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Review Article

SKIN KINETICS AND DERMAL CLEARANCE

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ABSTRACT

Availability of several therapeutic and cosmetic formulations for topical application has made the research on skin kinetics as a topic of current interest. Topical formulations are typically meant for local effect although there is always a chance that the low molecular weight chemicals are easily transported across the skin layer and make it available in the systemic circulation. Thus there is a major concern about the transport of chemical moieties following the topical application of cosmetics and therapeutic formulations and the real time measurement of the molecules in the skin layer has become obligatory. It is well known that the properties of both drug and the excipients have identical role in determining the skin permeability of chemical moieties. In the last decade several investigations have been carried out in this filed using several in vitro and in vivo models. This review provides a brief account on the basics of skin kinetics, parameters assessed, various techniques and methods adapted in skin kinetic studies. Moreover, we have also discussed about the micro-environment inside the skin layer and the possible mechanism of drug depot formation, skin metabolism and clearance of molecules from the skin layers.

Keywords: Dermatopharmacokinetics; Topical formulation; Stratum corneum, Permeability

INTRODUCTION

Several therapeutic and cosmetic formulations are applied on the skin surface to attain local effects. However, the major constrain is all about the permeability of chemicals from these topically applied formulations, as the skin being a permeable barrier. The pathways for the movement of drug molecules into the skin layers are well known. Development of drug delivery systems has introduced quite a few novel formulations in to the market, with the objective of enhancing the topical bioavailability[1]. Novel vesicular formulations such as microemulsions, liposomes, and nanoparticles have demonstrated the potential to enhance cutaneous drug delivery of both lipophilic and hydrophilic drugs. Alternatively, the possibility of targeting molecules in the skin layer is also being investigated. It is understood that the properties of both formulation and drug are equally important in deciding the movement of drug in the skin layers. Hence, it is likely that the individual drug molecule and excipient could exhibit different permeability behaviour[2]. In addition, presence of enzymes in skin causes alteration of the structure of the molecules and alters the topical bioavailability as well. Hence, the assessment of topical bioavailability remains unavoidable[3]. In this context, researchers in the last few decades have given much emphasis on the assessment of skin kinetics of topical formulation. An ideal method would be a technique allows non-invasive determination of rate and extent to which an active molecule attains its site of action on or within the skin. However, one should remember that the measurement of drug from the skin layers (target area) could be a difficult task.

Regulatory agencies like U.S. Food and Drug Administration (FDA) have been exploring different techniques to characterize drug's dematopharmacokinetics (DPK). Mathematical models have been designed to predict the topical bioavailability of the formulations. Numerous in vitro and in vivo approaches have been assessed to measure the topical bioavailability. Few approaches have been designed to measure the real time measurement of molecules in the skin layer. The fate of molecules including depot formation, clearance etc from the skin layers are being investigated. Recently, microdialysis technique has been introduced to dermatological research as a valuable *in vivo* tool[4,5]. This article discusses the issues and recent updates in skin kinetics studies and dermal clearance of topical formulations.

STRUCTURE OF HUMAN SKIN

Human skin is an extraordinary organ that allows terrestrial life by regulating water and heat loss from the body and at the same time preventing the entry of harmful chemicals or microorganism[6]. The structure of skin is well known; however a brief description of this barrier highly in relation to skin kinetics is provided herein. Skin covering an area of about 2 m^2 in an average human adult. It receives approximately 1/3 of all blood circulating through the body. Skin with a thickness of few millimetres separates the underlying blood circulation network from the outside environment. Anatomically, the skin has three major tissue layers: the epidermis, dermis and hypodermis[7]. The epidermis is the outermost layer of the skin and comprises of stratified keratinised squamous epithelium varies in thickness in different regions of the body[8]. Epidermis consists of different layers such as stratum corneum, stratum lucidum, stratum granulosum, stratum spinosum and stratum germinativum. Stratum corneum acts as a rate limiting barrier which restricts or control the inward and outward movement of chemical substances. It consisted of 15-20 layers of acutely flattened, metabolically inactive, polygonal cells having a dry weight density of 1.3-1.4 g/cm^3. Epidermis has no blood vessels or nerve endings, but its deeper layers are bathed in the interstitial fluid from dermis, which provides oxygen and nutrients and is drained away as lymph[6].

Dermis is tough and elastic, about 3-5 mm thick, consists of a matrix of connective tissue woven from fibrous proteins[8]. Dermis essentially consists of 80% of protein[7]. Collagen fibres bind water and give the skin its tensile strength[6]. Branches from the arterial plexus supply blood to sweat glands, subcutaneous fat, hair follicles and dermis. Sweat glands are found all over the skin and most numbers in the palms of soles of the feet, palms of the hand, axillae and groins. Hair follicles are found all over the skin surface except soles of the feet, palms of the hand, red part of the skin, and selected portion of the sex organ[8].

ROUTES OF DRUG PERMEATION THROUGH SKIN

There are three major routes by which skin absorption may occur (Figure1). Primarily, the chemical moieties are transported through the keratin-packed corneocytes via partitioning into and out of the cell membrane (transcellular). Secondly, the molecule is transported around the corneocytes in the lipid rich extracellular regions (intercellular). Thirdly,

the shunt transport supported by the sweat glands, sebaceous glands and hair follicles (transappendageal)[9].

The permeation potential of a permeant through intercellular or transcellular routes is highly dependent on their relative ability and partitioning in each phase. Thus, the hydrophilic and lipophilic molecules follow separate pathways to transport across the skin layers[10,11]. Further, the non-ionic and lipophilic compounds are easily permeated. Alternatively, skin appendages such as sebaceous gland, hair follicles and sweat glands act as a diffusional shunt through rate-limiting barriers, facilitating the absorption of topically applied molecules. Transappendageal absorption may be a dominant pathway of dermal permeation, in case of slowly diffusing molecules[10,12].

TRANSPORT MECHANISMS

Passive diffusion is the major process of absorption of drug molecules into the skin. The rate of drug transport across the skin layers obeys Fick's Law of Diffusion. Diffusion process stops when the concentration gradient reaches zero[13]. The following equation describes the drug flux following passive diffusion

$$J = \frac{DPA\Delta C}{h}$$

Where, J is the steady state flux across the stratum corneum, D is the diffusion coefficient / diffusivity of drug molecule (cm^2/sec), ΔC is the concentration gradient of drug across the stratum corneum (g/cm^3), P is the partition coefficient of drug between skin and formulation, h is the thickness of stratum corneum (cm), A is the surface area of stratum corneum (cm^2). According to this equation, the rate of drug passage depends upon its aqueous solubility, directly proportional to its partition coefficient (oil/ water), concentration of drug in formulation and the exposed surface area of the skin; and inversely proportional to the thickness of the skin. In reality, there is low concentration on the receiver side due to continuous blood stream. Thus, the donor side have comparatively high drug concentration; equation becomes[14]

$$J = P_M AC$$

Where, P_M is the permeability constant, C is the concentration of drug at the absorption site. However, the P_M can be determined by

$$P_M = \frac{DP}{h}$$

FACTORS AFFECTING PERCUTANEOUS ABSORPTION

Many factors govern the delivery of drugs and cosmetics into the skin from topically applied formulations. These include the size of the molecule, the lipophilicity of the component, type of formulation, presence of penetration enhancers, and physical state of the stratum corneum etc and are discussed below.

Molecular weight (size)

Percutaneous absorption is inversely proportional to molecular weight and particularly affects the diffusion coefficient. Molecules of size larger than 500 Daltons usually have more difficulty to penetrate through the stratum corneum[15].

Partition coefficient

For optimal permeability lipid/ water partition coefficient of 1 or greater is generally required. It may be altered by some chemical changes without influencing the pharmacological activity of the drug.

pH

Application of formulation which have very high or very low pH values can be destructive to the skin. With moderate pH values, the flux of ionisable drugs can be affected by changing the pH which alters the ratio of uncharged and charged molecules and their permeability.

Penetrant concentration

A proportional increase in flux is observed by increasing the concentration of the dissolved drug. At higher concentration than the solubility excess solid drug acts as a reservoir and maintains a constant drug level for a prolonged time[16-19].

Electronic structure and dissociation constant (pK_a)

Highly ionised molecules do not permeate very much.

Hydration

Skin occlusion with impermeable plastic films or wraps prevents the water loss from the skin surface and this causes increased level of hydration in the stratum corneum thereby decreasing the diffusional path length and protein network density. This increases skin penetration. Occlusion decreases in following order: occlusive film = transdermal patches > lipophilic ointments > w/o cream > o/w cream.

Temperature

For a large temperature variation, the penetration rate of material through skin change 10 fold, since diffusion coefficient decreases as temperature falls. Occlusive vehicles increases skin temperature by 2-3^{0}C resulting in increased molecular motion and permeability[20].

Skin metabolism

Skin can metabolize chemical carcinogens, steroid hormones and some other drugs. The therapeutic efficacy of topically applied compounds particularly prodrugs and carcinogenic responses in the skin may be determined by such metabolism. Skin can metabolize some 5% of topical drugs[8].

Release characteristics

Drug solubility in the vehicle determines the rate of release. The following factor affects the drug release mechanism:

- Partition coefficient of drug from delivery system to the skin[16-19].
- pH of the vehicle- Only non-ionized molecules are able to diffuse through the lipophilic intercellular regions of the stratum corneum. Ionized forms have been reported to permeate through the intercellular regions of the stratum corneum. Moreover, ions present in the skin and compound ions may form pairs which can lead to formation of neutral compounds[21].
- Whether the drug is suspended or dissolved in the delivery system.

Composition

The drug delivery system composition includes boundary layer, polymer, thickness, and vehicles affect not only the drug release rate but also the stratum corneum permeability by means of hydration, making with skin lipids or other effects which promotes the sorption[21].

PARAMETERS ASSESSED IN KINETICS STUDY

Evaluation of DPK of drug involves assessment of maximum quantity of drug active molecule in the stratum corneum (C_{max}), time to reach maximum level (T_{max}), and area under the curve (AUC)[22]. DPK method measures drug levels of topically applied formulation in the outer most layer of the skin (stratum corneum), in vivo as a function of time post application and post removal of the formulation, so as to generate stratum corneum concentration versus time profile from which maximum amount in the stratum corneum (C_{msx}), time to reach maximum concentration (T_{max}) and area under

the curve (AUC) can be obtained. The stratum corneum is removed by the repeated application of adhesive tape providing a minimum invasive technique to determine the drug concentration within the skin. The DPK method assumes that: (a) stratum corneum is the rate-limiting barrier to percutaneous absorption, (b) drug concentration in the stratum corneum directly related to drug's diffusion into the viable dermis and, (c) stratum corneum drug concentrations are more relevant for estimating local, dermatological efficacy than plasma concentrations[23]. It has also been possible to deduce drug's portioning and diffusion parameters which characterize the absorption process and which can subsequently be used to predict an entire absorption profile from a single short contact duration experiment[22].

TECHNIQUES AND METHODS USED IN SKIN KINETICS STUDIES

There are number of in vitro, in vivo methods of pharmacokinetic assessment of drug molecules which include;

- In Vitro Permeation Assessment
- Tape Stripping
- Microdialysis
- Vasoconstrictor Assay
- Confocal Laser Scanning

In vitro permeation assessment

The in vitro permeation experiments are performed to screen and optimize the topical formulation. *In vitro* measurements are useful in comparing the diffusion rates of different compounds and in obtaining concept of rate of transepidermal passage of substance prior to *in vivo* tests. It also provides a better understanding of the factors that affects percutaneous absorption *in vivo*[24]. The evidences suggest that rate of drug permeation from their formulations and temporal profiles may be similar[25,26]. The skin permeation studies are carried out in vertical Franz diffusion cell thermostated at 37^0C (Figure 2). The hairs of excised tissue are trimmed as close as possible to the skin surface. Then it was clamped between the donor and receptor compartment. The receptor compartment consisted of physiological buffer (pH=7.4) and the donor compartment with the formulation and covered[24]. The passage of test substance through the skin is determined either by the loss of test substance from one compartment, by its appearance in the other or by both combined methods.

Tape stripping technique

Tape stripping of stratum corneum is a quick and relatively non-invasive approach to quantify the amount of drug absorbed into the skin. Tape stripping is carried out by successively applying an adhesive tape strip onto the skin surface (Figure 3). Gentle force is applied to assure good contact and consequently the tape is removed by an upward movement[23]. The estimation of transepidermal water loss before the first tape strip and after a set number of tape strips using Aquaflux evaporimeter is used to determine the exact number of tapes applied. If TEWL attained 8 times the baseline value, the tape stripping is stopped. Eight times increase in TEWL value indicates approximately complete removal of the stratum corneum[27]. The tape strips need to be extracted to recover and assess the amount of absorbed drug.

Tape stripping is applied in various areas of cutaneous biology such as (a) to determine the barrier function, (b) to check out dermatophatologies (xerotic conditions, inflammatory, neoplastic disorders), (c) to observe gene expression, (d) to check pH profiles, (e) to inspect animal skin as a surrogate for human skin, (e) to assess the drug's local bioavailability whose target site is stratum corneum etc. The method is used to assess the drug or excipients level in the skin after topical dermatological application, either in the removed tape strips, or directly in the tape stripped skin. It presents an alternative for local bioavailability or bioequivalence assessment of topically applied drugs whose target is the underlying viable tissues. The fundamental resistance to the penetration of topically applied compounds is stratum corneum. It has been argued that drug level in the barrier should be correlated with those attained in the underlying viable components of the skin (sites of many dermatological diseases are manifest)[28].

Draft guidance published by US FDA in 1998 in which steps for conducting bioavailability/ bioequivalence study were described. The guidance included the following steps[28]:

- A drug product needs to be applied for given time, following application the excess of formulation should be removed by an appropriate method.
- Two consecutive tape strips of stratum corneum should be taken and rejected.
- Consecutively, 10 tape strips shall be taken from the application site, combine and extract with a solvent.
- Multiple sites should be used to perform the entire process following different time of application (absorption phase) and different period between longest application time and the time at which stripping is carried out (elimination phase).
- Total amount of drug in the stratum corneum is indicated as a function of time as called dermatopharmacokinetic profile, characterized by maximum amount, time at which maximum amount obtained and an area under the curve (Figure 4).
- Comparison of two formulations of same drug simultaneously, would allow a determination of local bioequivalence using the dermatopharmacokinetic parameters.

Microdialysis

Recently, microdialysis technique has been introduced to dermatological research as a valuable *in vivo* tool[4,5]. This technique can be used for determining endogenous and exogenous compounds in the extracellular spaces of tissues and for assessing pharmacokinetic and pharmacodynamic response[27]. This method is minimally invasive and enables assessment of local pharmacokinetic profile of topical drug penetration from each sampling site[4,29,30]. This technique consisted of placing an ultrathin, semipermeable hollow fibre structure (probe) in the dermis with a sterile buffer at a very slow rate through a microdialysis pump. In the dermis, the probe functions as an artificial vessel and thus exchanges small diffusible molecules from the probe to the tissue and vice versa according to law of simple diffusion[31]. Compounds can be separated or can be added to extracellular space by diffusion through dialysis membrane[27]. Samples obtained are protein free and easily analysed without any further analytical purification. Partitioning of drug molecule between perfusate and extracellular fluid depends on the composition of the perfusate and the hydrophilic/lipophilic properties of the extracellular fluid the microdialysis fibre. Recently, it has also been seen that microdialysis applied to assess the cutaneous drug delivery using iontophoresis; technique appears to be promising tool as iontophoresis mostly used with ionised and highly polar molecules[32]. However, relatively large difference in individual drug levels were observed in between individuals and between microdialysis

probes. Recovery of substance is influenced by drug clearance from the tissues surrounding the microdialysis membrane that is diffusivity of substance and capillary blood flow around the probe and partition coefficient between perfusates and tissues. These factors may subject to changes both during an experiment and between application sites[4,33].

Since the first report of dermal microdialysis, this technique has been applied successfully in human volunteers to study the cutaneous release of histamine in response to different topical stimuli and the penetration of a number of organic solvents (ethanol and isopropranol) to measure inflammatory mediators in dermis, to study skin metabolism, to determine the drug absorption or other agents in skin and as an alternative route of drug administration[34]. Currently microdialysis exercised in the field of clinical research for the monitoring of secondary ischemia in neurointensive care or glucose monitoring for long term metabolic control in patients with diabetes mellitus. It can give very detailed chronological pharmacokinetic data and simultaneously several sampling sites can be considered in the same volunteer. Sampling of very lipophilic and highly protein bound drugs can produce problems due to their low recoveries with this technique.

Vasoconstrictor assay

Human Skin Blanching Assay (HSBA) also known as vasoconstrictor assay is the original approach and well established surrogate method, used to assess topical products involve clinical studies in patients to assess the safety and efficacy. But this method is restricted to the bioequivalence assessment of topical corticosteroid formulations[35]. Vasoconstrictor assay method followed the staggered application with synchronized removal method of FDA Guidance Topical Dermatological Glucocorticoids – In vivo Bioequivalence[36]. A Hill Top Chamber of 1.2 cm is used for the vasoconstrictor assay. The system is affixed to the skin surface with adhesive tape and specified volume of formulation or sample was applied in a 1.2 cm diameter area of the chamber. Volar forearm regions are selected for drug application, at least 4 cm from the wrist and 4 cm from the antecubital fossa. Formulations were applied only at 8:00 h because of known circadian activity of TG.

The formulations are applied to the skin for fixed dose durations. The chambers are removed and excess formulation is cleaned off, then skin blanching response is measured over 24 h or designated intervals of time following removal of the formulation[37]. The visual assessment is based on the application of an intensity scale 0-4 where zero indicates no blanching and numbers 1-4 assigned to increase in degree of blanching observed. The blanching response can be assessed by one or more observers used are taken into consideration for data processing. The total possible score (TPS) expressed in percentage (Figure 5) and is determined as:[36]

Total possible score.

e.g. Number of subjects (V), Number of independent assessors 4, Number of sites/product/arm (S), Maximum score/site 5. TPS = 5x4xSxV

% TPS = actual score / TPS x 100

Laser scanning confocal microscopy

Confocal laser scanning microscopy is another important tool for the assessment of dermatophrmacokinetic parameters. This tool focuses a beam to a given depth within the tissue and to take the reading of the concentration of penetrant or drug molecule at the level of focus. Thus, this tool allows an investigator to generate a concentration profile following topical application of drug product[25,26]. The system using a LSM 510 Invert Laser Scanning Microscope, equipped with an argon laser and HeNe laser. A Plan-Neofluar 10 x /0.3 objective an EC Plan-Neofluar 40 x /1.30 oil DIC M27 objective and a Plan-Apochromat 63 x /1.40 oil DIC M27 objective were used. The confocal images can be obtained in the plane parallel to the sample surface or in the plane perpendicular[34].

CLEARANCE OF DRUG FROM SKIN

Any substance applied to the skin surface may permeate into deeper tissue layers and reach into the body's systemic circulation by entering lymphatic or blood vessels in the dermis. Following skin permeation clearance is the key process involved in the percutaneous drug absorption[38]. The pharmacological effects of topical drugs or substances are directly dependant on microvascular parameters such as blood flow rate. The skin circulation and its microenvironment are a dynamic part of a living system and they are constantly adapting, growing, responding and changing with time[39]. The skin has plenty of rich vascular network system that regulates temperature and blood pressure, supply nutrition for the tissue and aid in wound repair and immunological responses[40]. Cutaneous microvasculature is enclosed in the papillary dermis 1-2 mm below the epidermal surface[41]. The vasculature in the human dermis consists of two horizontal plexus[42]. The lower one is located near the dermal-hypodermal junction that give rise to arterioles and venules which is either connect with the upper plexus or branch into the lateral micro-network supplies dermal appendages. The perforating vessels from the underlying muscle and subcutaneous fat formed the lower plexus. It is found 1.2-1.5 mm beneath the face of the skin. The connecting vessels produce lateral branches that supply the sweat glands and hair bulbs[41]. The second plexus located approximately 1-2 mm below the skin surface represent the boundary between the papillary and reticular dermis[38]. Capillary loops arise from the terminal arteriole which is a part of upper horizontal papillary plexus[41,43]. Sizes of vascular vessels are presented in (Table 1).

Capillaries

Three types of capillaries are found in the human body-continuous, fenestrated and discontinuous. Continuous capillaries are located in the skin and skeletal muscle, and are least permeable. The dermal capillary may be assumed as a bent, capital lambda shaped cylinder with is ventral part being broader internally and externally. The capillaries are most important in relation to the clearance into the systemic circulation (a) first permeable parts of circulation encountered by a topically applied permeant, (b) they are most permeable and numerous microvessels. It has an internal radius of approximately 3×10^{-6} m and a total loop length ranges from 1.5×10^{-4} to 5×10^{-4} m[44]. The capillary bed in the papillary dermis in which capillaries are perpendicular to the skin surface with slight twisting and slanting. Usually, there is one capillary per papilla and their surface density is approximately 50 or 60-70 capillaries per mm^2 of the skin surface[38].

Lymphatics

Lymphatic clearance route has extremely high permeability which becomes important for macromolecules which are not able to cross the microvascular barrier due to their size. The drainage network of the lymphatic system has the function to take away the debris of daily wear and tear in a tissue. In the human skin, lymphatics first appear at the subpapillary dermis and their infrequent presence has been connected with the abnormal skin[38]. Initial lymphatic vessels are cylindrical

microtubules approximately 50 µm in diameter[45] and are consists of weakened endothelial cells with a irregular basement membrane surrounding them. They form a web like network with a mesh magnitude of 200-500 µm in the human scalp. The diameter of vessels increases with depth, while the density of the network decreases. On the average, lymphatic channels have the number density of 0.3-1.5 (μm^2)[38]. Large molecules such as proteins, large sugars are cleared entirely by lymph vessels when injected intradermally. The removal of these molecules gives an evidence of the lymphatic utility of the skin[46].

Capillary permeability

The transport is almost entirely occurred by physical driving forces- pressure (passive transport) and pressure gradients across the blood tissue barrier. *S*everal routes have been classified in the capillary wall structure as pathways available for exchange. Depending on the solutes size, nature and lipophilicity, they may prefer one or more of these pathways[47]. Interendothelial cell junctions are the preferred pathway for the transport of hydrophilic solutes and also important for the small lipophilic solutes. They resemble the pores or slits filled with extracellular phase. Secondly through the endothelial cells allows the transport of lipophilic solutes, water etc. Further, the lipophilic molecules may also through the endothelial cell membrane without any difficulty. In addition, the existence of endothelial cell vesicles on the endothelial wall has been directly linked with their permeability. These circular vesicles have their blood or tissue side open, which leads to ridge like formation that increases the endothelial surface available for penetration and reduces the transendothelial diffusion path. They can shuttle back and forth between the cell surfaces, and they often exchange contents with each other or fuse together to form transient channels[48].

Factors influencing dermal/ cutaneous clearance

Blood vessels

- Total area at a certain depth in the skin
- Average distance between blood vessels at a given depth in the skin
- Thickness (permeability) of wall at a given depth in the skin
- Blood flow profile in the skin

Drug

- Diffusivity in the aqueous and lipid media
- Diffusion route in the aqueous sub-region of the skin
- Diffusion route in the lipid sub-region/ cell membrane
- Portioning between aqueous and lipid compartments
- Concentration at a given distance in the skin

SKIN METABOLISM

Skin is the largest organ in the body and contains all the major enzymes which are found in the liver and other tissues. Thus enzymes in the skin can catalyse a number of metabolic reactions. Skin metabolism of the topically applied compounds result in altered pharmacological and toxicological effects[49]. But the activity of enzymes in skin has found to be less as compared to liver. Therefore, the skin can act as a gateway for the entry of drug molecules into the body[50]. There are number of chemical groups which are particularly susceptible to skin metabolism such as alcohols, acids, primary amines, esters etc. Boehnlein had reported that esters were hydrolysed to their parent alcohol and acids by esterase. Nathan and Boehnlein observed that conjugation and oxidation/reduction of alcohols and acids were common in skin. Nathan et al have investigated that the primary amines were acetylated through the skin during percutaneous absorption. The drug molecules or chemical substances may exhibit greater or lesser biological activity that undergoes significant skin metabolism than predicted simply from skin penetration studies. The in vivo measurement of skin metabolism is difficult task because biological specimens may also contain metabolites from other tissues[3]. The skin absorption and metabolism can be simultaneously evaluated by applying in vitro techniques, thus safety and efficacy of these compounds can be assessed. The viable dermis comprises of different drug metabolising enzyme include epoxide hydrolase, CYPs, transferases (N-acetyl transferases) glucuronyl transferases and sulfatases. White and Baron reported that retinoic acid was metabolised by a specific CYP isoform, CYP26A1. Human keratinocytes contains transporter proteins which influences influx or efflux of some xenobiotics as reported by Baron[50].

DRUG DEPOT FORMATION

Several researchers have described a depot of drug that is found in the epidermal region of the skin following iontophoresis of prednisolone, lidocaine, salicylic acid etc. The depot indicates the highest concentration of drug detected in the intracutaneous[51]. The drug's residence time in the different skin layers can be determined by tape stripping and skin extraction studies at various time intervals following drug application. The works of Srujana Siddoju et al., on acyclovir following in vivo iontophoresis observed the depot formation immediately after drug delivery would be beneficial for the effective treatment of viral infections by providing sustained release of drug over an extended period[52]. Topical drug deposition can be assessed quantitatively, especially a radiolabelled compound, by the tape stripping method. But tape stripping may not useful for determining the follicular delivery because follicular contents may be stripped away. This method may be useful for determining the follicular deposition when it is used in combination with visualization by microscopic methods[49].

Vesicular carrier systems facilitate localization of drug at the application site by forming depot and also reducing the effective dose, frequency of dosing and systemic side effects of conventional topical therapies[53]. Schaefer et al. reported that particle size determine the penetration of molecules into the skin. Particles less than 3 µm diameter can penetrate the stratum corneum across intercellular route, particles in between 3-10 µm range localized in the follicles and particles above 10 µm cannot able to penetrate the skin. Nevertheless, this rule is not constantly true. There are many other factors that may affect the skin penetration. Silver nanoparticles, quantum dots, functionalized fullerene etc. has been investigated for the measurement of skin penetration and provided some evidence for penetration into the deeper skin layers. Tan et al. exposed titanium dioxide particle for 2-6 weeks in the epidermis and dermis of patients to determine titanium concentration. Failure in protection by TiO2 against ultraviolet B immunosuppression as revealed by epidermal cell lymphocyte reaction suggested that TiO_2 particles penetrated those cells located in the viable dermis; however no such penetration could be observed by electron microscopy. Many standard cosmetic formulations consisting of TiO2 and ZnO but skin penetration was not evident according to existing data. NANODERM European project focused mainly on the nanosized TiO_2 and ZnO particles skin penetration into healthy skin from various sunscreen formulations. Data suggested that the penetration was only restricted to the upper region of the stratum corneum[54]. α-

tocopherol and its photostability can be enhanced considerably when applied non-occlusively in the form of transferosomes. Lipid microparticles with > 1 μm are able to retain on the skin surface or localized on the hair follicles surfaces as a result preventing skin permeation of molecules having a capability. Thus, they form a film layer on the skin surface which can be helpful in sunscreen formulations for the protection against UV radiation[55].

CONCLUSION

Several in vitro, in vivo and mathematical models have been assessed to determine the real time measurement of the molecules in the skin layer. Regulatory agencies like U.S. FDA are still exploring different techniques to characterize drug's dematopharmacokinetics. Several parameters such as maximum quantity of drug active molecule in the stratum corneum (C_{max}), time to reach maximum level (T_{max}), and area under the curve (AUC) are widely assessed in dermatokinetics studies. Different methods for assessing pharmacokinetic profile of topically applied drug molecules includes in vitro permeation assessment, tape stripping, microdialysis, vasoconstrictor assay, confocal laser scanning etc are reported. The fate of molecules including depot formation, clearance etc from the skin layers are being applied to the skin surface are likely to permeate into the deeper tissue layers and reach into the body's systemic circulation by entering lymphatic or blood vessels in the dermis. Following skin permeation clearance is the key process involved in the percutaneous drug absorption. The pharmacological effects of therapeutic and cosmetic topical formulations are directly dependant on microvascular parameters such as blood flow rate. Novel vesicular formulations are capable to facilitate localization of drug at the application site by forming depot and also reducing the effective dose, frequency of dosing and systemic side effects of conventional topical therapies.

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Table 1: Sizes of vascular vessels[38,43].

	Diameter (µM)		Wall Thickness (µM)
	External	Endothelial	
Terminal arteriole	10-26	7.5-12	1-3.5
Arterial or venous capillary	10-12	4-6	2-3
Postcapillary venule	18-35	10-15	3.5-5

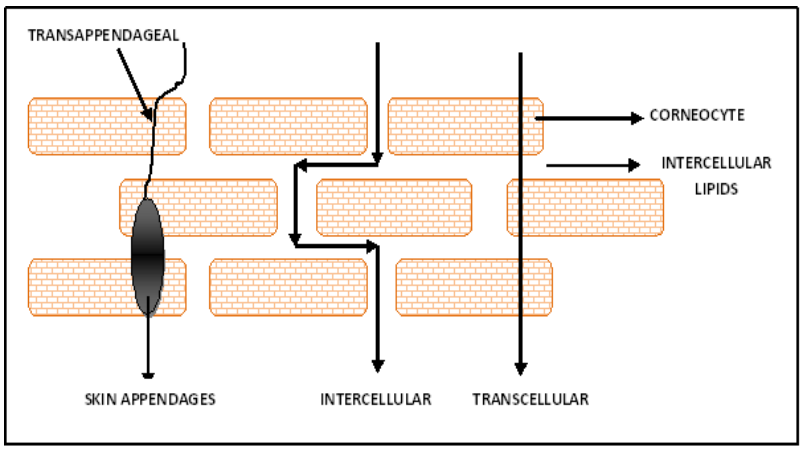

Fig 1: Routes of penetration into the skin.

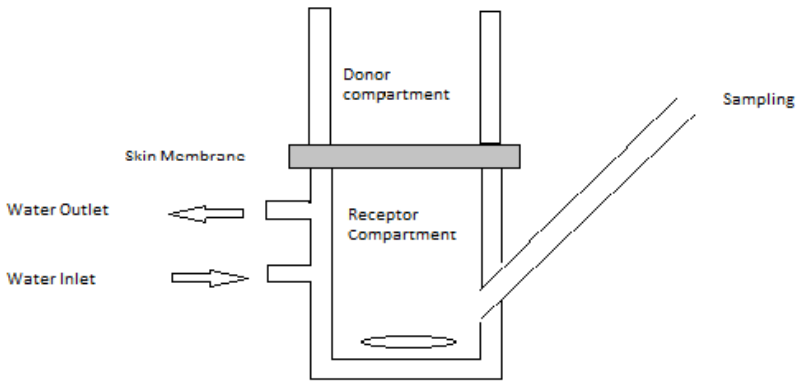

Fig 2: Schematic representation of a Diffusion Cell.

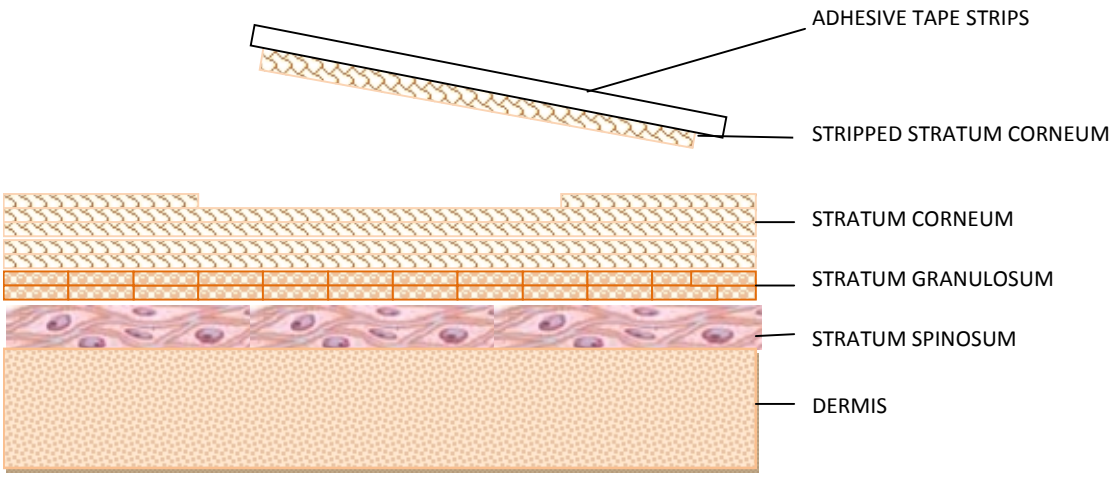

Fig 3: Adhesive tape stripping of stratum corneum.

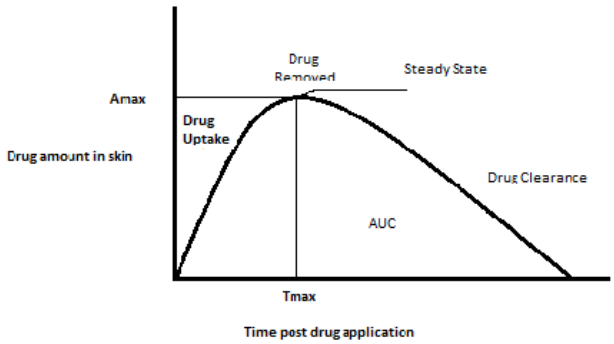

Fig 4: Schematic diagram illustrating the DPK drug analysis in the stratum corneum by tape stripping methodology recommended in FDA guidance 1998.

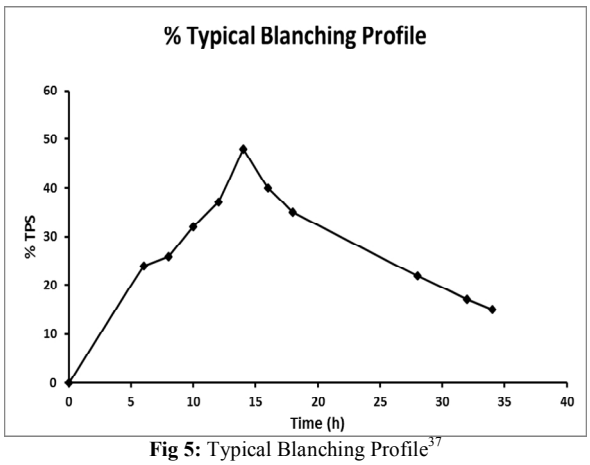

Fig 5: Typical Blanching Profile[37]

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