

Understanding Skin Penetration: Computer Aided Modeling and Data Interpretation

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Abstract: There has been considerable development in our knowledge about the mechanism of skin permeation. This has largely been brought about by the development of experimental techniques and increased computer technology, hardware and available software. The advanced computer technology and software have provided indications, relationships, at a molecular level, about routes of penetration and how the formulations can be formulated considering the effects of excipients and drugs on the barrier properties of skin layers. Available computer programs for molecular modeling have been used to calculate some molecular properties of the drug molecules such as surface area, partial charges etc. This publication reviews some of the mathematical models and techniques used some molecular descriptors and properties that have been constructed to predict and understand percutaneous penetration and transdermal delivery. The models are also useful for various enhancement strategies that can be used in dermal penetration and formulation development studies. If the appropriate biophysical techniques combined with the mathematical modeling and statistical analysis using computer, it can provide useful information for identifying the possible penetration processes when different classes of enhancers or excipients used in the formulation. Models are also useful for understanding which factors affect the penetration of molecules through skin and these factors/parameters can be used for the control of the penetration rate when effective transdermal delivery or therapy is required or targeted.

Keywords: Skin penetration, prediction, computer modeling.

1. INTRODUCTION

The skin, with an area of 1.8 to 2 m² [1], and a weight of almost 9 kg [2] making it the largest body organ, forms a unique interface layer between human body and outside world. It offers a number of opportunities as a route of administration of drugs, both for topical application in local treatment of skin disorders and for transdermal application of drugs for systemic effect; it is also highly susceptible to toxic penetrants. Transdermal application of drugs is increasing in popularity over the past decades. In many cases it has proved possible to produce therapeutic effects locally or systemically within the skin itself or, following transport by the blood, at remote target sites. Scopolamine, glyceryl trinitrate, clonidine, nicotine and steroids such as testosterone and oestradiol have been used clinically *via* the transdermal route [3,4]. Only limitation is the insufficient permeability. In many cases, the transdermal permeability needs to be increased or enhanced. The low permeability of the skin limits its usefulness to potent drugs, which exert their pharmacological effects at low concentrations [5]. Rational design of new transdermal systems or formulations requires an understanding of the process of penetration and the factors that determine it at the molecular level. Conversely, transdermal absorption is often undesirable. The disposal of industrial, agricultural, and household chemicals for well over a century has indiscriminately and seriously poisoned the ecosystems which sustain life. Human exposure has attracted considerable concern and the risks of transdermal contamination have been assessed [5].

Potentially risky activities may involve the whole body (bathing, swimming etc.) or partial-body (household cleaning, industrial or agricultural processes, boating etc.) exposure. The cosmetic industry is also interested in active delivery to the various strata of the skin. However, cosmetic industry often wants to reduce dermal uptake, for example in the use of UV filters.

It is important to be able to predict skin permeability and understanding mechanism of penetration for both transdermal drug application and understanding risk factor of pollutants and chemicals. Over the past decades many research groups have been examining dermal tissue using a range of sophisticated, sensitive biophysical and computational techniques to understand and predict skin permeability. The properties of skin and factors affecting skin permeability are being revealed and increasing in detail.

Permeation will be a function of the physicochemical properties of the active and their role needs to be understood in light of the mechanisms of penetration across the stratum corneum. Quantitative structure activity relationships and modeling are useful in predicting the behavior of novel compounds and may provide insights into the mechanisms of activity. Development in the computer science and software technology allows us to calculate sophisticated equations rapidly and create new models to understand effective parameters in permeability.

2. SKIN STRUCTURE AND ROUTE OF PENETRATION

Skin forms a protecting covering layer against the external environment and prevents water loss from the underlying tissue. It is flexible enough to resist permanent

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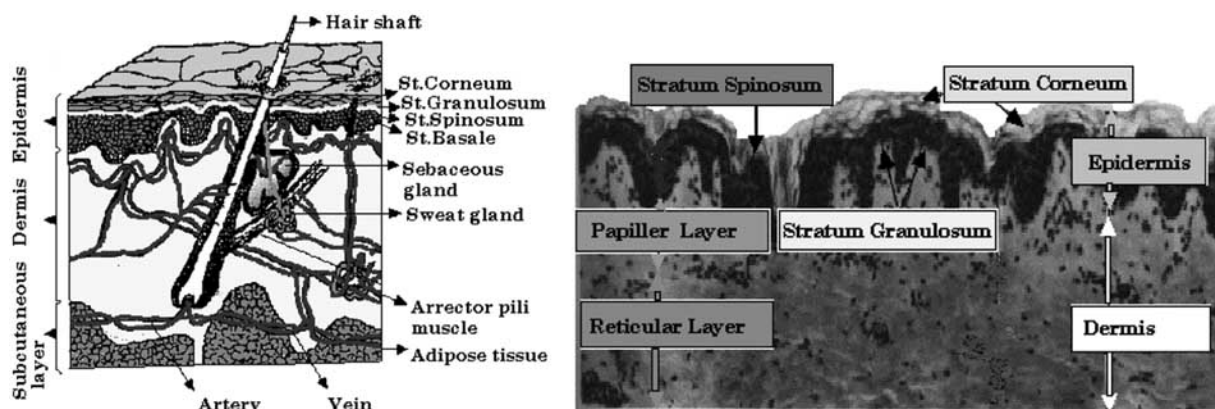


Fig. (1). Schematic structure and light micrograph showing the various layers of human skin (x 20).

distortion from movement and thin enough to allow the perception of stimuli. It also performs many ancillary functions such as synthesis and metabolism, and the production of sweat enables temperature control and excretion of waste products by means of sweating etc. [6,7]. It has been also reported that skin protects the body from antigenic stimuli by means of a part of the immune system known as skin associated lymphoid tissue (the SALT system [8]).

The skin can be considered to be composed of three layers: subcutaneous tissue, dermis and epidermis [2]. Some scientists consider that the discontinuous layer of sebum, a complex lipophilic fluid secreted by the sebaceous glands, constitutes a fourth, outermost layer.

Stratum corneum is the outermost layer of the epidermis. It consists of 10 to 25 layers of dead, elongated, fully keratinized corneocytes, which are embedded in a matrix of lipid bilayers [9, 10]. It has been shown that the stratum

corneum is the main barrier to penetration through the skin. When a topical formulation is placed on the skin, the active drug is required to penetrate through the stratum corneum into the viable tissue. The limiting factor for these processes is the slow diffusion through the dead horny layer of skin [11-14]. Stratum corneum behaves as a hydrophobic membrane. The rates of permeation of skin by low and high molecular weight organic non-electrolytes are mostly determined within the stratum corneum.

Many tape-stripping experiments have established that the stratum corneum plays an important role in resisting penetrant [15, 16]. Epidermis stripped of stratum corneum has little molecular specificity and its diffusional resistance is only about an order of magnitude smaller than that of water [5].

The stratum corneum lipids are important for the barrier function [17]. The hydrophobic chains in the ceramides, except ceramide 1, are straight and saturated. The double

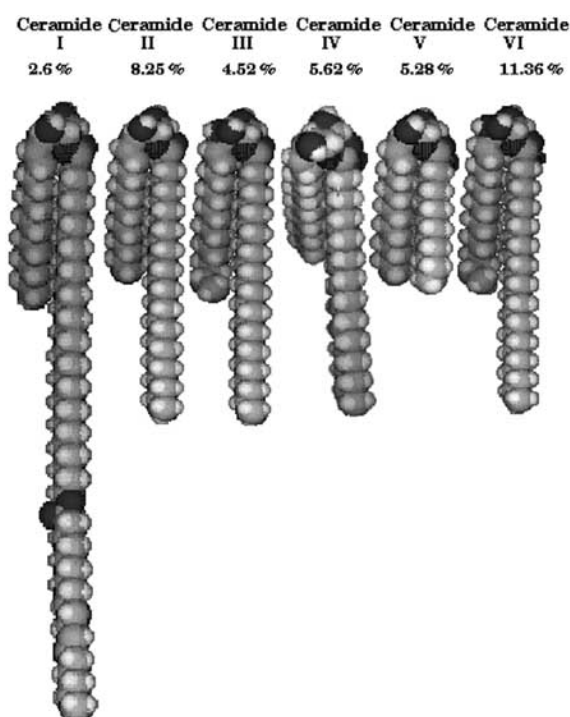


Fig. (2). Structures of ceramides found in human stratum corneum [23].

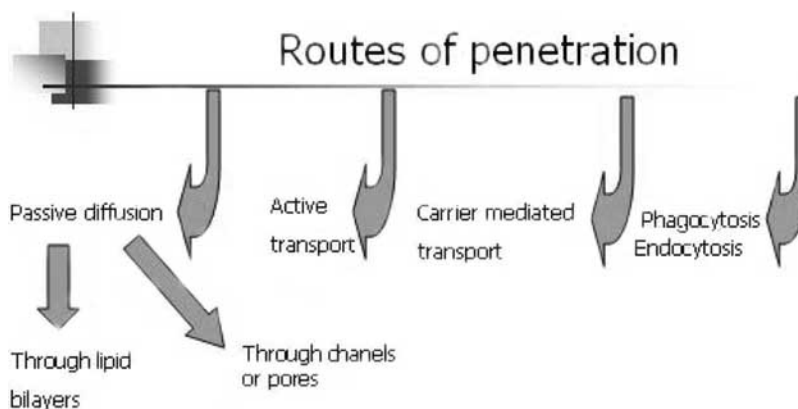


Fig. (3). The main routes of penetration.

bonds in the sphingosine chains are situated at the polar end of the lipid molecule, so they do not produce distortions in the aliphatic chains. The predominance of these neutral lipids in the stratum corneum is thought to be suited to the formation of a highly ordered impermeable barrier. The presence of long chain-saturated fatty acids indicates that the

The intercellular spaces contain structured lipids/proteins and a diffusing molecule has to cross a variety of lipophilic and hydrophilic domains before reaching to the stratum corneum and viable epidermis junction. Although the nature of the barrier is very heterogeneous, the diffusion through the skin can be described by simple Fick's laws [26].

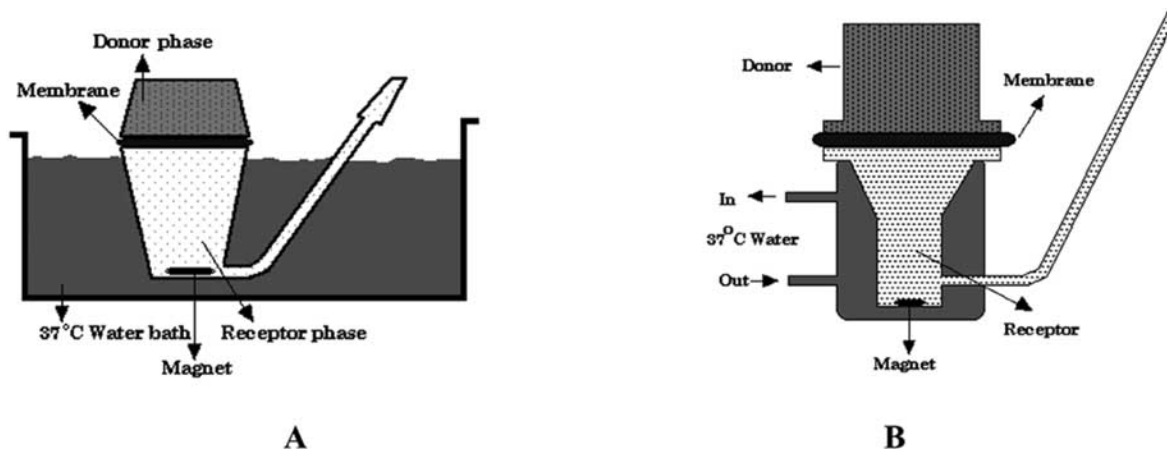


Fig. (4). A typical (A) and modified Franz type (B) Franz type diffusion cells.

intercellular lamellae exist in a gel rather than fluid crystalline state [18]. There is increasing evidence that ceramides play a major role in structuring and maintaining the (lipid) barrier function of the skin. As such they are considered to function as superior "emollients", rendering the skin soft and conferring water-retention properties on the stratum corneum [19-21]. Ceramide 1 is considered to be of special importance in barrier function. This acylceramide is one of the principal carriers of linoleic acid, an essential fatty acid necessary to maintain the barrier properties [22]. The molecular structures and appearance of the molecules can be examined using molecular modeling computer programs (Fig. 2).

There have been many discussions on the route of penetration (Fig. 3), but experimental results suggest that, under normal conditions, the main route is observed through the intercellular spaces or lipid bilayers [24, 25]. The diffusional path length is therefore much longer than simple thickness of the stratum corneum (20-30 mm). The penetration through skin is also affected by several biological factors such as skin age, body site, skin condition and diseases, water content of the skin or hydration.

3. DETERMINATION OF PENETRATION THROUGH SKIN

The most commonly used device for the *in vitro* diffusion work is the Franz type diffusion cell (Fig. 4a) or various modifications (Fig. 4b) in which a donor compartment containing the permeant is separated from a receptor compartment by a membrane. The increase in concentration of the permeant in the receptor phase is measured as it diffuses from its vehicle through the membrane. A review of the different types of diffusion cells has been given by Gummer and Friend [27, 28].

The flux of permeant is calculated from the permeation profile and permeability coefficient (k_p) can be calculated according to Fick's first law by the following equation:

$$K_p = \frac{J \text{ (flux.mg.cm}^2 \text{ / h)}}{C_{\text{vehicle}} \text{ (mg)}} \quad \text{Equation 1.}$$

J (Flux) can be calculated from the linear part of the diffusion-time profile, C_{vehicle} is the concentration at the donor phase.

Table 1. The Considered Descriptors of Previously Developed Models for Skin Penetration Prediction

Model	Considered descriptors	Reference
Flynn	Octanol/water partition coefficient (Log K _{oct})	5
Potts and Guy	Log K _{oct} , Molecular weight (MW)	44
Pugh <i>et al.</i> (Group contribution)	Number of atoms such as C, OH, H, Halide groups on the molecule and aromaticity of the molecule	45
Solvatochromic approach	Molecular volume (V), hydrogen bond acceptor (α) and donor (β) ability and dipole properties (π) of the molecule	46, 48
Pugh <i>et al.</i> (Solubility parameter)	Molecular volume, MW, cavity, melting point, bonding, Log K _{oct} , activity coefficient and solubility of the molecule	47
Pugh <i>et al.</i> (Partial electronic charge)	Log K _{oct} , MW, Sum of the partial charges on the atoms (sum of oxygen charges, sum of hydrogen charges etc.), hydrogen bond acceptor (α) and donor (β) ability of the molecule	39
Wilschut <i>et al.</i>	Log K _{oct} ., MW ^{-0.5}	49

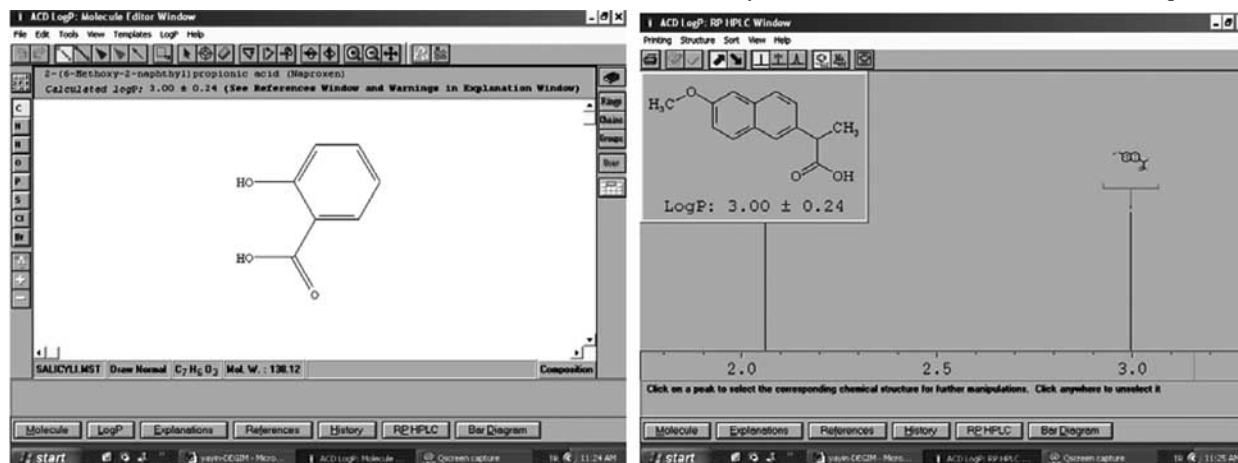
The rotating diffusion cells consist of rotating donor phase separated from static receptor phase by a membrane. This technique has been used as an *in vitro* model for membrane transport [29, 30].

Human *ex vivo* models and artificial membranes have been used for *in vitro* measurements of drug penetration. Although such models cannot fully replicate *in vivo* conditions, they can provide useful information. Human skin is often difficult to obtain but many studies have been conducted using human skin samples. Membranes prepared from a number of lipids such as isopropyl myristate (IPM) have been suggested [31], as well as a model membrane system based on using stratum corneum lipids prepared by fixing liposomes composed of ceramide, palmitic acid, cholesterol and its ester [32]. But because of their structural simplicity they cannot provide an adequate model of the heterogeneous nature of the intercellular channels [33]. Many studies have been done using different animal skins or perfused rabbit ear [34, 35] but, as mentioned before their properties are different from human skin in terms of enzyme content, thickness, composition of lipids and proteins etc. Polymer membranes such as polydimethylsiloxane (PDMS) and cellulose acetate have been used to investigate skin permeation. The physicochemical properties of a homologous series of alcohol across PDMS membrane have been investigated [36]. It was concluded that permeability

increases with alkyl chain length increases and molecular weight increases. The partition into the membrane was also important. The PDMS membrane has also been used by Jetzer *et al.* to interpret structure activity relationships between skin and nitrophenols [37]. The octanol / water partition coefficient was also considered and it was concluded that it was not a suitable solvent system to mimic the skin lipid partition behavior - hexane, methyl chloride or silicone rubber appeared to be more suitable.

4. MATHEMATICAL MODELS

Knowledge of molecular structure is one of the key to understand function and behavior of molecules. Intrinsic to chemistry is the concept and there is a relationship between bulk properties of compounds and their molecular structure and this determines the properties of matter. A change in the structure generally resulted in associated change in its properties. Finding some molecular descriptors that explain variations in physicochemical properties or biological activity has resulted in the development of linear free energy relationships (LFER) [38] and quantitative structure activity relationships (QSAR) [39]. Quantitative structure activity relationships (QSARs) are useful in predicting behavior of novel compounds and providing insights into mechanisms of activity. In transdermal studies, the technique is often

**Fig. (5).** Calculation of Log K_{oct} values using ACD Log P computer program (screen shots).

based on multiple regression analysis (MRA) of molecular features that determine an index of permeation such as the permeability coefficient, k_p , or the diffusion of permeant across some part of the skin. Earlier reports [40-43] were limited using small data sets then Flynn [5] published a collection of over 90 k_p and octanol/water partition coefficient ($\log K_{ow}$) values for compounds. This data set was then formed the basis of prediction of $\log k_p$ from molecular weight (MW) and $\log K_{ow}$, by Potts and Guy in 1992 [44]. The developed models were provided variable results for the complete data sets and have only limited statistical accuracy, possibly because of collecting data from several different sources associating with the risk of likely erroneous values.

The modeling studies for the prediction of permeability using functional group contributions [45], solvatochromic parameters [46] and Hildebrand solubility parameters [47] were continued to perform. Pugh *et al.* [48] criticized the use of the composite term, k_p , and reported the dependency of diffusion across the stratum corneum on MW and the scaled H-bonding values α and β , Wilschut *et al.* [49] questioned the reliability of some of Flynn's data and applied inclusion criteria for k_p values.

A current trend in quantitative QSAR studies is the use of theoretical molecular descriptors that can be calculated directly from molecular structure. Using computational methods to derive them is faster and more convenient. QSAR once quantified, can be used to estimate the properties of other molecules using computer program tools. Recently, a new model has developed and proposed for

diffusion across human stratum corneum in terms of molecular weight, H-bonding and electronic charge using computational techniques [39]. Table 1 summarizes the methods and considered descriptors of developed models.

5. CALCULATING THEORETICAL MOLECULAR DESCRIPTORS USING COMPUTER TECHNOLOGY

-Calculating Octanol/Water Partition Coefficients

Log K_{ow} values have been used as a one of the important predictors in many models and prediction equations as mentioned earlier. There are number of different computer programs available such as Medchem (Biobyte, Claremont, CA) and ACD Log P (Advanced Chemistry Development Inc., Ontario, CA). These programs are user friendly and it allows calculating Log K_{ow} values of molecules. Fig. 5 show an actual screen shots during Log K_{ow} calculation on computer.

-Calculating and Interpretation of Molecular Properties of Penetrants Using Molecular Modeling Programs

Computer molecular modeling provides a wide range of opportunities to investigate structure activity relationships by creating three dimensional representations of molecules. The electrostatic attraction and repulsion force were investigated by calculating distortion of electric charges, molecular orientations and minimum energy configurations and volumes to understand the effects of enhancers on permeation across ceramide membranes [50].

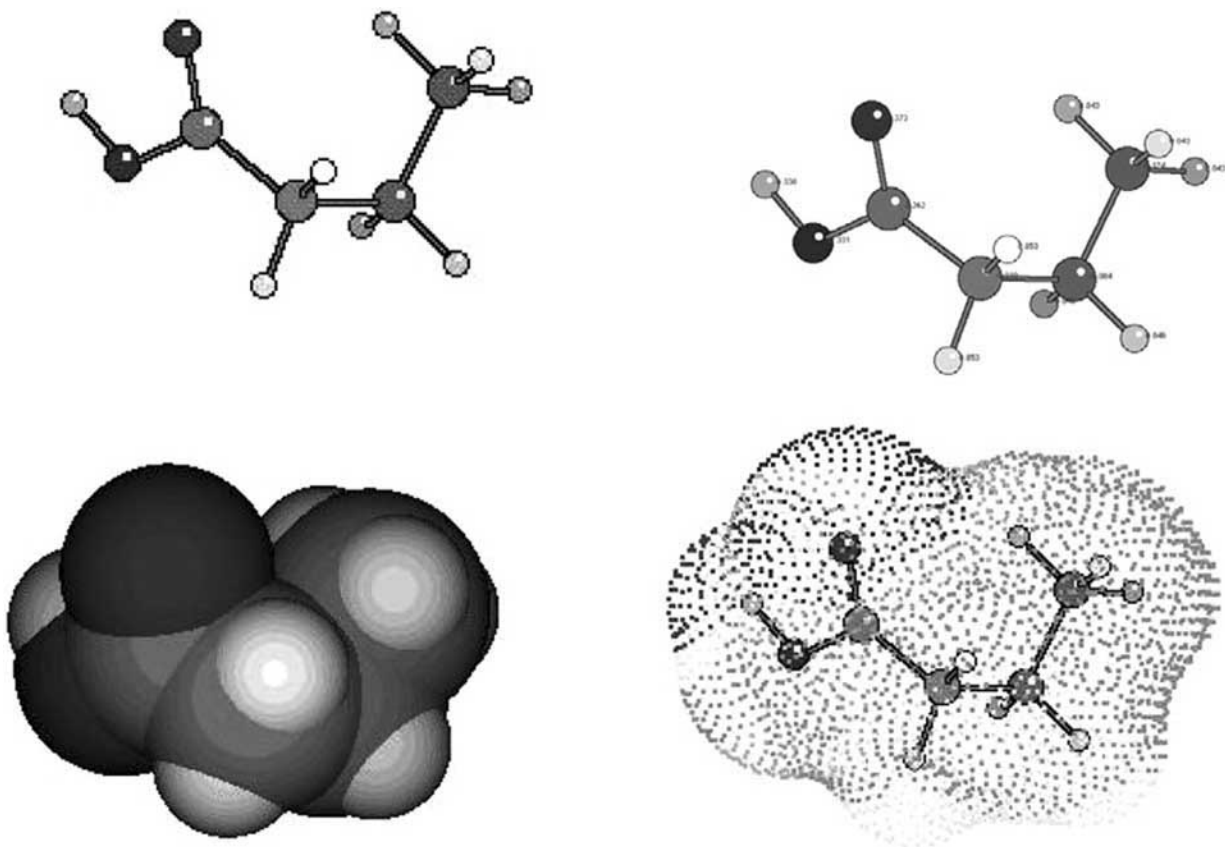


Fig. (6). Calculation of molecular properties of penetrants using Nemesis (actual screen shots for butanoic acid as an example).

Although there are number of different computer program available for the molecular modeling, in one attempt, molecular modeling was performed using the NEMESIS V1.0 package (Oxford Molecular, Oxford, UK) to calculate molecular properties of compounds for skin penetration modeling [39]. Conformation analysis using a step size of 30° was used to find the approximate energy minimum conformation, followed by optimization to identify the minimum energy conformation. This two step approach reduces the risk of finding a local minimal energy form. The program calculates partial charges on atoms on the basis of inductive effects in saturated molecules and Huckel molecular orbital calculations for p systems. The partial charges of the atoms (H, C, O, N and halogen) constituting the molecule were noted. Various combinations of the surface area, molecular length and the charges were tried by authors in the statistical analyses, but none gave superior results to the simple sum of the modulus of the charges [39].

Molecular graphics have also been useful in considering the structure of penetration enhancers such as Azone analogues. Azone is a well-documented penetration enhancer [51]. This substance appears to act by reducing the diffusional barrier of the stratum corneum by inserting itself into the structured lipids located in the intercellular

channels. The fluidity of the microenvironment of the lipids is reduced and diffusion improved. Inter atomic distances were measured and the distance along the longest axis of the compound was determined (17 \AA) [35]. Partial charge calculations have provided indications about the interaction of the polar region of the ring structures in the Azone derivatives and ceramide head groups [52]. The azone appears to act by reducing the diffusional barrier of the stratum corneum by inserting itself into the structured lipids located in the intercellular channels. The fluidity of the microenvironment of the lipids is reduced and diffusion improved.

According to Coulomb's law the potential energy of ionic bonding can be calculated by using relevant atomic charge [53]. If there is an interaction between penetrant molecule and the skin lipids during penetration, the charge of the skin lipids is probably constant in the range of normal variability while the charge of the penetrants may change. Coulombic interaction may be useful for the interpretation of the penetration process. A high partial charge on the penetrant will increase the intermolecular Coulombic forces between penetrant and skin lipids and retard penetration. The distance between molecule and skin lipids is unpredictable but the molecular width of the penetrants may reflect it [54]. Although some similarity was found between Coulombic

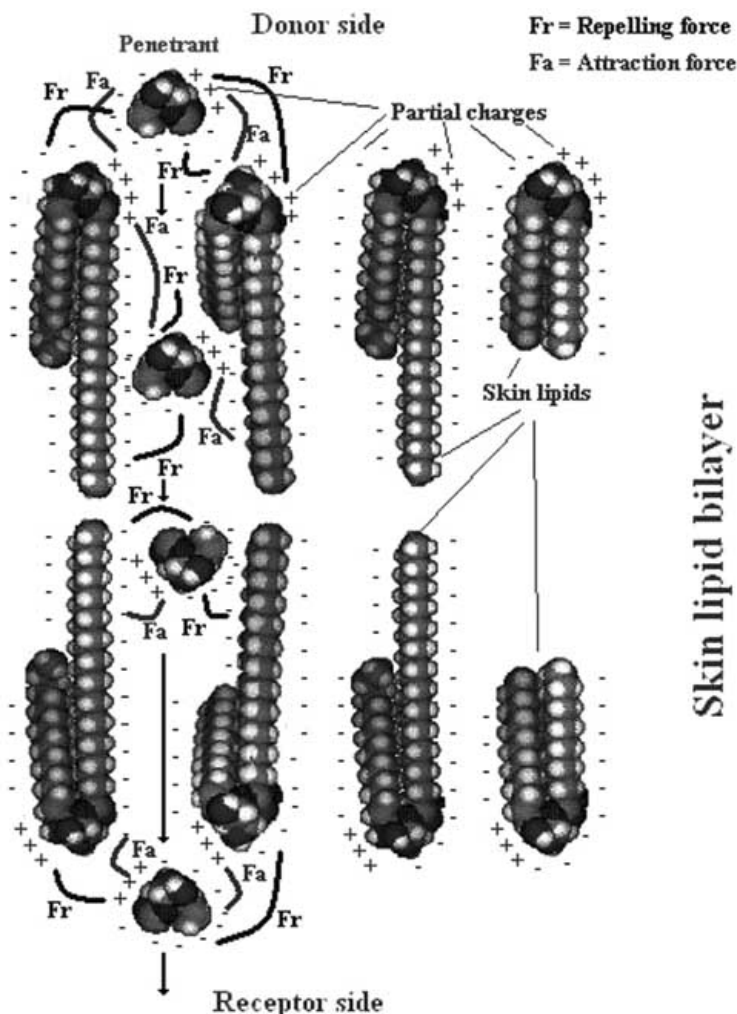


Fig. (7). The possible penetration process and intermolecular forces occurring during the penetration.

forces and skin penetration, the intermolecular forces are not easily calculable. Fig. 7 shows possible intermolecular interaction between penetrants and skin lipid bilayers during the penetration process.

While the Coulombic forces between penetrant and skin lipid retard the molecule, it is driven across the membrane by the concentration gradient.

The Coulombic interaction between penetrant and skin lipids between the donor and receptor compartments results in a complex interaction of attraction and repulsion effect. Compounds with higher values of partial charge on the molecule penetrate more slowly than the molecules with smaller values of partial charge.

6. MORE SOPHISTICATED INTERPRETATIONS

- Artificial Neural Network (ANN) Modeling

ANN is a biologically inspired computer algorithm designed to learn from data in a manner emulating the learning pattern in the brain. Most ANN systems are very complex multi-dimensional nonlinear information processing systems [55]. ANNs are composed of hundreds of single processing elements called artificial neurons. Artificial neurons are connected with coefficients (weights), which constitute the neural structure, and are organized in sets of layers, the input layer, output layer, and hidden layers between. Neural networks obtain their knowledge by detecting the patterns and finding the relationships from the data and trained through experience with appropriate learning exemplars. The input layer neurons obtain data and the output neurons produce the ANN's response. Hidden neurons communicate only with other neurons. They are part of the internal pattern and determine/produce solutions to solve problems and network learns dependencies in the model. Each hidden or output unit has a number of incoming connections from units in the preceding layer. The weighted sum of the inputs simulates activation of the neuron. Thus, what is learned in a hidden neuron is based on all the inputs taken together. The activation signal is passed through an activation function (transfer function) to produce a single output of the neuron. The behavior of a neural network is determined by the transfer functions of its neurons, by the learning rule, and by the architecture itself. In many studies, the supervised network with a back propagation learning rule has been used. In this type of model, information from inputs (molecular descriptors) is fed forward through the ANN to optimize the connection weights among neurons. The output of the neuron is related to the summed input by a sigmoid shaped transfer function. During training, optimization of the network weights is made by back propagation of error (e.g. difference between predicted and measured drug flux), and the inter unit connections are changed until the error in predictions is minimized across many data sets and until the network reaches a specified level of accuracy [55]. These connection weights store the knowledge necessary to solve specific problems.

A successful ANN modeling has been used to assess theoretically derived molecular descriptors of permeant penetration across polydimethylsiloxane membrane [55].

Neural Networks TM (StatSoft®) was used for building the QSPR model and CAChe Project leader Version 3.11 (Oxford Molecular Ltd.) was used to calculate molecular descriptors from the molecular structure for this study. A genetic algorithm was used to select important molecular descriptors and supervised ANN was used to correlate selected descriptors with the experimentally derived maximum steady-state flux through the polydimethylsiloxane membrane ($\log J$). Calculated molecular descriptors were used as the ANN's inputs and $\log J$ as the output. Developed model indicates that molecular shape and size, inter-molecular interactions, hydrogen-bonding capacity of drugs, and conformational stability could be used to predict drug absorption through skin. A 12-descriptor nonlinear computational neural network model has been developed for the estimation of $\log J$ values for a data set of 254 drugs [55].

ANN approaches have not been applied to skin permeability data since Degim *et al.* published prediction of skin penetration using ANN modeling results in 2003 [56]. This publication builds on the use of partial charge, $\log K_{oct}$, and MWdata to predict skin permeability using these factors as inputs into an ANN. Pythia is a computer program (Runtime Software LLC; Carson City, NV) for the development and design of neural networks. A special feature of the program is the evolutionary optimizer. This module of the software automatically generates suitable networks for a given training data set. The best network model was developed using the optimizer and the ANN that achieved the lowest square deviations. Fig. 8 shows the details of the developed ANN model [56] for the prediction of skin permeability. Although a successful relationships was found between predicted and experimental results (Fig. 9); there was no dependence found between descriptors indicating that a complex relationship exists between structure of the penetrant and skin penetration.

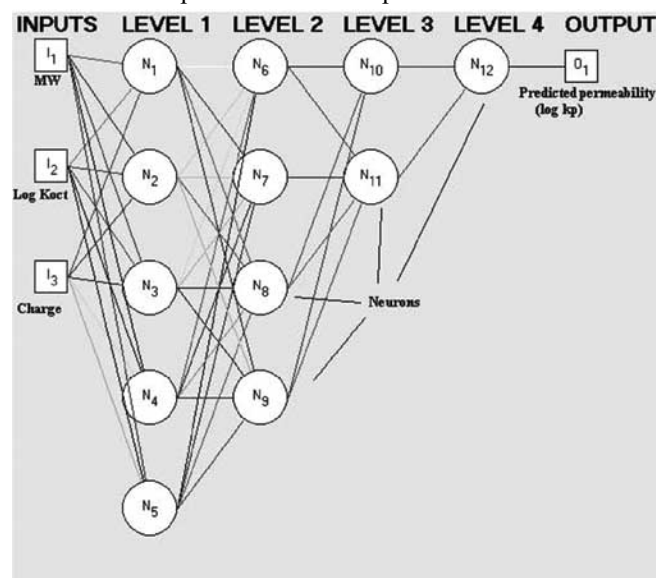


Fig. (8). The developed ANN model for predicting skin permeability [56].

The developed ANN models described herein concluded that this is not requiring any experimental parameters; it could potentially provide a useful and precise prediction of skin penetration for new chemical entities in terms of both

therapy and toxicity. It could reduce the need of performing penetration experiments using human skin or other model membranes. The results indicate that the simple predictors investigated to date can be capable of predicting skin penetration. If the predicted result present with its values among the training set, a better result can be obtained but, if it is too far from the training set values, an unexpected or unreliable result can be easily obtained. Other problem of the ANN modeling is the memorizing; if the program over trained this will be the problem.

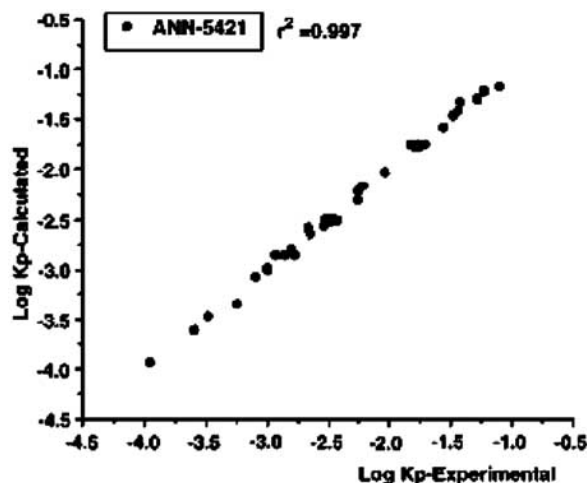


Fig. (9). The relationship between predicted log kp values and experiment results using the ANN model [56].

7. THE FUTURE OF SKIN RESEARCH

There are some physical techniques such as iontophoresis, electroporation and ultrasound have also a future to impact on transdermal drug delivery through the skin. Since the possibility of extracting some compounds from skin for diagnosis or monitoring was understood [57, 58], there are quite fast developments in non-invasive drug monitoring realized recently and following the one of the approach for monitoring the blood glucose level, GlucoWatch G2e Biographer device has been developed [59,60].

Monitoring of the urea level of patients with insufficient kidney function requires repetitive blood sampling. The potentially painful nature of blood sampling and the difficulty of venous access, particularly in premature neonates, as well as possible complications of needle injuries, create many disadvantages. A non-invasive technique using reverse iontophoresis has recently proposed to monitor blood urea levels without taking any blood sample and the possibility of producing a watch-type urea measuring/monitoring device has been discussed [61].

This kind of techniques may be very useful and will be available in the future for especially premature neonates, because their skin is known to be more permeable and it is difficult to obtain a blood sample by needle from these patients. Premature neonates have a very small skin surface, but this kind of devices could be easily applied to another part of the body such as the abdomen, if a larger area is needed. These results also suggest that reverse iontophoresis

could be applied to the monitoring of other substances in the blood. Further studies are undoubtedly necessary to minimize variability.

8. CONCLUSIONS

The past decades have given us a useful of information about how molecules pass across the skin. We learnt much about the skin penetration mechanism and effective parameters, the barrier function of the skin and enhancement strategies, choosing suitable drug candidates for dermal application etc. But we also understood that this process is still too complicated and interfered by several factors. Although all developments have been made because of understanding of skin penetration properties, it is still necessary to understand how the physicochemical properties of the penetrant penetration medium impact on the transport rate. This can be achieved in the future using even more sophisticated biophysical and computational techniques.

ABBREVIATIONS

ANN	=	Artificial Neural Network
C	=	Carbon
C _{vehicle}	=	Concentration at the donor phase
F _a	=	Attraction force
F _r	=	Repelling force
H	=	Hydrogen
IPM	=	Isopropyl myristate
J	=	Flux
k _p	=	Permeability coefficient
LFER	=	Linear free energy relationships
log K _{oct}	=	Octanol/water partition coefficient
MRA	=	Multiple regression analysis
MW	=	Molecular weight
N	=	Nitrogen
O	=	Oxygen
PDMS	=	Polydimethylsiloxane
QSAR	=	Quantitative structure activity relationships
V	=	Molecular volume
π	=	Dipol properties
α	=	Hydrogen bond acceptor ability
β	=	Hydrogen bond donor ability

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