawn and sink condition s maintained by replacing with equivalent amount of the same fluid.
Figure 1.11: Skin models used in in-vitro testing procedure
Figure 1.12. Schematic representation of *in vitro* drug diffusion study across rat skin using franz diffusion cell
In-vitro drug release profile modeling:

In-vitro drug release has been recognized as an important tool in drug development and as an important parameter in quality control. Under specified conditions, it can be used as replacement to assess or predict bioequivalence. (Banakar U. V, et al 1992)

Suitable evaluation test to study drug release is needed to evaluate the drug product and ensure batch-to-batch reproducibility and consistent pharmacological/biological activity and to evaluate scale up and post approval changes such as manufacturing site changes, component and composition changes. Drug release from sustained release formulation is controlled by several factors via different mechanisms like diffusion, erosion or osmosis. Various mathematical models are suggested by many researchers to explain the profiles of drug release from various systems. (Mario G., et al 2005; Higuchi T.,et al 1963; Udupa N., 1992) All the model dependent methods depend upon a curve fitting procedure. Various mathematical functions have been used to fit the observed data in appropriate model. Both the linear and non-linear models are being used in practice for dissolution modeling. Linear models include Zero order (Maincent P., et al 1986), Higuchi (Watnasirichaikul S.,et al 2002, Lambert G., et al 2000), Hixson-Crowell (Montasser I., et al 2002), where as the nonlinear models include First order (Mbela T.K.M., et al 1992, Couvreur P. et al, 2002) Korsmeyer- Peppas (Korsmeyer R W, et al 1983, Bouchemal K., et al 2006), Logistic etc.

The zero order rate equation 1.5 explains the systems when the drug release rate does not depend on its concentration.

\[ C = k_o t \] \hspace{1cm} (1.5)

Where \( C \) is drug concentration at time (t)

\( k_o \) is the zero-order release rate constant.

The first order equation 1.6 explains the release from a system when the drug release rate is concentration dependent.

\[ \log C = \log C_0 - \frac{kt}{2.303} \] \hspace{1cm} (1.6)

Higuchi explained the drug release from porous, insoluble matrix as a square root of time dependent process based on fickian diffusion as shown in equation 1.7.

\[ Q = Kt^{1/2} \] \hspace{1cm} (1.7)
The Hixson-Crowell cube root law equation 1.8 explains the drug release from systems when there is a change in surface area and diameter of particles.

\[ KHCt = Q_0^{1/3} - Q_t^{1/3} \text{ \ldots \ldots (1.8)} \]

To characterize the drug release mechanism, data obtained for drug release is plotted into all the equations. Korsmeyer peppa plot had shown linear graph and the regression coefficient is calculated using slope of the straight line.

Korsmeyer et al suggested a simple relationship to describe release of drug from polymeric system. To determine the mechanism of drug release, log cumulative % drug release is plotted against log time according to equation 1.9.

\[ \frac{M_t}{M_\infty} = K_{KP} t^n \text{ \ldots \ldots (1.9)} \]

Where \( \frac{M_t}{M_\infty} \) is fraction of drug released at time \( t \),

\( K \) is the rate constant and \( n \) is the release exponent.

\( n \) value is used to characterize different release mechanisms as the following:

<table>
<thead>
<tr>
<th>( n )</th>
<th>Diffusion mechanism</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.45</td>
<td>Fickian diffusion</td>
</tr>
<tr>
<td>0.45 &lt; ( n ) &lt; 0.89</td>
<td>Anomalous diffusion</td>
</tr>
<tr>
<td>0.89</td>
<td>Case-II transport</td>
</tr>
<tr>
<td>( n ) &gt; 0.89</td>
<td>Super case-II transport</td>
</tr>
</tbody>
</table>

### 1.3 Introduction to Inflammation

Inflammation is physiological response which occurs in response to various injurious agents (e.g. physical trauma, bacterial infection, chemicals or any other phenomenon) finally aiming to reduce the damage and promote tissue repair (Nathan C, 2002). Inflammatory processes are needed for optimal repair, immune surveillance and regeneration after injury (Vodovotz et al., 2008). Inflammation is nonspecific, defensive response of the body to tissue damage. The characteristic signs and symptoms of
inflammation include redness, pain, heat and swelling. Inflammation may lead to loss of function of damaged area depending on extent of injury (Tortora G J et al 2006). Inflammation is a major component of the damage occurred due to autoimmune diseases, and is a fundamental contributor of various infectious and non-infectious diseases such as cancer, diabetes, cardiovascular disease, rheumatoid arthritis, Alzheimer’s disease and arteriosclerosis. Depending on the intensity of this process, mediators generated in the inflammatory site can reach the circulation and cause fever (Lucas S M, et al., 2006; Kassuya C A, et al., 2009; Franklin P X et al., 2008). The inflammatory process protects body from diseases by releasing cells and mediators that combat foreign substances and prevent infection (Frank M M et al, 1991; El-Gamal M I, et al., 2010). Inflammation is a complex pathophysiological process mediated by a variety of signaling molecules produced by leucocytes, macrophages and mast cells undergoing various cellular responses such as phagocytic uptake, and the production of inflammatory mediators such as nitric oxide (NO), prostaglandin E2 (PGE2) and tumour necrosis factor (TNF)-α (Kinne R W et al., 2000; Yu et al., 2010), that lead to edema formation as a result of extravasation of fluid and proteins and accumulation of leucocytes at the inflammation site (White M, 1999).

1.3.1 Non Steroidal Anti-Inflammatory Drugs (Tripathi K D, 2008, Kadam S S et al 2008)

Non-steroidal anti-inflammatory drugs (NSAIDs) are commonly used to manage pain and inflammation in different arthritic and postoperative conditions. NSAIDs have four major activities, such as anti-inflammatory, antipyretic, analgesic and uricosuric. NSAIDs shows anti-inflammatory effect by virtue of their ability to inhibit the activities of cyclooxygenases; these are enzymes that mediate production of prostaglandins from arachidonic acid.
**Different classes of NSAIDs**

Depending on chemical structure NSAIDs are classified into different classes as shown below:

1. **Salicylic acid derivatives**: e.g. Aspirin and Diflunisal.
2. **Para-amino phenol derivatives**: e.g. Paracetamol (Acetoaminophen).
3. **Aryl acetic acid derivatives**: e.g. Indomethacin and Sulindac.
4. **Fenamates**: e.g. Mefenamic acid and Meclofenamates.
5. **Oxicam**: e.g. Lornoxicam, Meloxicam
6. **Propionic acid derivatives**: e.g. Ibuprofen and Ketoprofen.
7. **Coxib**: e.g. Celecoxib and Rofecoxib.

**Oxicam**

United States Adopted Names Council (USAN Council) describes oxicam as relatively new enolic acid class of 4-hydroxy-1, 2-benzothiazine carboxamides having anti-inflammatory and analgesic properties. Oxicam is a potentially growing class of non-steroidal anti-inflammatory agent belonging to the benzothiazinone dioxide series of heterocyclic molecules.

**Reasons for selection of oxicam**

1. Oxicam shows all diverse functions shown by other NSAIDs and oxicam is highly effective class of NSAIDs, mainly used in various arthritic condition and post operative inflammation.
2. These agents show good anti-oxidant property.
3. The rate of metabolism of oxicam is slow.
4. Gastro irritancy property of oxicam is less.

**Selection of drug candidate (Nayak S H et al 2004)**

The selection of drug is critical in the successful development of transdermal products. Meloxicam has the following properties:

1. Moderate oil and water solubility.
2. Low melting point (< 300°C) and low molecular weight (< 500da).
3. Daily dose is of few mg (< 10 mg/day)
4. It is not metabolized in the skin.
5. Non-irritating and non-allergic properties.

As Meloxicam satisfy all the criteria mentioned above, Meloxicam was used as model drug for the present research work.

1.4 Introduction to Cyclodextrins
Cyclodextrins (CD) comprises a group of cyclic oligosaccharides having hydrophilic outer surface and lipophilic central cavity. The cyclodextrin molecules are relatively large with a number of hydrogen donors and acceptors. Cyclodextrins are used in the pharmaceutical, agrochemical, food and cosmetic industries as "molecular cages" (Roux M et al, 2007). In the pharmaceutical industries they are used as complex forming agents to improve the aqueous solubility of poorly soluble drugs and thereby enhance their bioavailability and stability (Loftsson T et al 2007). Cyclodextrins reduce gastrointestinal irritation, convert liquid drugs into microcrystalline or amorphous powder and prevent drug–drug and drug–excipient interactions, etc (Pitha, J. et al, 1990).

Structure
In the structure of cyclodextrins, the primary hydroxyl groups are located on narrow side of the torus whereas the secondary hydroxyl groups are located on the wider edge as shown in Figure 1.13. The most common cyclodextrins are α-cyclodextrin, β-cyclodextrin and γ-cyclodextrin, which consist of six, seven and eight glucopyranose units, respectively. While it is considered that, due to steric factors, cyclodextrins with lower than six glucopyranose units doesnot exist. The reported cyclodextrins are designated δ-, ε-, ζ-, η- and θ-cyclodextrin containing nine, ten, eleven, twelve and thirteen glucopyranose units respectively (French, D. et al 1965).
Only δ-cyclodextrin amongst these large-ring cyclodextrins has been well characterized (Miyazawa, H., et al; 1995). Some of the physicochemical of the four most common cyclodextrins are given in table 1.2.

Table 1.2- Characteristics of α, β, γ and δ- cyclodextrin

<table>
<thead>
<tr>
<th>Parameter</th>
<th>α</th>
<th>β</th>
<th>γ</th>
<th>δ</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of glucopyranose units</td>
<td>6</td>
<td>7</td>
<td>8</td>
<td>9</td>
</tr>
<tr>
<td>Molecular weight</td>
<td>972</td>
<td>1135</td>
<td>1297</td>
<td>1459</td>
</tr>
<tr>
<td>Central cavity diameter (Å)</td>
<td>4.753</td>
<td>6.065</td>
<td>7.583</td>
<td>10.3112</td>
</tr>
<tr>
<td>Water solubility at 25°C (g/100 mL)</td>
<td>14.5</td>
<td>1.85</td>
<td>23.2</td>
<td>8.19</td>
</tr>
</tbody>
</table>

Melting points of α-, β-, and γ-cyclodextrin lies between 240 to 265°C (Nash, R. A.; 1994). The parent cyclodextrin viz. β –cyclodextrin has limited aqueous solubility and when it is complexed with lipophilic drugs or the compounds having limited aqueous solubility, shows B-type phase-solubility diagrams as explained by Higuchi (Higuchi, T.
et al., 1965). Solid drug-cyclodextrin complexes are obtained when the unmodified cyclodextrins are added into aqueous drug solutions or drug suspensions. It is observed that β- and δ-cyclodextrin form intramolecular hydrogen bonds between secondary OH groups, which detract from hydrogen bond formation with surrounding water molecules, resulting in less negative heats of hydration (Fromming, K. et al; 1994, Hashimoto, H.; 1991). The enhancement in solubility with these derivatives is due to the chemical manipulation of crystalline cyclodextrins into amorphous mixtures of isomeric derivatives. For example, by treating a base-solubilized solution of β-cyclodextrin with propylene oxide, (2-hydroxypropyl)-β-cyclodextrin is obtained, resulting in an isomeric system which has an aqueous solubility in excess of 60% (w/v) (Pitha, J. et al. 1986). The physicochemical properties of the derivatives including their ability to form drug complexes gets affected by the molar substitution, i.e., reaction between the average number of alkyl or hydroxyalkyl groups and one glucopyranose unit, and the location of the alkyl or hydroxyalkyl groups on the cyclodextrin molecule (Botsi A., et al. 1995, Bodor N., et al. 1995).

Industrial Applications
In the cosmetic industry cyclodextrins were assessed as stabilizers of chemically labile compounds, to prolong their action, to reduce local irritation and to reduce unpleasant odors. In food industry cyclodextrins were used as stabilizers for flavoring agents to reduce unpleasant odor and taste. In Japan, there is a tradition for industrial usage of natural products and the Japanese considered the parent cyclodextrins as natural materials originating from starch and thus as “non-toxic” natural products. By 1970, the Japanese have studied the chemistry and production of cyclodextrins. In early 1980s cyclodextrins were presented as industrial raw materials, mainly for the cosmetic and food industries (Hashimoto, 1991).

1.4.1 Inclusion complex formation
By molecular complexation cyclodextrins form solid inclusion complexes (host–guest complexes) with a wide range of solid, liquid and gaseous compounds (Villiers M A, 1891). The guest molecule is trapped within the cavity of the cyclodextrin host molecule.
to form complexes. The formation of complex is a dimensional fit between host cavity and guest molecule (Muñoz-Botella S, et al 1995). Complexes are formed because the lipophilic cavity of cyclodextrin molecules facilitates entry of appropriately sized non-polar moieties by providing a microenvironment into the cavity (Loftsson T, 1996). No breaking or formation of covalent bonds is observed during inclusion complex formation (Schneiderman E, et al 2000). The driving force of complex formation is the release of enthalpy-rich water molecules from the cavity (Szetjli J., 1998). The physicochemical properties of guest molecules are influenced when the inclusion in cyclodextrins is formed since the guest molecules are temporarily locked or caged within the host cavity giving rise to beneficial modifications of guest molecules. (Schmid G., 1989)

The properties are:

a) Improvement in solubility of highly insoluble guests,

b) Stabilization of labile guests against the degradative effects of oxidation, visible or UV light and heat,

c) Control of volatility and sublimation, physical isolation of incompatible compounds,

d) Modification of taste by masking unpleasant odours, bad flavours and

e) Controlled release of drugs.

1.4.2 Complexation techniques


a) Co-precipitation: The guest is added in an aqueous solution of $\beta$-cyclodextrin is with stirring. If the guest can tolerate higher temperatures, the concentration of $\beta$-cyclodextrin can be kept high about 20%. Before the precipitate is formed, solution of cyclodextrin and guest should be cooled while stirring. Formed precipitate is collected by decanting, centrifugation or filtration and using small quantity of water or other water-miscible solvent, the precipitate can be washed.

b) Slurry complexation: Aqueous solution of $\beta$-cyclodextrin (50–60%) is prepared with stirring. Guest is added to the cyclodextrin solution and form complex. As the water phase gets saturated with cyclodextrin complex, the complex will crystallize or precipitate out of the aqueous phase. The complex can be collected by the same procedure as mentioned in co-precipitation method.

c) Paste complexation: Guest is mixed with small amount of water and paste is formed by trituration. $\beta$-cyclodextrin is mixed using mortar and pestle on small scale and kneader is used on large scale. Type of guest influences the amount of time required. Obtained complex can be dried directly or it can be washed by small amount of water and collected by filtration or centrifugation. Depending on the type of guest and amount of water used, the complexed product may be in the form of fine powder a dry hard mass.

d) Damp mixing and heating: No water is used in this method. The water of hydration in the cyclodextrin is used to make the mixture with guest. The guest is mixed well with cyclodextrin and kept in a sealed container. Contents in the sealed container are heated to 100°C. The contents are taken out and dried.

e) Extrusion: Guest is mixed with cyclodextrin and water and is added to the extruder. In the barrel of extruder, degree of mixing, amount of heat and time is
controlled. Depending on the amount of water, the extruded complex dry and after cooling the complex is dried in an oven.

f) Dry mixing: By this guests are complexed with cyclodextrin by mixing. This method is suitable for oils and liquid guests. Depending on the guest, duration of mixing is variable. This method is performed at ambient temperature.

The possible structure of solid inclusion complex of drug with β-cyclodextrin is shown in figure 1.14.

**Figure 1.14** Structure of β-cyclodextrin inclusion complex