INTRODUCTION

Management of illness through medication has entered an era of rapid growth. Today, there are a host of drugs for combating virtually every disease or condition known to man and a variety of means by which these drugs are delivered to the human body for therapy such as tablets, capsules, injections, aerosols, creams, ointments, suppositories, liquids etc., often referred to as conventional drug formulations. Therapy with such formulations involves attainment and maintenance of drug concentration in the body within a therapeutically effective range by introduction of fixed doses of a drug, at regular intervals, into the body. After the administration of one dose, the drug concentration rises to high levels, system-wide, at least initially. With the passage of time, the concentration diminishes owing to natural metabolic processes and a second dose must be administered to prevent the concentration from dropping below the minimum effective level. The disadvantages of this kind of therapy are: (i) drug concentration in the body follow a peak and trough profile leading to greater chances of adverse effects or therapeutic failure, (ii) therapy is inefficient and costly since large amounts of drug are lost in the vicinity of the target organ and close attention is required to monitor therapy to avoid overdosing.

It is recognized that continuous intravenous infusion is a superior mode of drug administration as compared to the oral route not only to bypass hepatic “first-pass” metabolism but also to maintain a constant and prolonged drug level in the body. A closely monitored intravenous infusion can provide the dual advantage of direct entry of the drug into the systemic circulation and the control of circulating drug levels. However, such a mode of administration involves certain risks which necessitate hospitalization of the patient for close medical supervision of drug administration. The benefits of intravenous infusion could be closely duplicated without its hassles by using the skin as the port of entry of drugs. This is known as transdermal administration and the delivery systems are known as transdermal drug delivery system\(^1\).

1.1 TDDS

The TDDS are defined as self-contained, discrete dosage forms which, when applied to the intact skin, deliver the drug(s), through the skin, at a controlled rate to the systemic circulation\(^1\).
1.1.1 ADVANTAGES OF TDDS\textsuperscript{1,2,3}

The advantages of transdermal delivery over other delivery systems are as follows:

\begin{itemize}
  \item Transdermal medication delivers a steady infusion of a drug over an extended period of time.
  \item Adverse effects or therapeutic failures frequently associated with intermittent dosing can be avoided.
  \item Transdermal delivery can increase the therapeutic value of many drugs via avoiding specific problems associated with the drug e.g. gastro-intestinal irritation, low absorption, decomposition due to ‘hepatic first pass’ effect, formation of metabolites that cause side effects, short half-life necessitating frequent dosing etc.
  \item Due to above advantage, it is possible that an equivalent therapeutic effect can be elicited via transdermal drug input with a lower daily dose of the drug than is necessary, if e.g. the drug is given orally.
  \item The simplified medication regimen leads to improved patient compliance and reduced inter and intra-patient variability.
  \item At times the maintenance of the constant drug concentration within the biophase is not desired. Application and removal of transdermal patch produce the optimal sequence of pharmacological effect.
  \item Patients have difficulty in swallowing tablets and capsules and some patients are tempted to crush tablets to assist in swallowing which destroys any controlled release characteristics of the tablets.
  \item Greater flexibility of dosage in that dosing can be easily terminated by removal of the transdermal drug delivery system.
  \item Self administration is possible with these systems.
  \item It is of great advantage in patients who are nauseated or unconscious.
\end{itemize}

1.1.2 DISADVANTAGES OF TDDS\textsuperscript{1,2,3}

\begin{itemize}
  \item The drug must have some desirable physicochemical properties for penetration through SC and if the drug dosage required for therapeutic value is more than 10
mg/day, the transdermal delivery will be very difficult if not impossible. Daily doses of less than 5 mg/day are preferred.

✓ The skin’s low permeability which limits the number of drugs that can be delivered in this manner.

✓ Skin irritation or contact dermatitis due to the drug, excipients and enhancers of the drug used to increase percutaneous absorption is another limitation.

✓ Clinical need is another area that has to be examined carefully before a decision is made to develop a transdermal product.

✓ Many drugs with a hydrophilic structure permeate the skin too slowly to be of therapeutic benefit.

✓ The barrier function of the skin changes from one site to another on the same person, from person to person and with age.

1.2 SKIN AS A TRANSDERMAL ROUTE

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

1.2.1 SKIN ANATOMY AND PHYSIOLOGY

There are three major components of the skin (Figure 1.1).

(i) Hypodermis

The hypodermis is the deepest section of the skin. The hypodermis refers to the fat tissue below the dermis that insulates the body from cold temperatures and provides shock absorption. Fat cells of the hypodermis also nutrients and energy. The hypodermis is the thickest in the buttocks, palms of the hands, and soles of the feet.
(ii) Dermis

The dermis is located between the hypodermis and the epidermis. It is a fibrous network of tissue that provides structure and resilience to the skin. While dermal thickness varies, it is on average about 2 mm thick. The major components of the dermis work together as a network. This mesh-like network is composed of structural proteins (collagen and elastin), blood and lymph vessels, and specialized cells called mast cells and fibroblasts. These are surrounded by a gel-like substance called the ground substance, composed mostly of glycosaminoglycans. The ground substance plays a critical role in the hydration and moisture levels within the skin.

(iii) Epidermis

The epidermis is the outermost layer of the skin. Categorized into five horizontal layers, the epidermis actually consists of anywhere between 50 cell layers (in thin areas) to 100 cell layers (in thick areas). The average epidermal thickness is 0.1 millimeters, which is about the thickness of one sheet of paper. The epidermis acts as a protective shield for the body and totally renews itself approximately every 28 days.

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Figure 1.1: Diagram of the layers of human skin⁶
The first layer of the epidermis is the **stratum basale**. This is the deepest layer of the epidermis and sits directly on top of the dermis. It is a single layer of cube-shaped cells. New epidermal skin cells, called keratinocytes, are formed in this layer through cell division to replace those shed continuously from the upper layers of the epidermis. This regenerative process is called skin cell renewal. Melanocytes, found in the stratum basale, are responsible for the production of skin pigment, or melanin.

The second layer of the epidermis is the **stratum spinosum**, or the prickle-cell layer. The stratum spinosum is composed of 8-10 layers of polygonal (many sided) keratinocytes. In this layer, keratinocytes are beginning to become somewhat flattened.

The third layer is called the **stratum granulosum**, or the granular layer. It is composed of 3-5 layers of flattened keratin—a tough, fibrous protein that gives skin its protective properties. Cells in this layer are too far from the dermis to receive nutrients through diffusion, so they begin to die.

The fourth layer in the epidermis is called the **stratum lucidum**, or the clear layer. This layer is present only in the fingertips, palms, and soles of the feet. It is 3-5 layers of extremely flattened cells.

The fifth layer, or horny layer, is called the **stratum corneum (SC)**. This is the top, outermost layer of the epidermis and is 25-30 layers of flattened, dead keratinocytes. This layer is the real protective layer of the skin. Keratinocytes in the SC are continuously shed by friction and replaced by the cells formed in the deeper sections of the epidermis. In between the keratinocytes in the SC are epidermal lipids (ceramides, fatty acids, and lipids) that act as a cement (or mortar) between the skin cells (bricks) (Figure 1.2) This combination of keratinocytes with interspersed epidermal lipids (brick and mortar) forms a waterproof moisture barrier that minimizes transepidermal water loss to keep moisture in the skin. This moisture barrier protects against invading microorganisms, chemical irritants, and allergens. If the integrity of the moisture barrier is compromised, the skin will become vulnerable to dryness, itching, redness, stinging, and other skin care concerns.

The main structural components of this composite system are 1) The corneocytes: stacked up to 18–20 layers depending on the anatomic location in the body; these provide the physical barrier 2) Corneodesmosomes: functioning as “spot weldings” or “rivets” to
hold the corneocytes together. Desmosomes are programmed to go through a gradual degradation process so as to enable the orderly desquamation of outermost, worn-out corneocytes. 3) The mortar lipids filling the tortuous pathway between the stacked corneocytes: a highly complex mixture of about 13 species of ceramides, cholesterol, and free fatty acids in an equimolar ratio; these provide the permeability barrier. 4) A battery of lipolytic and proteolytic enzymes: involved in the processing of pro-barrier lipids and degradation of desmosomes, respectively, they contribute to ongoing biochemical activities in the SC, which was once thought to be inert and dead. 5) The secreted contents of epidermal lamellar bodies at the interface of SC and the stratum granulosum: these are the pro-barrier lipids which give rise to the multiple lipid lamellae of the SC and which are interspersed with the enzymes and anti-microbial peptides. All these components are crucial to the SC barrier, which is viewed as a challenge to transdermal drug delivery. Interfering with, or altering the functional properties of any one of these components can weaken the barrier. In the very outer layers of the SC, the moisture barrier has a slightly acidic pH (4.5 to 6.5).

![The “Bricks and Mortar” of the Stratum Corneum](image)

**Figure 1.2: Structure of SC⁹**

### 1.2.2 ROUTES OF DRUG PERMEATION ACROSS THE SKIN¹⁰

SC is the rate-limiting barrier to delivery for most molecules. There are essentially three pathways by which a molecule can traverse intact SC (Figure 1.3)
(i) Transcellular pathway (Intracellular pathway)

It was originally believed that transcellular diffusion mechanisms dominated over the intercellular and transappendageal routes during the passage of solutes through the SC. However, transport by the transcellular route would involve the repeated partitioning of the molecule between lipophilic and hydrophilic compartments, including the almost impenetrable corneocyte intracellular matrix of keratin and keratohyaline. The pathway is directly across the SC and hence the pathlength for permeation is usually regarded as the thickness of the SC.

(ii) Intercellular pathway

The intercellular lipid route provides the principal pathway for the small, uncharged molecules. The intercellular route is highly tortuous, with permeants moving through the continuous domains between the keratinocytes. The pathlength taken by the molecule is considerably greater than the SC thickness. Various estimates have been proposed for the intercellular permeation distance, ranging from 150-500 μm.

(iii) Transappendageal pathway

The penetrant transverses the SC via a ‘shunt’ pathway: e.g., a hair follicle or a sweat gland. These shunts are known to be important at short times prior to steady state.
diffusion. The available diffusional area of the shunt route is approximately 0.1% of the total skin area and therefore the contribution to drug permeation compared to the former is significantly less. Despite their small fractional area, the skin appendages may provide the main portal of entry into the subepidermal layers of the skin for ions and large polar molecules. The appendageal pathway has been reported to be the major contributor to the initial phase of SC permeation.

1.2.3 PERMEATION OF DRUG FROM THE FORMULATION

The processes which may occur after application of a transdermal formulation to the skin are illustrated in Figure 1.4. Initially, the drug must be released from the vehicle followed by partitioning into the SC. Molecules will subsequently diffuse (as a result of a concentration gradient) through the SC before a further partitioning process into the viable epidermis, and further diffusion through the viable epidermis towards the dermis. The vasculature and lymphatic vessels in the dermis will clear the drug from the skin. This process is efficient and essentially produces a very low active concentration in the layers of the skin below the SC.

![Figure 1.4: Schematic representation of the processes involved in drug transport into, and across, the skin from any transdermal applied formulation](image-url)
A number of drugs may interact with the different skin layers in the course of percutaneous penetration, resulting in limited absorption. These interactions may be in the form of reversible/irreversible binding to several structures in the biological tissue, such as the SC keratin and/or specific sites in the skin to produce a physiological response (e.g. therapeutic activity or allergic reactions). Drug binding is distinct from drug accumulation or retention in the different compartments as the latter results from relatively high drug partition slow drug diffusivity or drug crystallisation. A further possibility is that both processes may contribute to the reservoir capacity of the skin for certain compounds, e.g. steroids. In addition drugs may undergo metabolism during the process of permeation.

1.2.4 MATHEMATICS OF SKIN PERMEATION

(i) Fick’s first law of diffusion

In transport, the flow (or flux, \( J_i \) in mol cm\(^{-2} \) s\(^{-1} \)) is related to the velocity of molecular movement (\( \dot{v} \) in cm s\(^{-1} \)) and the concentration (\( C_i \) in mol cm\(^{-3} \)) of the molecules in motion in equation (1).

\[
J_i = C_i \dot{v}
\]  
(1)

A fundamental principle of irreversible thermodynamics is that the flow, at any point in the system, at any instant, is proportional to the appropriate potential gradient. It can be expressed mathematically for a species \( i \) as shown in equation (2) where \( \partial \mu_i / \partial x_i \) is the gradient and \( L_i \) is the proportionality constant.

\[
J_i = -L_i \left( \partial \mu_i / \partial x \right)
\]  
(2)

Equation (2) is the general form of Fick’s first law of diffusion.

If constant temperature and pressure is assumed equation (2) can be expressed as equation (3) where \( D_i \) is the diffusion co-efficient.

\[
J_i = -D_i \left( \partial C_i / \partial x \right)
\]  
(3)

Equation (3) is the classic form of Fick’s first law of diffusion.

(ii) Fick’s second law of diffusion

Fick’s second law relates the rate of change in concentration with time at a given point in a system to the rate of change in concentration gradient at that point.

\[
\partial C / \partial t = D \partial^2 C / \partial x^2
\]  
(4)
Fick’s laws are more applicable if certain parameters or boundaries are specified. Most of in-vitro experimental design aim to mimic as closely as possible the in-vivo situation. The most common in-vitro design is one where a membrane separates two compartments. One compartment contains the permeant in a vehicle (donor solution) and the other compartment contains a receptor solution that provided sink conditions. After sufficient time, steady-state permeation across the membrane is achieved when the concentration gradient of the permeant across the membrane is constant. Under these conditions, equation (4) can be simplified to (5), where \( Q \) is the cumulative mass of the permeant that passes through a unit area of a membrane in a time \( t \), \( C_0 \) is the concentration of the permeant in the first layer of the membrane and \( h \) is the thickness of the membrane.

\[
\frac{dQ}{dt} = J = \frac{DC_0}{h}
\]  

(5)

In practical terms, \( C_o \) (the concentration of permeant in the outer layer of the membrane), that is very difficult to measure. The value \( C_o \) is replaced with a term that links it to the concentration in the vehicle \( C_v \) through the partition co-efficient \( K \), which rearranges to give equation 6.

\[
\frac{dQ}{dt} = J = \frac{DKC_v}{h}
\]  

(6)

The term \( DK/h \) in equation 6 is called permeability coefficient \( (P) \). It can be substituted into equation 6 to give

\[
\frac{dQ}{dt} = J = PC_v
\]  

(7)

The permeation profile can be plotted of \( Q \), the cumulative amount of drug passing through a unit area of membrane against time \( t \). The slope of the straight line is flux \((dQ/dt)\). The lag time can be obtained from extrapolation of the pseudo-steady state portion of the permeation profile to the intercept on the time axis and can be related to the diffusion coefficient by equation (8)

\[
t_{lag} = \frac{h^2}{6D}
\]  

(8)

**1.3 INFRINGING BARRIERS OF DRUG PERMEATION**

One challenge in designing of TDDSis to overcome the skin's formidable barrier function. Many intensive researches in the development of strategies is to increase the delivery as well as to facilitate the extraction of molecules for monitoring and diagnostics purposes which are categorized as active and passive means. These are based on two strategies: increasing skin permeability and/or providing driving force acting on the drug.
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Since passive transdermal permeation of the majority of the drugs needs enhancement to achieve clinically relevant plasma concentrations, both chemical and physical enhancement methods have been developed. Figure 1.5 shows some penetration enhancement techniques.

Maximizing transdermal drug delivery

Figure 1.5: Various penetration enhancement techniques

1.3.1 PRONIOSOMES

Proniosomes are vesicular systems, in which the vesicles are made up of non-ionic based surfactants, cholesterol and other additives. Proniosomes are nowadays used to enhance drug delivery in addition to conventional niosomes. Proniosomes exists in two forms, i.e. semisolid liquid crystal gel and dry granular powder, depending on their method of preparation. Out of these two forms, the proniosome gel is mainly used for transdermal applications.

The active ingredients present in the formulation of drugs and permeate through the intercellular lipid matrix, i.e. intercellular and transcellular. However, vesicular delivery systems use three pathways for permeation of drugs in the tissues and they are, a) hair follicle associated with sebaceous glands, b) through sweat glands and c) across the continuous SC layer.
Semisolid liquid crystal gel (proniosomes) prepared by dissolving the surfactant in a minimal amount of an acceptable solvent, namely ethanol and then hydration with least amount of water to form a gel. These structures are liquid crystalline compact niosomes hybrids that can be converted into niosomes immediately upon hydration or used as such in the transdermal applications. Use of proniosome gel in transdermal delivery does not require hydration prior to application, but they can be applied as such or loaded on a base material of emulsion, gel, ointment, etc. prior to application. The base material helps in the application of the formulation to the skin and dilution of the active material^{15-18}.

Due to the limited solvent system present, the proniosomes formed were the mixture of many phases of liquid crystal, viz. lamellar, hexagonal and cubic phase liquid crystals as given in Figure 1.6.

![Schematic representation of various liquid crystalline phases](image)

**Figure 1.6: Schematic representation of various liquid crystalline phases**

Dissolution of most surfactants in water, leads to the formation of lyotropic liquid crystals rather than micellar solution. *Lamellar phase* shows sheets of surfactants arranged in bilayer form, whereas in *hexagonal phase* cylindrical units are packed in hexagonal fashion. *Cubic phase* consists of curved bio-continuous lipid bilayer extending in three dimensions, separating two congruent networks of water channels. These liquid crystals present an attractive appearance because of their, transparency and high viscosity, although in the beginning of its formation, a short range of less viscous compositions (so called liquid/gel compositions) appear in some cases. Addition of water leads to interaction between water and polar groups of the surfactant results in swelling of bilayers^{19-21}.

When the concentration of solvent is increased above a limited value, the bilayers tend to form random spherical structures, i.e., multilamellar, multivesicular structures. When
shaken with water i.e. the aqueous phase of water, complete hydration takes place leading to the formation of niosomes. The beauty of these proniosomes lies in their ability to rearrange as stable noisomal suspensions, on hydration with water.\(^\text{22}\)

1.3.1.1 Advantages of Proniosomes\(^\text{23}\)

- They are becoming popular due to their semisolid/liquid crystalline compact nature when compared to niosome dispersion.
- Proniosomal gels are generally present in transparent, translucent or white semisolid gel texture, which makes them physically stable during sterilization, storage and transport.
- Both the non-ionic surfactants and phospholipids in proniosomes can act as penetration enhancers and help in diffusion of the drug.
- Proniosomes have higher advantages such as additional convenience of dosing and distribution.
- They avoid the problems associated with either the aqueous niosome dispersion, such as problems of physical stability, aggregation, fusion, and leakage.
- Proniosomes also avoid problems associated with liposomes like degradation by hydrolysis or oxidation as well as sedimentation, aggregation or fusion during storage.
- Proniosomes not only offer a promising means of drug delivery, but also could enhance the recovery rate of the skin barrier.

1.3.1.2 Interaction Between skin and vesicles\(^\text{24-31}\)

There is a direct contact of proniosome formulation with skin after applies, so it is better to discuss the potential interactions between skin and vesicles formed in proniosome/niosome formulations. As we know that proniosomes or proniosomes derived niosomes are composed of non-ionic surfactants, and the vesicles are composed of these non-ionic surfactant only. So it is advisable to study the interactions between non-ionic surfactants and the skin. The non-ionic surfactants are amphipathic molecules consisting of a hydrophobic (alkylated phenol derivatives, fatty acids, long chain linear alcohols, etc.) and a hydrophilic part (usually ethylene oxide chains of variable length).
Nonionic surfactants are used widely in pharmaceuticals to increase their stability, solubility and permeation. There is a strong indication that the degree of interaction between vesicles and skin mainly depends on physicochemical properties of the surfactant molecules of which the niosomes or proniosomes are composed. Skin consists of a range of bioactive material like membrane phospholipids, proteins, amino acids, peptides, etc.

Vesicles prepared from cholesterol and polyoxyethylene alkyl ether surfactants were studied with isolated human SC incubated for 48 hours and for vesicle skin interactions. Fusion of liquid as well as gel state vesicles on the superficial layer of SC takes place, but liquid state vesicles induced perturbations in liquid organization, so water pool formation within the SC was observed. Stacks of lamellae and irregular structures were formed on the skin with fusion and adsorption of vesicles onto the SC surface. These structures and interactions strongly depend on vesicle composition and physiological properties. After a 12-hour pretreatment, permeation across span 60-treated skin was significantly higher than that across non-treated skin. Surfactant treated formulations were found to be superior to phospholipid treated and non-treated formulations in facilitating the permeation of enoxacin, as well as drug deposition into the skin. On the basis of results, two types of interaction were observed between vesicles and skin surface. First, interaction was the skin-formulation interface involving adsorption and fusion of vesicles of niosome or proniosomes on the SC surface, resulting in new structure formation. Secondly, vesicle-skin interactions found in the deeper layers of the SC, involve alteration of the bilayer ultra-structure. A fluorescence depolarization study indicated that alkanoyl-N-methylglucamide surfactants decrease the fluidity of dipalmitoyl phosphatidylcholine membranes. Non-ionic surfactants decreased the phase transition temperature of negatively charged dilauroylphosphatidic acid membrane. The interaction between surfactant molecules incorporated in the lipid membrane was also observed. Surfactants are known to increase the permeability of vesicles and phospholipid membranes, causing low molecular mass compounds to leak. The interaction between biological membranes and non-ionic surfactant tested for phospholipid composition and rate of biosynthesis of major phospholipid components indicate no significant change in the phospholipid composition, whereas biosynthesis and turnover rates of phospholipids
were increased two to four times. The available data suggests that the tested surfactant damaged the epidermis membranes. Surfactant cause modification in physicochemical characteristics of natural membranes and can disrupt artificial membranes but also. Nonionic surfactants have the ability to increase the permeability of sarcoplasmic reticulum. This phenomenon has been frequently exploited to extract and solubilize sparingly soluble proteins such as membrane proteins.

1.3.1.3 Vesicle formation in proniosomes

The ability of nonionic surfactant to form bilayer vesicles instead of micelles is not only depends on the hydrophilic-lipophilic balance values of the surfactant and the chemical structure of the components, but also on the critical packing parameter (CPP). In proniosomes the vesicle-forming tendency is similar to niosomes. The relationship between the structure of the surfactant including size of hydrophilic head group, and length of hydrophobic alkyl chain in the ability to form vesicles is described as

\[ CPP = \frac{y}{l_c a} \]  

where \( y \) = hydrophobic group volume, \( l_c \) = the critical hydrophobic group length and \( a \) = the area of the hydrophilic head group. A CPP of between 0.5 and 1 indicates that the surfactant is likely to form vesicles. A CPP of below 0.5 (indicating a large contribution from the hydrophilic head group area) is said to give spherical micelles and a CPP of above 1 (indicating a large contribution from the hydrophobic group volume) should produce inverted micelles, the latter presumably only in an oil phase, or precipitation would occur.

Addition of cholesterol suppresses the tendency of the surfactants to form aggregates and also provides greater stability to the bilayer membranes by increasing the gel liquid transition temperature of the vesicle and also attributes to the higher hydrophilic-lipophilic balance and smaller critical packaging parameters. Cholesterol addition also enables more hydrophobic surfactants to form vesicles. Apart from this addition of cholesterol also influences membrane permeability, encapsulation efficiency and bilayer rigidity.

Stabilization and permeability can also be enhanced by the addition of lecithin and by the addition of charged molecules like, diacetyl phosphate and stearyl amine to the bilayer.
1.3.1.4 Conversion of proniosome gel into niosomes 22, 36, 37

Proniosome gel is an intermediate state of formation of niosome. Minimum quantity of continuous phase, leads to the formation of liquid crystalline compact mass of proniosomes. Proniosome gel thus obtained has some advantages over conventional niosomes due to their compact gel nature, which helps in degradation, transportation and stability. The conversion of proniosome gel into niosomes can be achieved in two ways.

*Hydration by skin: The hydration is achieved by skin itself i.e. the water in the skin is used to hydrate the proniosome formulation and conversion to niosomes.

*Hydration by solvents: Aqueous systems i.e. purified water, saline solution and buffers are used to convert proniosomes to niosomes with or without agitation and sonication.

The proniosome gel system is directly being formulated in the patch for used in dermal and transdermal applications without the requirement of polymeric matrix for dispersion. The formulation takes water from the skin and converts into niosomes. The addition of aqueous phase from outside also leads to the formation of niosomes. After the addition of aqueous phase, agitation and sonication leads to formation of niosomes with small size vesicles. The addition of water into compact mass of proniosome leads to the swelling of bilayers as well as vesicles due to the interaction of water with polar groups of the surfactant. Due to the inclusion of water in the bilayers, the stacked structures tend to separate. Above a limiting concentration of solvent bilayers tends to form spherical structure which gives rise to unilamellar to multilamellar vesicular structures. Addition of shaking step in hydration process leads to complete hydration and formation of niosomes.

1.3.1.5 Mechanisms for permeation of vesicles through skin 22, 32, 38-43

Proniosome gel is a liquid crystalline compact mass, which upon hydration leads to unilamellar to multivesicular, multilamellar and spherical shaped niosomes. The drug is entrapped into the vesicles (derived niosomes self-assembly of non-ionic surfactant). SC is considered to be a particularly impermeable barrier, so there is need to elucidate the mechanism through which the drug into vesicles is delivered to the deeper layers.

Proniosomes contain both non-ionic surfactant and phospholipids, both can act as penetration enhancer and useful in increasing permeation powers of many drugs. A single mechanism is not sufficient to describe the permeation of drug containing vesicles into
the skin. Many hypotheses exist relating to the permeation of vesicles through skin for drug release in deeper layers. The ability of vesicles (present in many delivery systems) to modulate drug transfer across skin can be explained by several mechanisms.

- Adsorption and fusion of vesicles onto the surface of skin leading to a high thermodynamic activity gradient of drug at the interface, which is the driving force for permeation of lipophilic drugs.
- The penetration enhancers effect of vesicles to reduce SC barrier properties.
- The bilayers present in niosomes acts as rate-limiting membrane barrier for drugs.

Modification in the structure of SC is also one of the possible mechanisms for the permeation of the vesicle-encapsulated drug. Intracellular lipid barrier in the SC was found dramatically looser and more permeable after treating with liposomes and niosomes.

1.3.2 IONTOPHORESIS

Iontophoresis can be defined as “the permeation of ionized drug molecules across biological membranes under the influence of electrical current” or a technique that involves the transport of ionic or charged molecules into a tissue by the passage of direct or periodic electric current through an electrolyte solution containing the ionic molecules to be delivered using an appropriate electrode polarity.

Ions in solution are transferred through the skin by passing DC electrical current between two electrodes. Iontophoresis implies the use of a small amount of physiologically acceptable electric current (0.5 mA/cm² or less) to drive ionic (charged) drugs into the body by using an electrode of the same polarity as the charge on the drug. The drug is driven into the skin by electrostatic repulsion. Interposition of moist pad between the electrode plate and skin is necessary for making a perfect contact, preventing any skin burns, overcoming skin resistance and protecting the skin from absorbing any caustic metallic compound formed on the metal plate surface. The technique has been observed to enhance the transdermal permeation of ionic drugs by several folds and has expanded the horizon of transdermal control drug delivery for systemic medication. Beside the usual benefit of transdermal drug delivery system, iontophoresis presents a unique opportunity to provide programmed drug delivery. This is because the permeation rate is
proportional to the current density, which can be readily adjusted. Such dependence on current may also make drug absorption via iontophoresis less dependent on biological variables. While all these enhancers have been individually shown to enhance transdermal drug transport, nowadays their combinations have been hypothesized to be more effective compared to each of them alone.

1.3.2.1 Historical background of iontophoretic process

Iontophoresis, derived from the Greek “ionto” meaning ‘ion’ and “phoresis” meaning ‘to bear,’ is a process that allows increased penetration of ionized molecule across or into the tissue by application of low electric current. Clinical application of current can be traced back to the ancient time of the golden age of the Greek civilization and was probably originated by Varatti in 1747. The idea of applying electric current to increase the permeation of electrically charged drugs into surface tissues was probably originated by Pivati in 1747. In the eighteenth century Galvani and Vota combined the knowledge that electricity can move different metal ions and the movement of the ions produce electricity. In beginning of twentieth century Leduc introduced the term ionototherapy and formulated laws regarding this process.

1.3.2.2 Iontophoretic Research & Drug Delivery

Since most of the drugs showed less than adequate skin permeability in passive studies, iontophoresis was proposed to be a physical technique for enhancement of skin permeation. The release characteristics of drug from delivery system and the composition of drug delivery system can largely affect the transdermal permeation of drug molecules. Organic solvents and surface active agents can also alter the permeability of skin and enhance the percutaneous absorption. The transdermal permeation rate increases as the drug release rate from the drug delivery system increases. Major mechanisms of enhancing drug flux through skin are:

- Iontophoresis (electrorepulsion, electromigration or Nernst plank effect)
- Electroosmotic flow
- Damage effect (current induced increase in skin permeation)
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Iontophoresis enhances drug delivery across the skin by two principal mechanisms: electrorepulsion and electroosmosis. Electrorepulsion is the direct effect of the applied electric field on a charged permeant. The second mechanism, electroosmosis, results from the fact that the skin supports a net negative charge at physiological pH.

Iontophoresis is a non-invasive method used to boost high concentration of a charged substance, generally medication or bioactive agents, transdermally by repulsive electromotive force using a small electrical current applied to an iontophoretic chamber containing a similarly charged active agent and its vehicle.

For effective delivery via iontophoresis, the positively charged chamber, termed anode, will repel a positively charged chemical, while the negatively charged cathode, will repel a negatively charged chemical into the skin. In the presence of an electric field, electromigration and electroosmosis are the dominant forces in mass transport.

These movements are measured in units of chemical flux, commonly $\mu$mol/cm$^2$ h. This technique is based on the general principle that like charges repel each other. Thus, during iontophoresis, if delivery of a positively charged drug (D+) is desired, the charged drug is dissolved in the electrolyte surrounding the electrode of similar polarity, i.e. the anode in this example. On application of an electromotive force the drug gets repelled and moves across the SC towards the cathode, which is placed elsewhere on the body. Communication between the electrodes along the surface of the skin has been shown to be negligible, i.e. movement of the drug ions between the electrodes occurs through the skin and not on the surface. When the cathode is placed in the donor compartment of a Franz diffusion cell to enhance the flux of an anion, it is termed cathodal iontophoresis, and for anodal iontophoresis the situation would be reversed. Iontophoresis uses a low current, and patients’ have little or no sensation during the procedure.

The basic mechanisms of ionic/molecular transport across the skin by iontophoresis are illustrated in Figure 1.7. charges repel each other, hence the charged ion is repelled by a similarly charged electrode and absorbed through the skin. The skin being negatively charged at physiological pH acts as a cation selective membrane and favours movement of cations through anodal iontophoresis. Anodal iontophoresis also causes convective motion of the solvent occurring in response to movement of counter ions. This process of electroosmosis is involved in the motion of neutral compounds as well as positively
charged ions. Because of the complex nature of iontophoretic delivery, a number of attempts have been made to define the rate of iontophoretic delivery.

![Figure 1.7: Basic mechanism of iontophoresis](image)

Abramson and Gorin derived an equation to compare the iontophoresis flux to electric mobility, electroosmosis and simple diffusion. The increased flux during iontophoresis would include:

- Flux due to the electrochemical potential gradient across the skin;
- Change in the skin permeability due to the electric field applied; and
- Electro-osmotic water flow and the resultant solvent drag.

\[ J_{\text{ionto}} = J_{\text{electric}} + J_{\text{passive}} + J_{\text{convective}} \]

- Flux due to electric current application; 
- The flux due to passive delivery through the skin; and 
- The flux due to convective transport due to electro osmosis. The Nernst-Planck equation has been used with modifications to predict iontophoretic enhancement ratios (ratio of steady state flux in presence of electric potential and in absence of potential) as the original equation lacks a term for convective electroosmotic flow studied the contributions of osmotic flow and incorporated this fact into several equations.

### 1.3.2.3 Pathways of Molecular Transport in Iontophoresis

Percutaneous absorption may take effect simultaneously by 3 main pathways (Figure 1.3):

- Intercellular (paracellular) between the comeocytes;
• Transcellular (intracellular) through cells;
• Appendageal (shunt pathway) hair follicles, sweat ducts, secretary glands.

Ions prefer the routes of shunt pathway. Physiochemical properties of drug molecules affect the drug distribution. Hydrophilic molecules tend to localize the drug in hair follicles. On the other hand, lipophilic molecules are distributed mostly in lipid intercellular regions of the lipid membrane of epidermal keratinocytes and SC. Therefore, Transdermal iontophoresis should be termed as electrically assisted transdermal delivery.

Electroosmotic flow is a flux or bulk fluid induced by a voltage difference across a charged membrane; it is a one way flow of counter ions i.e. from anode to cathode. Therefore, cathodic delivery of anions is hindered, and thus anodic delivery of cations is assisted by it. When the delivery of large anion from the anodic compartment is more efficient than delivery from cathode, this is called wrong-way iontophoresis. The electrorepulsion effect gives the largest enhancement to flux of small lipophilic cations.

1.3.2.4 Types

Voltage drop across a membrane driving force for the flux of ions through it opens up new type of approaches to mode transport of ionic drugs across skin. Iontophoresis is usually defined as either anodal (+) in which the positive anode is placed in the solution applied to the epidermis and negative cathode is placed in the solution applied to the epidermis and negative cathode is placed in the dermal receptor solution, or cathodal (-), in which the electrode location are reserved. Anodal (+) introphoresis is facilitated by the movement of a caution from the donor to the receptor, whereas cathodal iontophoresis implies the movement of an anion from the donor to receptor.

1.3.2.5 Merits

✓ It is a non-invasive technique could serve as a substitute for chemical enhancers.
✓ It eliminates problems like toxicity problem, adverse reaction formulation problems associated with presence of chemical enhancers in pharmaceuticals.
✓ It may permit lower quantities of drug, results in fewer side effects.
✓ TDDS of many ionized drug at therapeutic levels was precluded by their slow rate of diffusion under a concentration graduation, but iontophoresis enhanced flux of ionic drugs across skin under electrical potential gradient.
✓ Iontophoresis prevent variation in the absorption of TDDS.
✓ Eliminate the chance of over or under dosing by continuous delivery of drug programmed at the required therapeutic rate.
✓ Provide simplified therapeutic regimen, leading to better compliance.
✓ Permit a rapid termination of the modification, if needed, by simply by stopping drug input from the iontophoretic delivery system.
✓ It is important in systemic delivery of peptide/protein based pharmaceuticals, which are very potent, extremely short acting and often require delivery in a circadian pattern to simulate physiological rhythm, eg. Thyrotropin releasing hormone, somatotropine, tissue plasminogen activates, interferons, etc.
✓ Provide predictable and extended duration of action.
✓ Reduce frequency of dosage. Self-administration is possible.
✓ A constant current system automatically adjust the magnitude of the electric potential across skin which is directly proportional to rate of drug delivery and therefore, intra and inter-subject variability in drug delivery rate is substantially reduced. Thus, minimize it.
✓ An iontophoretic system also consists of a electronic control module which would allow for time varying of free-back controlled drug delivery.
✓ Iontophoresis turned over control of local anesthesia delivery in reducing the pain of needle insertion for local anesthesia.
✓ It prevents contamination of drugs reservoir for extended period of time.

1.3.2.6 Physico-chemical parameters

The movement of drug ions across the skin is dependent not only the magnitude of apparent electric field, but also upon the concentration of solution, the molecular size of drug to be passed, as well as charge and valence of ion.

(i) pH:

The iontophoretic drug delivery rate is dependent on the ionic form of drug delivery, which is extremely effected by the pH of the system, when the skin is maintained at a negative charge by exposing the solution with pH 4 or higher, it facilitate the transdermal delivery of cationic drugs.
(ii) **Species variation:**

The vide differences in physical characteristics such as appendages per unit area, thickness and structural changes between human and laboratory rodent display a variation in penetration of drugs. The average penetration of drugs is in order of rabbit > rat > guineas pig > human. Human skin is very much less permeable than other rodents but iontophoretic delivery of drug is 7-fold greater in human skin consists of greater negative charge/or greater area fraction of negative pores.

(iii) **Characteristics of Penetrants:**

The rate of penetration of substances through the intact skin depends on the size, charge, and configuration of molecules and relative solubility of the compound in lipid, water, in the horny layer and on the vehicle in which the compound is presented to the skin. The iontophoresis gives uncertain drug delivery rate for an ionic solute of molecular weight 8000 to 12,000. For a negatively charged species, the size dependent flux enhancement neutralizes the influence of electric field. Conversely, positive charged species becomes increasingly important to affect the electric field as the size of permeant increases.

(iv) **Concentration:**

The concentration dependent iontophoretic delivery has not been fully investigated, some of the authors reported that as the concentration of drugs increase in reservoir system then permeation of drug also increases. In some cases the flux of solute was non-linearly proportional to its concentration.

(v) **Buffer Systems:**

It is important to optimize the concentration of buffer species in the system and should be sufficiently high to maintain good buffer capacity but should not reach an extent such that the current is mostly carried by the buffer species instead of drug special which may result the low efficiency of iontophoretic permeation.

(vi) **Ionic Strength:**

The ionic strength of a drug delivery system is directly related to the iontophoretic permeation of drugs. Some authors reported that increasingly the ionic strength of the system decreases the permeation rate of drug, and has no significant effect on penetration up to the 0.5 V.
(vii) Electrodes:

The electrode materials used for iontophoretic delivery are to be harmless to the body and sufficiently flexible to apply closely to the body surface. The most common electrodes are aluminum foil, platinum and silver/silver chloride electrodes used for iontophoretic drug delivery. A better choice of electrode is silver/silver chloride because it minimizes electrolysis of water during drug delivery. The positioning of electrodes in reservoir depends on the charge of the active drug. The distribution of drug within the skin depends on the size and position of electrodes. They are usually selected according to individuals needs. Larger electrode areas introduce the greater amounts of drug but lesser current density is tolerated to the skin in a non-linear manner. Metal electrodes touching to the skin produce burns with much lower current in composition to padded electrodes. A loose contact of electrodes and skin also produce burn due to uneven distribution of current. The safe current density varies with the size of electrodes.

(viii) Temperature:

The penetration of drug through skin is affected by dual effect of both humidity and temperature. The iontophoretic delivery follows the Arhenious equation and enhances drug permeation with temperature.

1.3.2.7 Electrical parameters

(i) Current:

The extent of charged molecules, which may penetrate through the skin, are theoretically proportional to the intensity of current and the duration of treatment for a transdermal iontophoretic delivery. The relationship between the drug delivery rate (D) and current (I) follows the given question: \[ D = \frac{ItM}{Zf} \]

where, \( t \) is the fraction of current carried by drug ions or transference number, \( M \) is the molecular weight of drug ion, \( Z \) is the molecular charge per drug ion and \( F \) is Faraday’s constant.

(ii) Voltage:

The ionic flux due to an applied voltage drop across a membrane is based on the fundamental thermodynamic properties of the system. The diffusion of drug during iontophoresis follows Nerst-Plank equation. It states that the flux of the ionic drug due to applied electric filed is directly proportional to the voltage drop and charge of the ion.
(iii) **Resistance:**

The electrical resistance of the skin varies widely with iontophoretic drug delivery. The resistance of the skin during iontophoretic application was much lower on sweat pores, especially when they discharge sweat. A slight fall in resistance occurs when electrode was interested in to the epidermis.

(iv) **Frequency/Impedance:**

The frequency of the applied current charges especially in man, variability of frequency dependent impedance of human skin ranges from 10 KHzs to 100 Khzs. The impedance of the skin decreases at higher frequencies less time is available to accumulate the charge on the skin surface during an applied pulse. The theoretical relationship between impedance of skin and frequency follows this equation: \( \frac{1}{Z_T} = \frac{1}{Z_R} + \frac{1}{Z_C} \)

(v) **On/Off Ratio:**

The on/off ratio of electricity effects the relative proportion of polarization and depolarization of skin, which results the efficiency of transdermal iontophoretic drug delivery. The number of on/off cycles in each second is shown as frequency. For example the on/off ration 1:1 at frequency 2000 Hzs (0.5 ms/cycle) provides 0.25 ms depolarization period and same time for the polarization.

(vi) **Wave Form:**

The waveform also affects the iontophoretic delivery of drug. The insulin delivery was highest at sinusoidal waveform than square and triangular waveform.

### 1.3.2.8 Operational parameters

(i) **Duration of Application:**

The transport of drug delivery depends on the duration of current applied in iontophoretic drug delivery. The iontophoretic penetration of drug linearly increased with increasing application time.

(ii) **Mode of Current:**

Direct current (DC) iontophoretic dosing of drug inevitably develops a skin polarization potential, which reduce the efficiency of iontophoretic delivery and cause skin irritation, burning and redness. But pulsed DC dosing pattern is effective for drug transport, the same time average voltage because it faces lower skin resistance in comparison to simple DC application in flux enhancement.
1.3.3 OTHER APPROACHES 42, 51-55

1.3.3.1 Prodrug

Prodrug strategy generally involves addition of a pro-moiety to increase partition coefficient and solubility to increase the partition coefficient and solubility to increase the transport of drug in the SC. Upon reaching the viable epidermis, esterase releases the parent drug by hydrolysis.

1.3.3.2 Thermodynamic activity

The maximum skin penetration rate is obtained when a drug is at higher thermodynamic activities in case of supersaturated solution. This increases the concentration gradient (C₀-Cᵢ) in the Fick’s law and thus forces the active ingredient out of formulation and in to across the SC.

1.3.3.3 Ion pair and coacervates

This strategy involves adding an oppositely charged species to the charged drug, forming an ion-pair in which the charges are neutralised so that the complex can partition into and permeate through the SC. A coacervate is a tiny spherical droplet of assorted organic molecules (specifically, lipid molecules) which is held together by hydrophobic forces from a surrounding liquid. The coacervate partitions into SC, where it behaves as ion pairs, diffusing, dissociating and passing into viable tissues.

1.3.3.4 Eutectic system

According to regular solution theory, the lower the melting point, the greater the solubility of a material in a given solvent, including skin lipids. The melting point of a drug delivery system can be lowered by formation of a eutectic mixture.

1.3.3.5 Liposomes

Liposomes are colloidal particles formed as concentric biomolecular layers that are capable of encapsulating drugs. These lipid molecules are usually phospholipids with or without some additives. Cholesterol may be included to improve bilayer characteristics of liposomes; increasing microviscosity of the bilayer, stabilizing the membrane and increasing rigidity of the vesicles.
1.3.3.6 High velocity particles

The term is also called as needle less injection. Transdermal delivery is achieved by firing the liquid or solid particles at supersonic speeds through the outer layers of the skin using a suitable energy source. Some of the needle free injector under development are: intraject, implaject, jet syringe, iject, miniject, crossject, powerject devices.

1.3.3.7 Ethmosomes

These are liposomes with a high alcohol content (up to 45%) capable of enhancing penetration to deep tissues and to systemic circulation. It is purposed that the alcohol fluids is the ethosomal lipids and SC bilayer lipids thus allowing the soft malleable ethmosomes to penetrate.

1.3.3.8 Transferosomes

Transferosomes are vesicles composed of phospholipids as their main ingredients with 10-25% surfactants and 3-10% ethanol. The driving force for penetration into the skin is the “Transdermal gradient” caused by the difference in water content between the respectively dehydrated skin surface (approximately 20% water) and the aqueous viable epidermis (close to 100%).

1.3.3.9 Solid - Lipid nanoparticles

These are made up of solid lipids. Their size ranges from 50- 1000 nm. They can also be stabilised by surfactants or polymers. There are mainly three structures: homogenous matrix, drug enriched shell and drug enriched core. They can protect active components against chemical degradation and modulate compound release. It is thought their enhanced skin penetration is primarily due to an increase in skin hydration caused by the occlusive film formed on the skin surface by it.

1.3.3.10 Aspasomes

Ascorbyl palmitate formed vesicles (Aspasomes) in presence of cholesterol and charge inducer dicetyl phosphate, encapsulating azidothymidine solution. The antioxidant potency of aspasome was much better than that of ascorbic acid. Thus, it can find applications as drug delivery system in disorders implicated with reactive oxygen species.
1.3.3.11 Hydration

Water is the most widely used and safest method to increase skin penetration of both hydrophilic and lipophilic permeants. The water content of the SC is around 15 to 20% of the dry weight but can vary according to humidity of the external environment. Additional water within the SC could alter permeants solubility and thereby modify partitioning from the vehicle into the membrane.

1.3.3.12 Permeation enhancers

Mainly classified as chemical enhancers and biochemical enhancers. These are compounds which promote skin permeability by altering the skin as a barrier to the flux of a desired penetrant. Penetration enhancers may act by one or more of three main mechanisms: 1) Disruption of the highly ordered structure of SC lipid, 2) Interaction with intercellular protein, 3) Improved partition of the drug, coenhancer or solvent into the SC.

1.3.3.13 Skin stretching

The authors claim that a tension of about 0.01 to 10 mP results in the reversible formation of micro pathways.

1.3.3.14 Microneedles array

Microfabricated microneedles are devices which are hybrids of the hypodermic needle and transdermal patch through the use of microscopic needles that can deliver the drug effectively (like a hypodermic needle). Their small size offers the potential advantages of delivering large molecules across the SC without extreme pain to the patients. The microneedles concept employs an array of micron-scale needles that can deliver drug into the epidermis and dermis, which ultimately leads to uptake by the capillaries for systemic delivery but not so far that microneedles hit the nerves.

1.3.3.15 Follicular delivery

Recent studies have re-examined the long held assumption that the follicles occupy approximately 0.1% of the surface area of human skin. Approximately 13.7% of the surface area of the forehead is available as follicles and a number of hydrophilic drugs can be delivered by this route.
1.3.3.16 RF Microchannels

RF MicroChannels are created by placing against the skin an array of closely spaced, tiny electrodes of very precise dimension. The alternating electrical current passing through the microelectrodes ablates the cells underneath each electrode, forming microscopic passages in the epidermis and outer dermis.

1.3.3.17 Macroflux

The system incorporates a titanium microprojection array that creates superficial pathway through the skin barrier layer to allow transportation of therapeutic proteins and vaccines or access to the interstitial fluids for sampling.

1.3.3.18 Microblades

The need for such a device existed because it was hypothesized that once a drug penetrated through SC with the aid of the device, permeation through the remaining layers could proceed readily. The apparatus basically consists of a cutter having a plurality of microprotrusions having a height chosen with respect to the layer of skin that is to be disrupted and stop for preventing the apparatus from penetrating the skin beyond a predetermined distance.

1.3.3.19 Ultrasound

It is typically defined as sound waves whose frequency is too high for perception by the human ear. Ultrasound (sonophoresis, phonophoresis and ultraphonophoresis) is a technique for increasing the skin permeation of drugs using ultrasound (20 KHZ to 16 MHZ) as a physical force. The mechanism of transdermal skin permeation involves disruption of the SC lipids, thus allowing the drug to pass through the skin.

1.3.3.20 Electroporation

This method involves the application of high voltage pulses to the skin, which has been suggested to induce formation of transient pores. The mechanism of penetration is the formation of transient pores due to electric pulses that subsequently allow the passage of macromolecules from the outside of the cell to the intracellular space via a Combination of possible processes such as diffusion and local electrophoresis.
1.3.3.21 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.

1.3.3.22 Medicated Tattoos

Medicated Tattoo is a modification of temporary tattoo which contains an active drug substance for transdermal delivery. Medicated tattoos are applied to clean, dry skin in the same manner as traditional temporary tattoos.

1.3.3.23 Complexes

Complexation of drugs with cyclodextrins has been used to enhance aqueous solubility and drug stability. As flux is proportional to the free drug concentration, where the cyclodextrins concentration is sufficient to complex only the drug that is in excess of its solubility, an increase in flux might be expected. Skin penetration enhancement has also been attributed to extraction of SC lipids by cyclodextrins.

1.3.3.24 Thermophoresis

Heat is expected to enhance the transdermal delivery of various drugs by increasing skin permeability, body fluid circulation, blood permeability, rate-limiting membrane permeability, and drug solubility. According to Kligman, diffusion through the skin, as elsewhere, temperature-dependent process, so raising the skin temperature should add thermodynamic.

1.3.3.25 Metered-Dose Transdermal Spray

It is a topical solution made up of a volatile cum non-volatile vehicle containing the drug dissolved as a single-phase solution. A finite metered-dose application of the formulation to intact skin results in subsequent evaporation of the volatile component of the vehicle, leaving the remaining non-volatile penetration enhancer and drug to rapidly partition into the SC during the first minute after application, resulting in a SC reservoir of drug and enhancer.
REFERENCES


