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Perspectives on Transdermal Drug Delivery

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ABSTRACT

Due to its numerous advantages, transdermal drug delivery has attracted considerable attention in recent times. The protective barrier nature of the skin however limits the absorption of most drugs across the skin. A variety of strategies are available for the modification of the drug and/or vehicle or the composition of the stratum corneum (SC), the outermost layer of the skin, to improve the absorption of most drugs. Furthermore, various techniques are available for studying the effectiveness, mode of action and skin toxicity of the penetration enhancers. Most of the earlier reviews have focused on a certain selected aspect of transdermal drug delivery. This review presents a comprehensive account of various aspects of drug delivery by transdermal route including the various chemical and physical penetration enhancers. It also discusses the in vivo and in vitro methods of evaluating chemical enhancers , their mode of action and safety.

Key word: Chemical penetration enhancers; Permeation studies; Physical penetration enhancers; Transdemal drug delivery.

INTRODUCTION

The concept of application of medicinal substances in the form of liquid, semisolid or solid to the skin to treat diseases has been as old as humanity. However, over the last two decades there has been renewed interest in the skin as a portal of entry of medicaments to the systemic circulation. Systemic effect results from transdermal delivery or percutaneous absorption of sufficient drug through the skin to the vasculature to produce therapeutic systemic concentrations. This involves the transfer of drug from the skin surface into the stratum corneum(SC), under the influence of

concentration gradient and its subsequent diffusion through the SC and underlying epidermis, through the dermis, and into the microcirculation [1].

In fact, the introduction of the first transdermal patch, Transdermal -Scop (developed in 1981 by Alza, Mountain View, CA, USA), containing scopolamine (hyoscine) for motion sickness brought about a tremendous interest in the usage of intact skin as a portal of entry into the systemic circulation of the body in recent years. Several transdermal products followed into the market place including transdermal devices containing nitroglycerin, clonidine , fentanyl, nicotine, oestradiol, testosterone, lidocaine and diclofenac [2]. Additionally, this interest can be reflected in the large size of the market. As at year 2000 for instance, the worldwide transdermal market was worth US$2billion [2] representing the most successful non-oral systemic drug delivery system. More interesting is that this market was dispersed amongst only eight approved active agents.

Transdermal delivery of drugs offers a lot of advantages over other routes of drug delivery which led to this burgeoning interest in this route in recent years. The merits include among others, non-invasiveness, better patient compliance and potential for continuous or controlled delivery. It provides a convenience route of administration for a variety of clinical indications and there is reversibility of drug application. Presently a lot of drugs are under development for transdermal drug delivery. Patients are eagerly waiting for many of the common drugs in form of transdermal systems. Apart from the patches, it may be possible in the near future that patients will be seen wearing transdermal systems in the form of disposable, battery operated wrist watches that will be operated and controlled by microchips to deliver the drugs at the desired rates.

Despite the interests and the merits in this drug delivery system, only very few drug candidates have been approved for transdermal delivery. Besides skin toxicity of the drug or drug excipients, the major obstacle facing this route of delivery is the barrier nature of the skin which limits the number of molecules permeating it to only few that can meet certain criteria. Such molecules should possess appropriate physicochemical properties such as low melting point (< 150 □C), low molecular weight (<500 D) and intermediate lipophilicity (log P= 1-3) as well as high potency (total daily dose < 10 mg). Only few drugs meet these criteria. Consequently, several approaches have been established in an attempt to overcome the barrier properties and deliver most medicaments through the skin. They include both the chemical and physical enhancement strategies. The former strategy involving chemical methods include penetration enhancers, pro-drugs, colloidal formulations, and supersaturated systems. The latter strategy involves physical methods, including phonophoresis, electroporation etc. More researches in recent years have therefore been devoted towards investigating the effect of numerous chemical or physical or the combination of both enhancers on the skin permeability of most of the common drugs especially those drugs that already have problems at their present route of administration. Several percutaneous research strategies are available including *in vivo* and *in vitro* permeation studies.

In this review article, various aspects of transdermal drug delivery including various enhancers and permeation studies are considered. Methods of studying enhancer mechanisms and skin toxicity of enhancers are also discussed.

2. Drug delivery across the skin

Skin structure

The skin basically consists of three anatomical layers (Figure 1)*:*

- The epidermis, which is a thin, dry and tough outer layer, itself made of several layers consisting of two main parts: the stratum corneum (SC) and the stratum germinativum; the most superficial layer is the SC which is formed and continuously replaced by the basal layer of the stratum germinativum;
- The dermis, which is the thick sensitive layer of skin or connective tissue beneath the epidermis that contains blood, lymph vessels, sweat glands and nerve endings;
- The subcutaneous fatty layer, which contains fatty layer and act as an insulator and depot of calories.

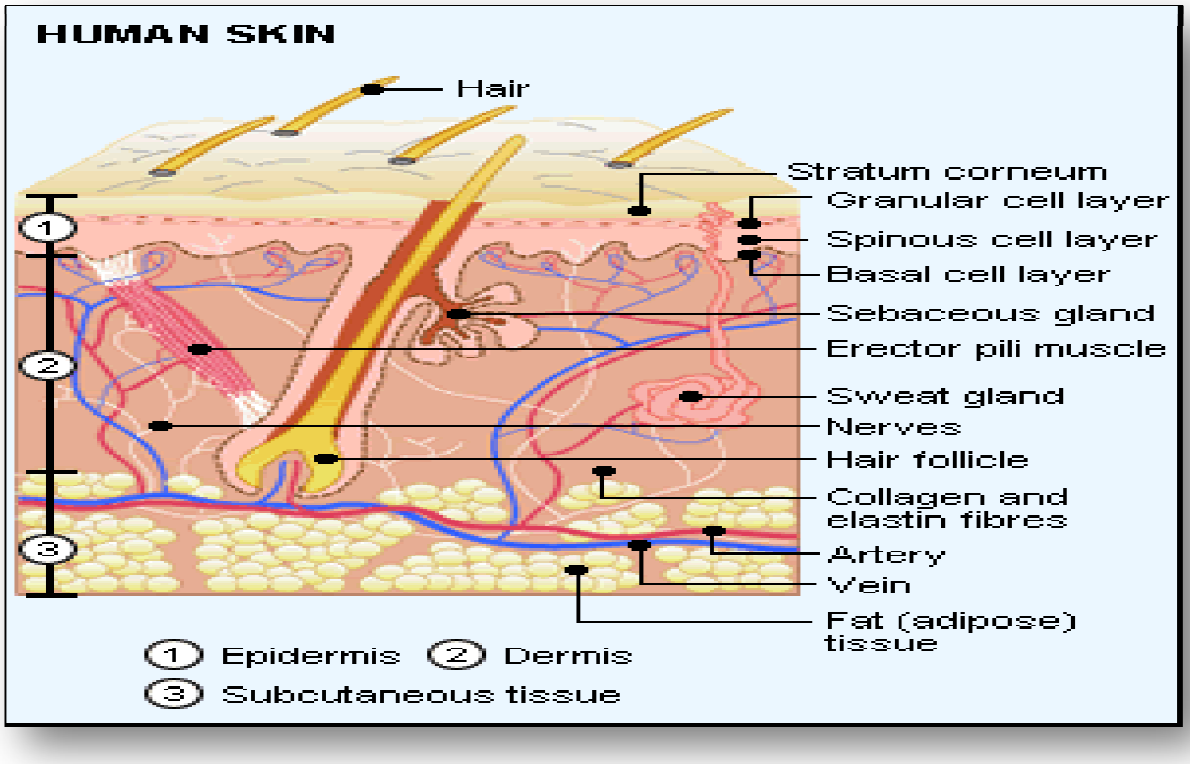

Figure 1. Diagrammatic representation of the cross-section of the human skin.

Skin as a barrier to drug transport and routes of penetration across the skin

The SC, viable epidermis and dermis offer barriers to penetrating molecules. For a drug penetrating across the skin, the greatest resistance is met in the SC. It consists of keratinized, flattened remnants of once actively dividing epidermal cells. It is hygroscopic but impermeable to water and behaves as a tough, flexible membrane [1]. The SC is a compositionally and morphologically unique biomembrane forming a lamella of keratin-filled corneocytes and anchored in a lipophilic matrix. The lipids are tightly packed as bilayers due to the high degree of hydrogen bonding. The lipids of this extracellular matrix are distinct in many respects [3]:

(1) they provide the only continuous phase (and diffusion pathway) from the skin surface to the base of the SC; (2) the composition (ceramides, free fatty acids and cholesterol) is unique among

biomembranes especially the absence of phospholipids; (3) despite this deficit of polar bilayer-forming lipids, the SC lipids exist as multilamellar sheets; (4) the predominantly saturated, long-chain hydrocarbon tails facilitate a highly ordered inter digitated configuration; (5) the formation of gel-phase membrane domains as opposed to the more usual (and more fluid and permeable) liquid crystalline membrane systems.

There are three potential pathways through which drug molecules in contact with the skin surface can penetrate:- through the sweat ducts, through the hair follicles and sebaceous glands (collectively called the shunt or appendageal route) or directly across the SC. It is generally agreed that as the appendages comprise a fractional area for permeation of approximately 0.1%, their contribution to steady state flux of most drugs is minimal. Thus the SC is the most important route for most drug skin penetration. Conventionally, it is thought that lipophilic compounds transfer preferably into the lipoidal intercellular phase of the SC while relatively more hydrophilic compounds transfer into the intracellular domain. However, more recent data [4] have shown that the intercellular route is now considered to be the major pathway for permeation of most drugs across the SC. Thus most of the techniques to optimize permeation to drugs across the skin are directed towards manipulation of solubility in the lipid dormain or alteration of the ordered structure of this region.

3. Factors affecting percutaneous absorption

Fick's first law

Drug permeation across the SC obeys Fick's first law (Equation 1). Thus the equation helps in identifying the ideal parameters involved in the diffusion of drug across the skin [4].

$dm/dt = J = DC_o P/H$…… Equation 1,

where dm/dt or J is the steady-state flux, D is the diffusion coefficient of the drug in the SC, H is the diffusional path length or membrane thickness, P is the partition coefficient between the SC and the vehicle, C_o is the applied drug concentration which is assumed to be constant.

Molecules showing intermediate partition coefficients (log P octane/water of 1-3) have adequate solubility within the lipid domains of the SC to permit diffusion through this domain whilst still having sufficient hydrophilic nature to allow partitioning into the viable tissues of the epidermis. Furthermore, optimal permeability of drug across the SC, according to the equation, is influenced by diffusion coefficient which has been demonstrated to be related to low molecular size and low melting point which is related to solubility.

Other factors which affect percutaneous absorption are discussed below.

Hydration and temperature

Skin occlusion with wraps or impermeable plastic films prevents the loss of surface water from the skin and this causes increased level of hydration in the SC thereby decreasing the protein network density and the diffusional path length. This increases skin penetration. Occlusion of the skin surface also increases skin temperature by 2-3^oC resulting in increased molecular motion and skin penetration.

Biotransformation of drug in the skin
If the penetrating drug is subject to biotransformation during skin permeation (in fact, catabolic activity of the viable epidermis is substantial), local and systemic bioavailability can be affected drastically. This point was taken advantage of when Sloan and Bodor reportedly synthesized 7-acyloxymethyl derivative of theophylline that diffuse through the skin far more efficiently than theophylline itself but are biotransformed rapidly to theophylline [1]. Thus transdermal delivery of theophylline can be enhanced this way.

Dermal clearance of drug
Blood flow limits the absorption of the drug from the dermis. For instance, vasoconstrictor drug administered through other routes can significantly affect blood flow to the dermis hence dermal clearance of the drug into the general circulation.

4. Selection of drug/ vehicle for transdermal delivery
As stated earlier, the choice of drug is based on the drug meeting the criteria of proper physicochemical properties and high potency. In addition, the drug should be non irritant to the skin. Generally, drugs that have problems in their present route of administration can be considered for transdermal delivery [2].

Apart from the drug, the vehicle in which the drug is presented to the skin is very important in the transdermal delivery of the drug. In many clinical situations, the rate-limiting step is the diffusion of the drug from the formulation to the skin surface. Diffusion of the drug from its vehicle depend on the same diffusion parameters as given in Equation 1, hence there is need for appropriate formulation of the drug in order produce a transdermal product that has excellent controlled release profile.

The factors to be considered in the selection of vehicles for transdermal drug delivery include: stability of the vehicle; the vehicle should be such that it will not chemically react with the drug to alter the nature of the drug; the vehicle must be non-toxic or antagonistic to the host skin; vehicle should allow a regulated and proper diffusion and controlled release of the drug.

When a drug does not possess the ideal physicochemical properties, manipulation of the drug or vehicle to enhance diffusion becomes necessary.

5. Drug/vehicle modification for improved transdermal delivery
Drug modification approaches
Various approaches have been investigated for the modification of the drug molecule for improved drug delivery. Some of them are discussed as follows:

Pro-drug approach
This generally involves addition of a promoiety to increase partition coefficient and hence solubility and transport of the parent drug in the SC. Upon reaching the viable epidermis, esterases release the parent drug by hydrolysis thereby optimizing solubility in the aqueous epidermis [5].

Formation of ion-pairs

Charged drugs could be made to penetrate easily through the skin by formation of lipophilic ion-pairs. This involves adding an oppositely charged species to the charged drug, forming an ion-pair in which the charges are neutralized so that the complex can partition into and permeate the SC. These ion pairs dissociate in the aqueous viable epidermis releasing the parent drug which can diffuse within the epidermis and dermal tissues. A 16-fold increase in the steady- state flux of ibuprofen ion- pairs across a lipophilic membrane has been reported [4].

Drug complexation:

Complexation of drugs with cyclodextrins is another attempt at modification of drug molecules to enhance percutaneous absorption. Cyclodextrins of pharmaceutical relevance contain 6, 7, or 8 dextrose molecules (α, β, y- cyclodextrin) bound in a 1, 4 -configuration to form rings of various diameters. The ring has a hydrophilic exterior and lipophilic core in which appropriately sized organic molecules can form non-covalent inclusion complexes resulting in increased aqueous solubility and chemical stability.

Thermodynamic activity of the drug in vehicle

It has been reported that maximum skin penetration rate is obtained when a drug is at its highest thermodynamic activity as is the case in a supersaturated solution [4]. Supersaturated solutions can occur due to evaporation of solvent or by mixing of cosolvents or by the addition of co-excipients like phospholipids. However, the most common mechanism in clinical practice is evaporation of solvent from the warm skin surface which probably occurs in many topically applied formulations.

Eutectic systems

Eutectic system is a mixture of two components which, at a certain ratio, inhibit the crystalline process of each other, such that the melting point of the two components in the mixture is less than that of each component alone. Thus melting point of a drug delivery system can be lowered by formation of a eutectic system. The lower the melting point, the greater the solubility of a material in a given solvent, including skin lipids, and hence skin penetration. This follows from a regular solution theory. A number of eutectic systems containing a penetration enhancer as the second component have been reported, for example: ibuprofen with terpenes, menthol and methyl nicotinate; propranolol with fatty acids [4].

Vehicle modification approaches/formulations

In addition to the above methods investigated, a variety of encapsulating/carrier systems have been evaluated for possible delivery of drugs transdermally. These include, among others, liposomes, niosomes, ethosomes, and solid lipid nanoparticles.

Liposomes

Liposomes are colloidal particles formed as concentric bimolecular layers that are capable of encapsulating drugs. Phosphatidylcholine from soybean or egg yolk is the most common composition. Cholesterol is usually added to the composition to stabilize the structure thereby generating more rigid liposomes. Most effective liposomes are those composed of lipids similar to SC lipids, which are likely to most readily enter SC lipid lamellae and fuse with endogenous lipids.

Niosomes
Noisomes are vesicles composed of non-ionic surfactants. They have been evaluated as carriers for cosmetic applications and for transdermal delivery of a number of drugs with promising results [4].

Ethosmes:
Ethosomes are liposomes with high alcohol content capable of enhancing penetration to deep tissues and the systemic circulation. It is proposed that the alcohol fluidises the ethosomal lipids and SC bilayer lipids thus allowing the soft, malleable ethosomes to penetrate.

SLN and NLC
Solid lipid nanoparticles (SLN) have recently been investigated as carriers for enhanced skin delivery of sunscreens, vitamin A and E, triptolidine and glucocorticoids [6]. It is thought that their enhanced skin penetration is primarily due to an increase in skin hydration caused by the occlusive film formed on the skin surface by the SLN which reduces trans-epidermal water loss (TEWL).

Apart from a nonspecific occlusion effect on penetration, penetration might also be affected by the SLN and NLC (nanostructured lipid carriers) themselves, the high specific surface area of nanometer sized SLN and NLC facilitates contact of encapsulated drugs with the SC [7].

Microemulsion and nanoemulsion
Microemulsions and nanoemulsions are thermodynamically stable, transparent (translucent) dispersions of oil and water stabilized by an interfacial film of surfactant and cosurfactant molecules with microemulsion having droplet size of sub micron and nanoemulsion of the droplet size of less than 100 nm [8-9]. These systems are shown to improve drug solubility and bioavailability. Surfactants are necessary to reduce the hydrophobic interaction between the phases and maintain a stable emulsion [8]. Reports have also shown that co-surfactants (usually short chain alcohols) are necessary to maintain a single phase [10].

6. Chemicals methods for the modification of stratum corneum barriers

The above approaches have focused more on the modification of the drug or the vehicle (formulations) to enhance the drug penetration through the SC. However, there is variety of chemicals and methods to reduce barrier capability of the SC in order to promote skin permeation.

Chemical enhancers-ideal properties
Penetration enhancers are as substances that are capable of promoting penetration of drugs into or through skin, by reversibly reducing the skin barrier resistance. An ideal penetration enhancer should have, among others, the following properties [11-14]:

- It should be pharmacologically and chemically inert, and chemically stable, non-toxic, non-irritant.
- It should have a rapid onset of action, predictable duration of activity, as well as a reproducible and reversible effect.
- After it is removed from the skin, the SC should rapidly and fully recover its normal barrier property.

- It should have a solubility parameter similar to that of skin.

No substance has been found to possess all the ideal properties in spite of the fact that a variety of compounds have been proposed as skin penetration enhancers.

Classification of enhancers

Several classification systems have been used in the literature but classification of chemical enhancers based on their chemical structures can be considered as the most promising system in comparison with the other categorizations. Table 1 shows the types of chemical enhancers classified using functional groups.

Table 1. Types of chemical penetration enhancers classified by functional groups and chemical structure [11]

Types	Examples
Water	water
Sulfoxides and similar compounds	dimethylsulfoxide, dimethylacetamide, dimethylformamide
Pyrrolidones	2-pyrrolidone, *N*-methyl-2-pyrrolidone, 1-lauryl-2-pyrrolidone
Alcohols	ethanol, 1-octanol, 1-hexanol, 1-decanol, lauryl alcohol,
Glycols	propylene glycol, butane-1, 2-diol, polyethylene glycol 400
Urea and derivatives urea	1-dodecylurea, 1-dodecyl-3-methylurea,
Azone and derivatives	Azone (laurocapram; 1-dodecylazacycloheptan-2-one),
Enzymes	Acid phosphatase, calonase, papain
Iminosulfu	*S, S*-dimethyl-*N*-(5-nitro-2-pyridyl) iminosulfurane,
Cyclodextrins	2-hydroxypropyl-ß-cyclodextrin,
Fatty acid esters	cetyl lactate, butylacetate, isopropyl myristate
Fatty acid	alkanoic acids, oleic acid, lauric acid, capric acid
Surfactant	sorbitan monopalmitate, sodium lauryl sulfate
Terpenes	limonene, nerolidol, farnesol, carvone, menthone
Polymers	ß-*D*-gluco pyranosyl-terminated oligodimethyl siloxanes, 1-alkyl-3-ß-*D*-gluco pyranosyl-1,1,3,3-tetra methyl disiloxanes
Monoolein	monoolein
Oxazolidinones	4-decyloxazolidin-2-one, 3-acetyl-4-decyloxazolidin-2-one

Some of these classes of enhancers are discussed here.

Fatty acids

Fatty acids consist of an aliphatic hydrocarbon chain and a terminal carboxylic acid group. Fatty acids differ in their aliphatic chain length, which is either saturated or unsaturated, in the number, position, and configuration of double bonds and may have branching and other substituents. A wide variety of long chain fatty acids have a potential utility as skin permeation enhancers. Most studies on fatty acid penetration enhancers have focused on oleic acid {CH_3 $(CH_2)_7CH=CH$ $(CH_2)_7COOH$}, a monounsaturated fatty acid with a characteristic lard-like odor.

Fatty acids have the potential to cause skin irritation, which has led to limitation in their use. The extent of skin irritation depends on concentration and type of fatty acids used [15-17].

Surfactants

Surfactants play an important role in many products, including pharmaceuticals, cosmetics, and food formulations as solubilizers, detergents, wetting agents, adhesives, emulsifiers and

suspending agents [18]. They generally consist of a lipophilic alkyl or aryl chain together with a hydrophilic head group. They can be classified into four main categories according to the presence of formally charged groups in the head; anionic (e.g. sodium laurylsulfate), cationic (e.g. cetyltrimethyl ammonium bromide), nonionic (e.g. polyoxyethylene sorbitan monopalmitate) and amphoteric (e.g. *N*-dodecyl-*N*, *N*-dimethylbetaine) [19]. It is generally recognized that nonionic surfactants possess the least toxicity and skin irritation potential [20], and therefore they have been widely investigated as skin penetration enhancers. Generally, the investigation of enhancing abilities of nonionic surfactants has been focused on five principal series of surfactants, which are polysorbates, sorbitan esters, polyoxyethylene alkylethers, polyoxyethylene alkylphenols and poloxamers [19]. The enhancing ability of surfactants is governed by several factors, including their functional groups, hydrocarbon chain length, degree and position of unsaturation, physicochemical properties of permeants, nature of the vehicles, and whether the surfactants are used alone or in combination [21-23].

Terpenes

Terpenes have received considerable attention as penetration enhancers because they appear to have high percutaneous enhancing abilities, with low skin irritancy and low systemic toxicity [24]. Terpenes are a series of naturally occurring compounds that are composed of hydrocarbons and possibly oxygenated derivatives such as alcohols, aldehydes, phenols, ketones, oxides, and esters. They are frequently found in plant essential oils and are composed of units of isoprene, C_5H_8, in a head-to-tail orientation to form linear chains or rings [25]. Example of terpenes include *p*-menthane, menthone, and menthol, terpinen-4-ol, verbenone, carvone, cineol, geraniol, thymol, cymene, limonene, and nerolidol. The chemical structures of terpenes and the physicochemical properties of the drugs play an important role in the enhancing activity of terpenes, as described by Aqil *et al*. [26]. In terms of skin toxicity, most terpenes are generally recognized as safe (GRAS), a status granted by the U.S. Food and Drug Administration (FDA). Okabe *et al*., [27] has demonstrated the safety of terpens on the skin.

Polymers

A polymer is a large molecule consisting of the repetition of small, simple chemical units, called monomers [28]. Based on their sources, polymers can be classified into two types, synthetic'or natural polymers. The synthetic polymers include polyethylene glycol, polyvinyl alcohol, polypropylene etc. while the natural polymers include casein, gelatin, dextran and starch. Many of them have been investigated for transdermal delivery of drugs. Owing to their large molecular weight, polymers enhancers are mostly retained in the SC and do not significantly penetrate deeper into the skin. Therefore, side effects such as inflammation or skin irritation are limited [29].

Because of their safety, various types of polymers have been synthesized and investigated for their enhancing activity. These have included benzalkonium chloride and hexadecylpyridinium bromide type polymers [29], polyethylene glycol/polydimethylsiloxane (PEG/PDMS) block copolymers with a cationic end-group [30-31]. These polymers scarcely penetrated beyond the SC of the skin, hence they are safe.

Chemical retarders
Apart from the chemical enhancers, retarders have also been identified. Penetration retarders may be useful in formulation where it is advantageous to minimize systemic absorption, such as insect repellents and sunscreens. Examples of some retarders include some of Azone analogues.

Mechanisms for enhancer activity
Barry *et al*., have reportedly devised the lipid-protein partitioning (LPP) theory to describe the mechanisms by which enhancers effect skin permeability [4]. These include:
- Improvement of partitioning of a drug, co-enhancer, or co-solvent into the SC;
- Disruption of the intercellular bilayer lipid structure;
- Interaction with the intracellular proteins of the SC.

Lipid disruption by chemical penetration enhancers
Many enhancers, such as Azone, DMSO, alcohols, fatty acids and terpenes, have been shown to increase permeability by disordering or 'fluidising' the lipid structure of the SC [4]. The enhancer molecules form microcavities within the lipid bilayers hence increasing the free volume fraction and subsequent diffusion of the drug.

Interaction with keratin
In addition to their effect on SC lipids, chemicals such as DMSO, decylmethylsulphoxide, urea and surfactants also interact with keratin in the corneocytes. The interaction and binding with the keratin filaments may result in a disruption of order within the corneocyte which causes increase in drug permeability. The enhancer molecules may also modify peptide or protein material in the lipid bilayer domain to enhance permeability.

Increased partitioning and solubility in SC
A number of solvents increase permeant partitioning into and solubility within the SC, hence increasing P in Fick's equation (Equation 1). Such solvents include ethanol, propylene glycol, Transcutol® and N-methyl pyrrolidone.
Other mechanisms of enhancer action reported include [4]:

Hydration
Water is the most widely used and safest method to increase skin penetration of both hydrophilic and lipophilic permeants. Increased skin hydration could alter permeant solubility and thereby modify partitioning from the vehicle into the membrane and may also swell and open the structure of the SC leading to an increase in penetration. Hydration can be improved by occlusion with plastic films, paraffins, oils from emulsions etc. can improve hydration and hence penetration rate. Drug delivery from many transdermal patches benefits from occlusion.

Combined mechanisms
Fick's law (Equation 1) shows that a combination of enhancement effects on diffusivity (D) and partitioning (K) will result in a multiplicative effect. Synergistic effects have been demonstrated for many combinations of enhancers. Some enhancers act inherently by multiple mechanisms.

Generally, enhancement in the permeability of the drug can be achieved by altering any or all of the three parameters *D*, *K*, or *h* from the Fick's law. The improvement of the drug permeation

could be due to an increased diffusion within the skin, an increased partitioning or a decrease in diffusion path length. Some enhancers might affect the diffusion coefficient, whereas others might affect the partition coefficient or the diffusion path length while some enhancers might affect both diffusion coefficient and partition coefficient.

Enhancement ratio (ER)

This is a term used to describe the activity of an enhancer. It can be used to compare the activities of different enhancers which are used for the same drug in a similar condition.

ER = drug permeability coefficient or flux after enhancer treatment/drug permeability coefficient or flux before enhancer treatment.

7. Physical methods of penetration enhancement

Beside the chemical modifications of the drug, vehicle or SC, a number of physical methods of penetration enhancement have seen evaluated. These are as follows.

Iontophoresis

Iontophoresis is the process of enhancing the permeation of topically applied therapeutic agents through the skin by the application of electric current [32].The drug is applied under an electrode of the same charge as the drug, and an indifferent counter electrode is positioned elsewhere on the body. The active electrode effectively repels the active substance and forces it into the skin [33]. Other mechanisms have been proposed [34-35]. Transdermal iontophoresis has a wide application including treatment of diseases and extraction of analytes (such as glucose) from the body [36-37]

Electroporation

Electroporation is another electrical enhancement method which involves the application of short (microsecond or millisecond), high voltage (50-1000 volts) pulses to the skin. 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 processes such as diffusion and electrophoresis. Macromolecules that have been delivered by electroporation include: insulin,vaccines, oligonucleotides and microparticles [38].

Microporation

Microporation involves the use of micro needles that are applied to the skin so that they pierce only the SC and increase skin permeability. Micro needles are needles that are 10 to 200 µm in height and 10 to 50 µm in width [38]. Micro needles do not stimulate the nerves, so the patient does not experience pain or discomfort. They are usually drug coated projections of solid silicon or hollow, drug filled metal needles.

Heat

Heat enhances the skin permeation of drugs by increasing body fluid circulation, blood vessel wall permeability, membrane permeability, and drug solubility, thus facilitating drug transfer to the systemic circulation. When heat is applied, the kinetic energy of drug molecules, proteins, lipids, and carbohydrates is known to increase in the cell membrane. Also, drug solubility both in the patch and within the skin may increase with a rise in temperature. Heat may also cause

changes in physiochemical properties of patches, sweating, and increased hydration of skin, thus increasing the permeation of drugs [39].

Sonophoresis

Sonophoresis involves the use of ultrasonic energy to enhance skin penetration of active substances [40] especially at low frequency regimes (20 KHz < f <100 KHz). Ultrasound parameters such as treatment duration, intensity, pulse length, and frequency are all known to affect percutaneous absorption though frequency is the most important [Mitragotri, 2004]. The mechanism of transdermal skin permeation involves the disruption of the SC lipids by the formation of gaseous cavities, thus allowing the drug to pass through the skin [41]. Several antibiotics have been delivered through this technique [42].

Needleless injection

This technique involves firing the liquid or solid particles at supersonic speeds through the SC [43]. It is a pain-free method of administration of drugs to the skin. The mechanism involves forcing compressed gas such as helium or nitrogen through the nozzle with the resultant drug particles entrained within the jet flow, reportedly traveling at sufficient velocity for skin penetration [44].

Pressure wave

Pressure waves generated by intense laser radiation, can render the SC as well as the cell membrane permeable to molecules. Pressure wave is only applied for a very short time (100ns-1µs) and it is thought that the pressure waves form a continuous or hydrophilic pathway across the skin due to expansion of lacunae domains in the SC. Application of pressure waves does not cause any pain or discomfort and the barrier function of the SC always recovers. Insulin and caffeine have been successfully delivered through this technique [45-46].

Magnetophoresis

The term, "magnetophoresis" was used to indicate application of a magnetic field and acts as an external driving force to enhance drug delivery across the skin. It induces alteration in the skin's structure that could contribute to an increase in permeability [47].

Radiofrequency

Radiofrequency involves exposure of the skin to a high frequency alternating current of 100 KHz that results in the formation of heat-induced micro channels in the cell membrane. The drug delivery rate is controlled by the number and depth of micro channels formed, which depends on the properties of the microelectrodes in contact with the skin during treatment [48]. Granisetron HCl has been delivered by this means [49].

Most of the above devices are still been investigated for possible clinical application in transdermal delivery of many drugs which otherwise cannot penetrate the skin e.g. charged species like amino acids, macromolecules etc. These new transdermal formulation technologies have changed the paradigm that there are only a few drug candidates for transdermal drug delivery.

8. Forms of transdermal products

This section gives an overview of the different forms in which the final transdermal product can be presented to the patient. In practice, the forms of transdermal products include: transdermal patch, ointments, colloidal formulations such as transdermal gel, emulsions, liposomes. Others are electrically modulated devices, and mechanically modulated devices.

While electrically and mechanically modulated devices have been discussed in earlier sections, transdermal patches are discussed here.

Transdermal patches

The major products currently marketed for transdermal absorption are the patches or the transdermal drug delivery systems (TDDS). The emergence of adhesive TDDS permitted skin residence times to increase from hours to days quite unlike ointments or gels. The matrix or reservoir formulations employed in these TDDS also provided for the maintenance of relatively uniform concentrations of diffusible drug to ensure relatively constant drug release rates. Skin occlusion from water-impermeable backing film of TDDS further facilitates TDDS systemic efficacy by increasing skin hydration and temperature with a corresponding increase in the rate and extent of skin permeation. The inclusion of skin penetration enhancers also serves to increase transport across the skin.

The functional parts of a patch, proceeding from the visible surface inward to the surface apposed to the skin are:-

a. an impermeable backing
b. a reservoir holding the active ingredient, together with release- controlling materials
c. an adhesive to hold the patch in place on the skin
d. a protective cover that is peeled away before applying the patch.

Most patches belong to one of two general types -the reservoir system, and the matrix (or drug-in-adhesive) systems.

Reservoir system

In the reservoir system, the drug reservoir is embedded between an impervious backing layer and a rate controlling membrane. The drug releases only through the rate-controlling membrane which can be micro porous or nonporous. In the reservoir compartment, the drug can be in the form of solution, suspension, or gel or dispersed in a solid polymer matrix. The scopolamine patch is an example of reservoir type.

Matrix system

In the matrix type, the active ingredient is dispersed entirely in the adhesive. Estradiol patch (Climara ®) is a typical patch of the matrix type. Another example for this type is Transtec ®, bupremorphine containing slow-release matrix patch for the treatment of intermediate to severe pain. The patch is able to control the drug delivery rate and produce stable plasma concentration [50].

9. Nature of transdermal studies

Transdermal studies can take the form of determination of partition coefficients and prediction of permeation constants from the values obtained. It can equally involve the actual permeation

studies either by *in vitro* or *in vivo* techniques to determine: (a) the permeation profile of the permeating chemical (b) the effect of the chemical enhancers or formulations or physical methods on the permeation profile of the permeant (c) the mechanism of action of the enhancers (d) the skin toxicity of the enhancers.

Prediction of permeation constants from partition coefficients

Permeability experiments have shown that hydrated SC has an affinity for both lipophilic and hydrophilic compounds. The bifunctional solubility arises from the hydrophilic corneocytes and the lipid- rich lamellar structures in the intercellular space. Thus attempts to predict permeability constants from oil: water or from solvent: water partition coefficients have had limited success [1]. There is therefore, need to carry out actual percutaneous absorption studies to ascertain the permeability profile of the drug being investigated.

In vivo transdermal studies

The last phase in the transdermal absorption studies of a drug is the *in vivo* clinical studies on humans. The conduct of *in vivo* studies in volunteers is closely regulated (Declaration of Helsinki, 1964 as amended in 2004; the ICH Guideline for Good Clinical Practice, 1996) [11]. The study protocol should be approved by an ethical committee and the subjects have to give written informed consent.

However, before this stage, *in vivo* studies are carried out using experimental animals. Any evaluation of a study of percutaneous absorption in animals must take cognizance of species variation. A group of researchers [1] has investigated percutaneous absorption and found a decreasing order of permeation, thus, rabbit>rat> swine>man. Nevertheless, other studies have shown that that data from these lower animals are comparable to that of man [1] even though such studies in animals either *in vivo* or *in vitro* can only be useful approximations of activity in man. Thus most preclinical transdermal studies make use of small animals such as rats, mouse because of their availability and ethical consideration in human volunteers and human skin.

In vivo methods/techniques

Several methods are available for *in vivo* percutaneous studies. These are discussed below.

Plasma/ excreta measurement

In the *in vivo* studies, the substance is applied during a certain period to a specified area of the skin site of volunteers/animal. The percutaneous absorption can be assessed by analyzing the parent chemical and/or its metabolite(s) in the skin layers or in biological media such as plasma, urine or exhaled air [51-54]. The amount of a chemical measured after dermal exposure is compared to that after a reference exposure with a known dose via, for example, intravenous administration or inhalation.

Microdialysis:

Microdialysis (MD) is a technique that measures a dermally applied chemical in the extracellular space beneath the exposed skin site. The principle is based on the passive diffusion of a chemical across the semi permeable membrane of a MD probe that is introduced into the dermis parallel to the exposed skin surface. The probe is slowly perfused with a tissue-compatible sterile buffer (the perfusate) which mimics the blood flow. Molecules able to pass the probe membrane will

diffuse across the membrane into the perfusate, which is collected at timed intervals for the analysis [55-56].

Tape stripping method (TS)

In vivo studies in recent years have made use of the skin-stripping method, which permits the estimations of the concentration or amount of the penetrating species as a function of depth of the SC. Generally a predetermined area of the skin is exposed to a chemical for a certain period of time. At the end of exposure, SC layers are removed sequentially by cellulose adhesive tape strips. The amount of recovered substance in each tape strip is determined with an appropriate analytical technique. It has been reported that the amount of the chemical in these SC layers gave good estimate of the total amount of the chemical absorbed into the systemic circulation [57-58].

Spectroscopic methods

A variety of spectroscopic methods have been applied in studying *in vivo* penetration of chemicals, and the vast majority of them are based on IR and Raman vibrational spectroscopy [59-60]. Presently, Fourier Transform Infra Red Attenuated Total Reflection (FTIR-ATR) is the most used spectroscopic technique for studying penetration kinetics, and different skin analyzers based on this spectroscopic technique have become commercially available. In these devices, an IR beam is emitted through an IR transparent crystal, which is in contact with the skin sample. During passage through the crystal, IR beam penetrates into the sample before returning. As a result, the radiated skin absorbs IR at frequencies corresponding to absorption spectrum of the substance [61]. A penetrating chemical can be quantified by measuring IR spectrum directly on the skin [62]. FTIR-ATR has been extensively used to quantify the absorption of various drugs, pesticides, and other chemicals [63].

In contrast to FTIR-ATR techniques, Raman spectroscopy represents a more recent *in-vivo* approach for non-invasive determination of percutaneous penetration of chemicals. Development of Confocal Raman Microspectroscopy (CRM) is of particular importance for wider application of this technique.

In vitro/ permeation studies

Actually, the evaluation of new chemical entities (NCEs) and other drugs of which their toxicities are indeterminate mandates their *in vitro* testing. The studies of Scheuplein and Rose and of Franz [1] demonstrate that *in vitro* studies of transdermal absorption under controlled conditions are relevant to *in vivo* drug penetration. It would be advantageous to use human cadaver skin for *in vitro* permeation studies but, in practice, for most investigators, human cadaver skin is not readily available. Thus animal skin is frequently used for *in vitro* studies. Weanling pig skin (skin from a pig that has recently been weaned) is recognized as the closest alternative to human cadaver skin in its permeability and lipid composition.

The equipment frequently used for the *in vitro* permeation studies is the diffusion cell. Several types of diffusion cells made out of glass, stainless steel or Teflon® have been designed. These include vertical diffusion cells (side-by- side), Franz diffusion cells and flow-through diffusion cells. Among these three types, the Franz cell is the most popular model for studying the diffusion of permeant across the skin. In this system, the intact skin or the epidermis is treated as a semi permeable membrane separating two fluid media. The transport rate of a particular drug is

evaluated by introducing the drug in solution on the SC side of the membrane, then measuring penetration by periodic sampling and analysis of the fluid across the skin membrane. Permeant analysis could be done using suitable analytical techniques which include: high performance liquid chromatography (HPLC), liquid scintillation counting (if radiolabelled drug is available) and ultraviolet (UV) or fluorescence spectroscopy.

Properties of the receptor solution such as temperature and buffer composition can have significant effect on drug permeation through the skin. Typically, physiological saline or a phosphate-buffered solution maintained at 37 □C is used, which simulates the temperature of the human skin. Generally, antibiotics and preservatives are added to the receptor solution to prevent microbial growth, enzymatic degradation and to stabilize the skin.

Permeation studies using the diffusion cell can be exceptionally labor intensive, time consuming, and costly. As a result, novel techniques have been developed and proved to be valuable in identifying new potential skin penetration enhancers. These techniques include high-throughput methods [64] and electrical resistance-based methods [65]. These two techniques can identify the potential chemical enhancers by determining the changes in electrical conductance of the skin or the changes in electrical resistance of the skin, respectively. The effects of chemical enhancers on the barrier properties of the skin can be elucidated by measuring these electrical changes in the presence of potential penetration enhancers. It has been have demonstrated that the high-throughput technique was over 100-fold more efficient than the more commonly used Franz diffusion cell method in the discovery of penetration enhancer mixtures [11].

Concern with the use of excised skin

One concern in the use of excised skin, whether animal or human is the variability in barrier properties of excised skin. Factors responsible for this variability are the source and characteristics of the donor skin e.g. elapsed time from death to harvesting of the skin, age and gender of the donor, health of the skin prior to the donor's death, exposure of the skin to chemicals or mechanical treatment (e.g. shaving or clipping prior to harvesting of the skin) etc. Also it has been shown that there is a regional variation on skin permeability such that for diffusion of a simple molecule through the skin: planter<palmar<arms<legs, trunk, dorsum of hand <scrotal and post auricular<axillary<scalp [1]. These factors are considered in carrying out transdermal studies using the excised skin.

Skin models

Apart from the animal or human skins, artificial skin models have been developed for percutaneous studies. These include:-

Living skin equivalents and epidermal equivalents

Recently, the use of living skin equivalents and epidermal equivalents has become popular for transdermal permeation and *in vitro* toxicity studies. These comprise a bilayered system of human dermal fibroblasts in a collagenous matrix upon which human coenocytes have formed a stratified epidermis. These skin equivalents have many advantages, including the ability to eliminate animal experimentation. Also, they use human skin cells, which provide skin properties similar to those found in native human skin. All the lipids found in the native human skin are found in skin equivalents, but in reduced quantities. These skin models have diverse

permeability characteristics depending on the tissue culture protocols. A group of researchers compared the penetration of drugs through a living skin equivalent, Wister rat skin, and human cadaver skin and found the following order: living skin equivalent> Wister rat skin> human cadaver skin[66-67].

Other artificial membranes

Besides the above skin models, polymeric substance and other artificial membranes have been used for transdermal experiments even through these membranes lack the complex histological structures present in the human skin.

Use of computer for percutaneous studies

Advances in the use of computer have given rise recently to *in numero* modeling or computer simulation of percutaneous absorption. Here, with the aid of a computer, the formulator can anticipate the effect of variables such as the thickness of the applied (vehicle) phase, alterations in drug partitioning between the vehicle and the SC, and the frequency of reapplication on the overall appearance of drug systemically as a function of time following topical application [1].

Methods for studying mechanism of action of enhancers

Effects and mechanisms of action of chemical enhancers have been investigated using a variety of techniques. These include permeation studies, vasoconstrictor assay, differential scanning calorimetry (DSC), infrared spectroscopy, X-ray diffractometry, and electron spin resonance spectroscopy (ESR). Permeation studies have been discussed in section previous section.

Differential Scanning Calorimetry (DSC)

A typically DSC thermal profile of hydrated human SC is composed of four major endothermic transitions, namely T_1, T_2, T_3, and T_4. The endotherm T_1 (35-42 □C) is attributed to the melting of lipid/fat contamination of the samples and is not important in elucidating the mode of action of chemical enhancers [11]. The endotherms T_2 (60-77 □C) and T_3 (70-90 □C) are attributed to the melting of bilayer lipids, whereas the endotherm T_4 (95-120 □C) is associated with protein conformation [68-70]. Differences between lipid transition temperatures of humans and animals have been reported [70].The interaction between the chemical enhancers and the skin can be examined by measuring the thermal transitions in the presence of the enhancers. The mode of action of a variety of chemical enhancers (e. g. surfactants and terpenes) has been evaluated by the DSC technique [71].

Fourier transform infrared spectroscopy

Apart from its use *in vivo* evaluation of the effect of chemical enhancers on human volunteers, FTIR spectroscopy can also be useful for studying the interaction between chemical enhancers and the SC in excised animal skins. Many of the infrared (IR) spectral bands of the SC can be attributed to the lipid or protein molecular vibrations. Some spectral regions of interest are the IR peaks near 2850 cm^{-1} and 2920 cm^{-1} owing to symmetric and asymmetric methylene group (H-C-H) stretching, respectively. It has been suggested that the main contribution to the C-H stretching peaks of the SC was the absorbance of the hydrocarbon chains of the SC lipids [68]. The FTIR spectral parameters that can be used as indicators of relative lipid acyl chain disorder are a blue shift of C-H stretching absorbances, a bandwidth at 70% height of the C-H stretching absorbances [71] and a ratio of the intensities of the C-H asymmetric and symmetric absorbances

[72]. Furthermore, the heights and areas of these C-H asymmetric and symmetric stretching absorbance peaks correspond to the amount of the lipids present in the SC [11]. Thus any extraction of the SC lipids by chemical enhancers results in a decreased peak height and area of these absorbances [73].

While changes in the IR spectrum provide information at the molecular level, transitions in the DSC thermal profile provide information at the macroscopic level. These two techniques can provide independent and complementary information about the structure of the SC [69,74].

Electron spin resonance spectroscopy (ESR)

Electron spin resonance spectroscopy (ESR) is a form of absorption spectroscopy used for studying a variety of biological membranes. Like most biological membranes, the SC has no paramagnetic components. Thus, a molecule with a stable paramagnetic group, known as spin-labeling agent, has to be specifically incorporated with the lipid or the lipid part of the biological membrane [11]. Generally, 5, 7, 12, and 16-doxyl stearic acids, which are fatty acids with a nitroxide free radical group, have been used as lipid spin-labeled reagents [75]. ESR technique can provide information about phase transitions and polarity of microenvironments surrounding the spin labels. In addition, molecular interactions within the SC can be obtained by using this technique. The action of chemical enhancers on the SC can be examined by measuring changes in the ESR spectra of membrane-incorporated spin labels. ESR has been used to investigate the mechanism of action of several penetration enhancers in human SC such as Azone [75] and surfactants [76].

X-ray diffractometry

Small and wide angle X-ray diffraction is valuable as a tool for studying molecular interactions and packing of molecules within the SC. It provides information about the structure and organization of biological lipid assemblies. X-ray techniques have been used to elucidate the mode of action of a variety of chemical enhancers. These include terpenes, Azone, and its derivatives [77].

Vasoconstrictor assay

This is a vasoconstrictor or blanching test previously used to evaluate the activity and bioavailability of corticosteroid formulations but presently has been applied for study the action of chemical enhancers. The test has been used for drugs that can elicit a local vasoconstriction effect, and therefore only a limited number of drugs (e. g. corticosteroids) can be assessed by this means [78-79]. As with FTIR spectroscopy, the blanching test can be applied to *in vivo* evaluation in human volunteers [11].

Methods for skin toxicity testing

The potential toxicity of some chemical enhancers limits their uses in dermatological or cosmetic preparations. The skin may interact with the chemical enhancers to induce several skin responses, such as irritation, rashes, and inflammation when penetrating through the viable epidermal layers of the skin. The skin irritation potential, and possible damage produced by the application of the chemical enhancers can be assessed by several techniques, including *in vivo* Draize test method, *in vivo* histopathological examination, *in vivo* laser Doppler velocimetry, *in vivo* bioengineering methods such as transepidermal water loss (TEWL) and electrical

capacitance [11]. The *in vivo* methods can be carried out in human or animal models. The main problem of human testing is the high cost. In the past decade, animal testing has been criticized by animal-rights activists for being inhumane. Thus *in vitro* cell culture techniques have been developed as alternative procedures for assessing the skin irritation responses. Several high quality culture systems (i.e. EpiDerm and Episkin) have been constructed and evaluated [11, 80].

CONCLUSION

The delivery of drugs to the systemic circulation through the skin has been shown to be a convenient means of drug administration. This review has presented several facets of this drug delivery route and the various studies involved. It is hoped that many current drugs as well as those that have been abandoned due to problems associated with their delivery route could be considered for possible transdermal delivery based on the information presented in this review article.

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