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New tools and approaches for predicting skin permeability

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This article reviews some new mathematical models and techniques used to predict and understand percutaneous penetration and transdermal delivery. These models are also useful for various enhancement strategies that can be used in dermal-penetration and formulation development studies. If appropriate, biophysical techniques can be combined with these new mathematical models and statistical analyses and it will be possible to understand the factors affecting penetration of molecules through skin. These factors, or parameters, can then be used to control the penetration rate when effective transdermal delivery or therapy is required or targeted.

Skin is the largest organ of the body with a surface area ~1.8–2.0 m^2 [1] and a weight of almost 9 kg [2]. It forms a unique and flexible interface between our internal milieu and the external environment and possesses sensory, thermoregulatory [3], metabolic [4] and immunological [5] functions. It is flexible enough to resist permanent distortion from movement and thin enough to allow stimulation. It also performs many ancillary functions, such as metabolism, and the production of sweat enables temperature control and excretion of waste products [3,6].

The skin is composed of three layers, subcutaneous tissue, dermis and epidermis (Figure 1) [2]. The discontinuous layer of sebum, a complex lipophilic fluid secreted by the sebaceous glands, is sometimes considered to be a fourth, outermost layer.

The stratum corneum is the outermost layer of the epidermis. In humans it consists of between 10 and 25 layers of dead, elongated, fully keratinised corneocytes that are embedded in a matrix of lipid bilayers [7,8]. This layer is only 6–10 μm thick [9] in most regions of the body but 0.4–0.6 mm thick in the palms of the hands and soles of the feet [10]. The stratum corneum consists of ~40% protein of which 80% is keratin. Keratin is a group of α-helical polypeptides ranging in size from 40,000–68,000 daltons [11]. The type and amount of lipid in the stratum corneum depends on body-site and, currently, it is generally accepted that skin permeability is affected by stratum corneum lipids [12].

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Penetration through skin

Although the skin has barrier function, some chemicals are able to penetrate it. The mechanism of penetration has been widely studied. The main resistance between stratum corneum and the epidermis cell layers for transdermal transport was first hypothesized by Rein in 1924 [13]. Schuplein and Blank [6] later established that transdermal penetration was limited by the stratum corneum itself, and that molecular impermeation was a passive process. Subsequently, Michaels *et al.* [14] showed that several drugs had significant permeability and determined their stratum corneum diffusion coefficients.

In the past 30 years, the hope of delivering therapeutic agents into the body through the skin has become a clinical reality. Starting with the first approval of scopolamine patches in 1979, transdermal delivery is now a viable way of delivering drugs. The rational design of new transdermal systems or formulations (and determination of risk assessments of transdermal exposures to chemicals) also requires an understanding of the process of penetration and the factors that determine it at the molecular level [15].

When a topical formulation is placed on the skin, the active drug has to penetrate from the stratum corneum into viable tissues (Figure 1). The main limiting factor for this process is the slow diffusion through the stratum corneum, which is known to be a dead layer [14,16–21]. The stratum corneum lipids are important for the barrier function [20,22]. 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

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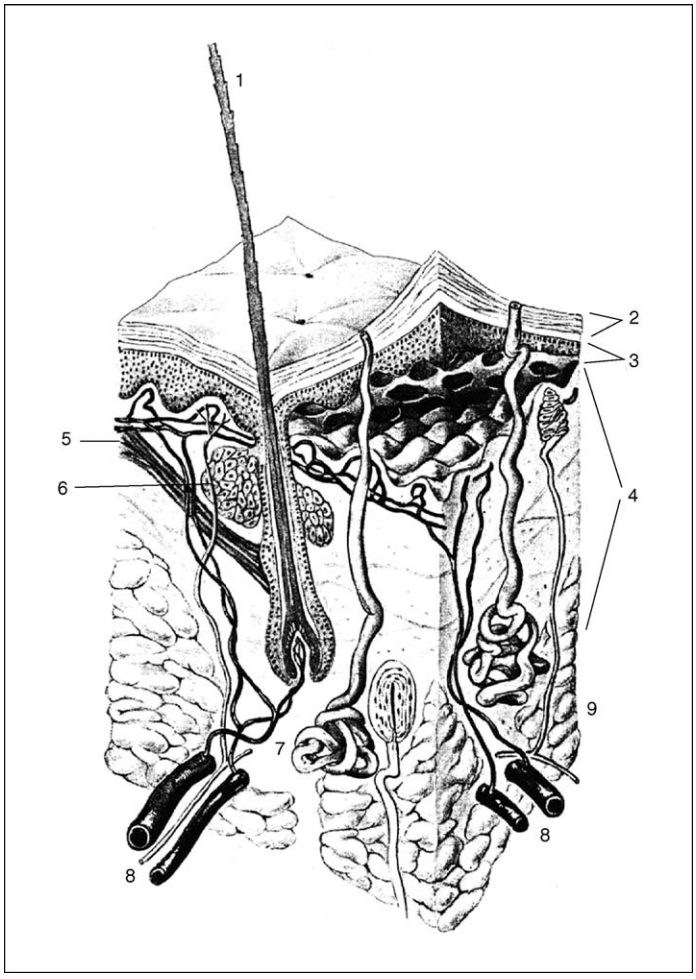

FIGURE 1

Schematic cross sectional view of skin structure. In this scheme the following structures are labelled: hair shaft (1); epidermis having an outermost layer, stratum corneum (2), and sequential inner layers, stratum granulosum, stratum spinosum and stratum basale (3); dermis (4); arrector pili muscle (5); sebaceous gland (6); sweat gland (7); blood vessels (8); and adipose tissue (9).

function as emolients, rendering the skin soft and conferring water-retention properties on the stratum corneum [23–26].

There are three main pathways that exist for passive transport of chemicals through the skin to the vascular network [6,27]: intercellular diffusion through the lipid lamellae; transcellular diffusion through the keratinocytes and lipid lamellae; and diffusion through appendages (hair follicles and sweat ducts).

Determination of penetration through skin

The most commonly used device for *in vitro* diffusion work and for determining penetration through skin is the Franz-type diffusion cell, or modifications of it. A donor compartment containing the permeant is separated from a receptor compartment by a membrane (excised human skin) [28–30].

Isolated and perfused whole rabbit ear or excised ear skin have also been used to determine the penetration of compounds through skin (*ex vivo* or *in vitro* experiments) [30–32]. Microdialysis experiments have also been performed to determine penetration of compounds through human skin under *in vivo* conditions [33].

Modelling of transport through the skin

Existing models

Several investigators have used the published human stratum corneum permeability coefficient (K_p, often expressed as log K_p) to predict skin permeability and they have examined the effect structural parameters of penetrants have on permeability [34–38]. This has led to the development of models. Using molecular descriptors that explain variations in physicochemical properties or biological activity of penetrants has resulted in the development of linear free-energy relationships (LFER) [39] and quantitative SAR (QSAR) [40]. QSARs are useful in predicting behaviour of novel compounds and provide insights into mechanisms of activity. Michaels *et al.* [14] have developed the concept of the stratum corneum being a two-phase region consisting of 'bricks and mortar', where the aqueous protein phase in the keratinocytes represents the bricks and the intercellular lipid phase represents the continuous mortar. The transport of the compound through the stratum corneum was assumed to be the sum of the diffusion through the lipid and protein. It was then concluded that diffusion through the lipid phase was ~500 times slower than diffusion through the protein phase [14]. Flynn [34] later stated that the density and compactness of the intracellular protein in the keratinocytes of the stratum corneum makes it almost thermodynamically and kinetically impossible for compounds to cross. It is now generally accepted that intercellular diffusion through the lipid lamellae is the predominant mode of transport. Flynn interpreted data from the literature in terms of a risk assessment and he concluded that penetration through the skin is related to the octanol–water partition coefficient (K_{oct}, often expressed as log K_{oct}). He proposed that a rough prediction of the skin permeability coefficient is sufficient to estimate the risk factor.

Earlier models and/or reports about the relationship between skin permeability and permeant properties [41–44] were inconclusive because they used small datasets; however, Flynn [34] published a collection of >90 log K_p and log K_{oct} values for compounds. This dataset formed the basis of a model for the prediction of log K_p from molecular weight (MW) and log K_{oct} [35]. These developed models provided variable results and have only limited statistical accuracy, possibly because of using data from different sources.

Other models developed for the prediction of permeability have used functional-group contributions [37] [model uses number of atoms (such as carbon, hydrogen, hydroxyl, etc.) and groups such as halide and amide, as well as aromaticity of the molecule, as predictors], molecular parameters [45] (model uses molecular volume (v), H-bond acceptor (α) and H-bond donor (β) ability and dipole properties (π) of the molecule as predictors) and Hildebrand solubility parameters [38] (model uses v, MW, cavity, melting point, bonding, log K_{oct}, activity coefficient and solubility of the molecule as predictors). Pugh *et al.* [46] 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.* [47] have used log K_{oct} and $MW^{-0.5}$ as predictors and have questioned the reliability of some of Flynn's data.

A current trend in QSAR studies is the use of theoretical molecular descriptors that can be calculated directly from molecular structure. Using computational methods to determine them is fast

and convenient. A new model was developed using epidermal permeability–penetrant structure relationships, using MW, H-bonding and electronic charge of the molecule, and computational techniques [48]. Pugh *et al.* [46] used a molecular modelling computer program called NEMESIS V1.0. The program calculates partial charges on atoms. The partial charges of the atoms (H, C, O, N and halogen) constituting the molecule have been noted and, consequently, the summed charges have been calculated. It was found that partial charges and MW of the penetrant are equally important predictors of diffusion across the stratum corneum.

After considering all the predictor models and methods published so far, none of them appears to be capable of explaining the entire penetration mechanism precisely. Some compounds appear as outliers in many methods, such as naproxen and atropine, whereas others, such as nicotine, fitted better with some methods than with others. Also, some experiment results and some permeability values reported in the literature were found to be inappropriate [49]. Although re-determination of these permeability values helped to increase fitting values for the prediction equations [49], developed models were not found to be capable of explaining the permeability process thoroughly. Therefore, new and more-complex models have started to be developed.

New approaches

Principal components analysis

Principal components analysis (PCA) has been used to assess the predictors of permeant diffusion across the human stratum corneum [48]. Log (D/h) values were estimated by Pugh *et al.* [48] and used instead of log K_p values, considering the diffusion coefficient (D, cm^2/h), diffusional path length (h, cm), permeability coefficient (K_p, cm/h) and K_{oct} [48]. MWs with scaled H-bonding parameters (α and β) or a summed modulus of partial charge from molecular modelling (sum of oxygen charges, sum of hydrogen charges on the molecule, etc.) were tested as predictors of (D/h). PCA detects relationships called principal components (PCs) among the variables in a table (matrix) that account for the data variation. The eigenvalue is a statistically calculated parameter which shows discriminating or important values (or parameters). Pugh *et al.* [48] considered the PCA relating log (D/h), MW, α and β as an example and the sum of the eigenvalue was reported as the number of PCs. The authors evaluated the contribution of log (D/h) and it was found to have an important role in the PC (or mechanism) [48]. PCA analyses were performed considering log (D/h), MW, α and β (Table 1). The sum of eigenvalues was the number of PCs (4) [48]. The eigenvalue of a PC shows the proportion of the total variation in the matrix attributable to that PC. Thus, if log (D/h) was completely determined by a single process involving MW, α and β, PC1 would have an eigenvalue of 4 and PCs 2, 3 and 4 would all be zero. This proportion for PC1 was 0.82 (i.e. 3.27:4). Within PC1 the eigenvector of a variable indicates the degree to which variations in data are attributable to that variable. The communality, defined as the sum of the squares of the eigenvectors [48], is shown in Equation 1.

$$(0.54^2) + (-0.54^2) + (-0.44^2) + (-0.48^2) = 1 \qquad \text{[Eqn 1]}$$

The contribution of log (D/h) was 0.29 (i.e. 0.54^2), which meant that it plays an important role in the PC (or mechanism). It was reported that the PCs with eigenvalues >0.75 indicate that PCA, on the basis of H-bonding and charge, indicated a single and dominant mechanism. PC1 of log (D/h), MW, α and β accounted for 82% of data variation, and the eigenvector signs showed inverse relationships between log (D/h) and both size and H-bonding. The eigenvector sign was also reported to be significant; a negative PC1 suggests a mechanism involving log (D/h) inversely related to MW and H-bonding. The similarity of eigenvectors for MW (−0.58) and charge (−0.56) in PC1 (Table 2) was found to be important for these two factors regarding penetration through skin [48].

Finally, PCA was found to be a useful tool for analyzing penetration mechanisms and important factors involved in skin penetration.

TABLE 1

Principal component (PC) analysis results: matrix of log (D/h), molecular weight (MW), scaled H-bonding values α and β, and calculated parameters

Eigenvectors	PC1	PC2	PC3	PC4
Log (D/h)	+0.54	+0.10	+0.22	+0.81
MW	−0.54	+0.01	−0.12	+0.53
α	−0.44	+0.79	+0.65	+0.07
β	−0.48	−0.61	+0.43	+0.24
Eigenvalue	+3.27	+0.58	+0.10	+0.05
Proportion of variation	+0.82	+0.14	+0.02	+0.01
Cumulative proportion of variation	+0.82	+0.96	+0.98	+1.00

Considering lateral free-volume diffusion and diffusion through pores and shunts

An analytical expression has been used to predict skin permeability of hydrophilic and hydrophobic solutes [50]. Solute permeation through four possible routes in the stratum corneum, including free-volume permeability through lipid bilayers (K_p^{fv}), lateral permeability along lipid bilayers ($K_p^{lateral}$), permeability through pores (K_p^{pore}) and permeability through shunts (K_p^{shunt}), has been analyzed by Mitragotri [50] (Equation 2).

$$K_p = K_p^{fv} + K_p^{lateral} + K_p^{pore} + K_p^{shunt} \qquad \text{[Eqn 2]}$$

The scaled particle theory [51] assumes that hydrophilic solutes permeate across the skin through imperfections in the lipid bilayers, modelled as pores. It was used to determine the contribution of free-volume diffusion through lipid bilayers. The contribution of lateral lipid diffusion was determined from the literature [50]. The contribution of pores was estimated using the hindered

TABLE 2

Principal component (PC) analysis result: matrix of log (D/h), charge and molecular weight (MW), and calculated parameters

Eigenvectors	PC1	PC2	PC3
Log (D/h)	+0.59	−0.34	+0.74
Charge	−0.56	−0.82	+0.07
MW	−0.58	+0.45	+0.67
Eigenvalue	+2.64	+0.24	+0.13
Proportion of variation	+0.88	+0.08	+0.04
Cumulative proportion of variation	+0.88	+0.96	+1.00

transport theory. Finally, the contribution of shunts (solute permeability through hair follicles and sweat ducts) was determined using a simple diffusion model. The model yielded a series of equations to predict skin permeability, based on solute radius and K_{oct}. Model predictions compared well with experimental data [50].

The most important finding of this model is that solute permeation can take place through four different pathways and that the contribution of each pathway can be estimated in a consistent manner. The predominant pathway used by a solute was determined by a combination of the molecular radius and hydrophilicity [50]. It was found that permeation of low MW hydrophilic solutes (e.g. water) can be entirely explained by diffusion through intercellular lipid bilayers. However, larger hydrophilic solutes, such as sucrose, permeate by diffusion through pores. Diffusion of macromolecules, such as dextran, was consistent with diffusion through shunts.

Probabilistic analyses

A probabilistic, transient three-phase model of percutaneous absorption of chemicals was developed [52] to assess the relative importance of uncertain parameters and processes of the penetration that might be important for the dermal risk-based exposure assessments. Three penetration routes through the skin were modelled as intercellular diffusion, aqueous-phase diffusion through sweat ducts and diffusion through hair follicles. Uncertainty distributions have been developed for this model and a Monte Carlo analysis was performed to simulate probability distributions of mass fluxes through each of these routes. Sensitivity analyses, using stepwise linear regression, were also performed to identify model parameters that were most important for the simulated mass fluxes at different times. This probabilistic analysis of percutaneous absorption (PAPA) method has been developed to improve risk-based exposure assessments and transdermal drug-delivery analyses where parameters and processes can be highly uncertain [52]. Results indicate that, at early time points before steady-state conditions had been established, transport through the sweat ducts provided a significant amount of drug flux into the bloodstream. Because of the uncertainty in the input parameters, a large range of permeant fluxes were simulated through each of the three routes at this early time. After the system had reached the steady state the uncertainty was reportedly reduced, and the relative importance of the pathways was also reduced [52]. The most important parameters for the simulated mass flux were identified and the relative importance of each parameter was quantified through the incremental coefficients of determination and semipartial correlations. These kinds of mechanistic models of multiphase heterogeneous transport through the skin coupled with probabilistic analysis can shed additional insight into how these methods can be improved through identification and refinement of important parameters and processes [52].

Artificial neural network modelling

Artificial neural network (ANN) modelling has been used to predict skin permeability [53,54]. ANN is a biologically inspired computer algorithm designed to learn from data in a manner emulating the learning pattern in the brain. Neural networks obtain their knowledge by detecting patterns and relationships from training data. Most ANN systems are highly complex, multidimensional, nonlinear, information processing systems [54]. The input layer neurons obtain data and the output neurons produce the ANN response. Hidden neurons communicate with other neurons. 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 behaviour of a neural network is determined by the transfer functions of its neurons, by the learning rule and by the architecture itself. During training, optimization of the network weights are made by the back-propagation of error and the inter-unit connections are changed until the error in predictions is minimized across many datasets and until the network reaches a specified level of accuracy [53]. These connection weights store the knowledge necessary to solve specific problems. Degim *et al.* [54] have successfully applied ANN approaches to skin permeability data. This work builds on the use of partial charge, log K_{oct} and MW data, predicting skin permeability using these factors as inputs into an ANN (Figure 2). Experimental permeability values were successfully predicted using the ANN model (Figure 3). There was no direct relationship found between descriptors and permeability indicating that a complex relationship exists between structure of the penetrant and skin penetration.

Fuzzy modelling

Fuzzy logic is a tool that has been successfully used for modelling, control systems, pattern recognition, image processing, among other applications. This modelling methodology has been proposed to predict skin permeability coefficients [55]. The difference between models previously published and the one using Fuzzy modelling is simply the method used to map the input to the output. Fuzzy models were developed by Pannier *et al.* [55] using

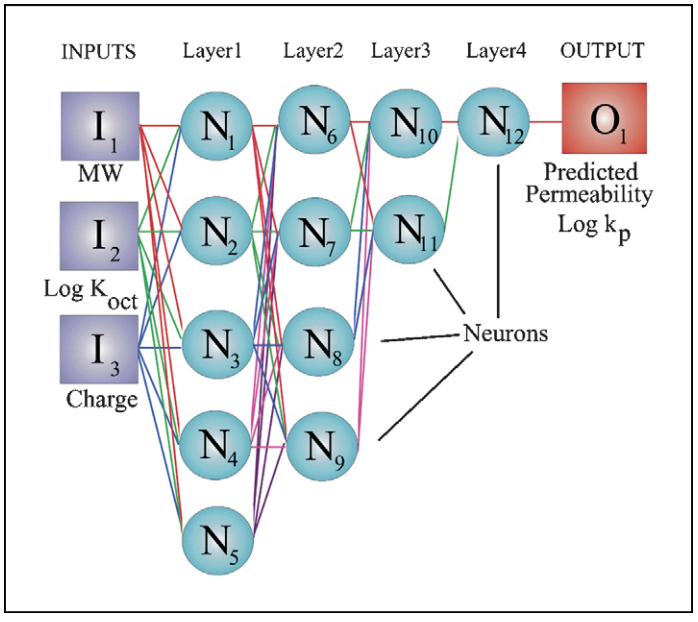

FIGURE 2

The neural network structure of the developed artificial neural network (ANN) model for predicting skin permeability. The program produces output (O_1, predicted permeabilities log K_p) from input neurons (I_{1-3}) using hidden neurons (N_{1-12}) at four layers [54].

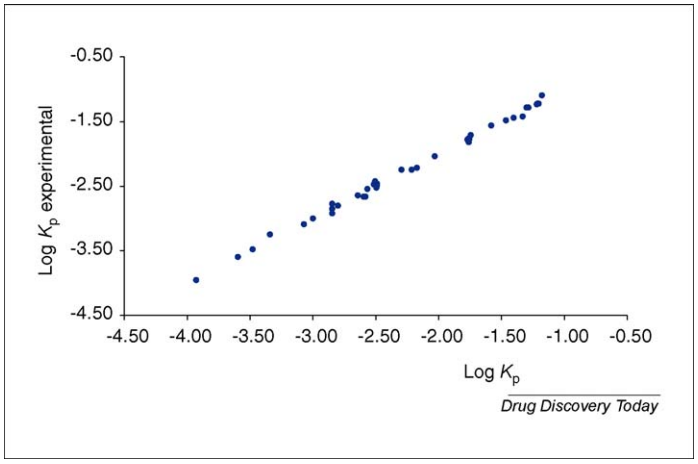

FIGURE 3

The relationship between predicted log K_p values and experiment results using the artificial neural network (ANN) model. All dots lying on the line indicate a high correlation ($r^2 = 0.997$) between experimental and predicted values, this also shows the capability of the prediction method of ANN modelling [54].

the adaptive neural Fuzzy inference system (ANFIS) [56], the MatLab computer software, as well as Flynn's [34], Potts and Guy's [35] and Abraham's [57] databases that include MW, log K_{oct} and molecular parameters for the compounds, such as H-bond donor activity (solute summation H-bond acidity, $\sum \alpha_2^H$), H-bond acceptor activity (solute summation H-bond basicity, $\sum \beta_2^H$) and dipolarity or polarizability (π). Three Fuzzy inference models were developed using subtractive clustering to define natural structures within the data and assign subsequent rules. The numeric parameters describing the rules were refined through the use of an ANFIS implemented in the MatLab program [55]. Each model was evaluated using the dataset. The data were divided into two subsets, defined as the training and checking sets, which were used to train the model and then to prevent over-fitting the data. If it is over-trained, the program can memorize data. The model was then evaluated by running the entire dataset through it and the output data were compared with the published experimental data. All databases produced Fuzzy inference models that successfully predicted skin permeability coefficients [55]. Fuzzy rule-based models are a realistic and promising tool that can be used to model and predict skin permeability coefficients as well as (or better than) previous algorithms with fewer inputs.

Biopartitioning micellar chromatography

Quantitative structure–permeability relationships (QSPRs) have been developed and these mathematical expressions are related to the logarithm of the permeability coefficient (log K_p) and several physicochemical parameters such as the log K_{oct}, molecular size descriptors (i.e. molecular mass, molecular volume) and H-bond descriptors. The use of chromatographic parameters in QSPRs (instead of molecular descriptors) gives rise to the quantitative retention–permeability relationships (QRPRs) [58]. One advantage of the QRPR models is that it is easy to predict the effect variables, such as pH, ionic strength, temperature and addition of modifiers and/or enhancers, have on the permeability of drugs. This is of great interest for the pharmaceutical industry, particularly when the effect these variables have on the permeability of compounds needs to be determined to optimize the vehicle features [58]. Several QRPRs for predicting skin permeability have been reported in the literature, including the use of immobilized artificial membrane (IAM) columns [59] and immobilized keratin stationary phases [60]. The success of biopartitioning micellar chromatography (BMC) in constructing these models could be attributed to the similarities between the BMC system and biological barrier and extracellular fluid interphases [61]. The biomicellar partition coefficient is given in Equation 3 [58,61].

$$k_{BMC} = \frac{k_{HA}Kh + k_A}{1 + Kh} \quad \text{[Eqn 3]}$$

In this equation, h represents the retention and the proton concentrations at each pH values and k_{HA} and k_A are fitting parameters representing the BMC retention of the protonated and deprotonated forms of the compound, respectively. K is the fitting parameter corresponding to the protonation constant in this experimental condition. For acidic compounds k_{HA} and k_A correspond to the retention of the neutral and anionic form, respectively, whereas for basic compounds these parameters represent the retention of the cationic protonated and neutral deprotonated base. Finally, a successful model was obtained by using the three significant variables, log k_{BMC}, melting points and molecular weights, as predictor variables. The model successfully predicted skin permeability coefficients [58].

BMC is proposed to be a very useful technique for predicting the effect pH has on the skin permeability of drugs. Using this approach, it is possible to estimate the permeability constants of the ionized and neutral forms of drugs. It was reported that the ionized forms of the compounds contribute to the overall permeability, although the contribution of the neutral forms is approximately one order of magnitude greater [58,61]. The proposed BMC methodology was found to be fast, reproducible, simple and economical, and provides similar results to the conventional *in vivo* approaches that use human skin in compound studies.

Almost all studies concerned with predicting skin permeability have focused on skin permeability coefficients (K_p, cm/h) from aqueous solutions, because permeability values were mostly obtained using saturated aqueous solutions. However, if the maximal flux (J_{max}) for a solute is known its flux from all vehicles can be estimated using its fractional solubility in the vehicle after accounting for vehicle-induced changes in skin permeability [62]. Interestingly, relatively few studies have examined J_{max} and structure relationships using human skin data [63]. The maximum dose of solute able to be delivered over a given period of time and area of application is defined by its J_{max} (per cm^2/hour) from a given vehicle. Recent studies have been performed to calculate J_{max} values from aqueous solutions across human skin. Epidermal K_ps were correlated with the permeant K_{oct}, these (as well as MW) were found to be the dominant determinants of J_{max} for the dataset [63] (Equation 4).

$$\log J_{max} = -3.90 - (0.0190 \times MW) \quad \text{[Eqn 4]}$$

($n = 87$, $r^2 = 0.847$, $p < 0.001$)

Estimated solubility in octanol (S_{oct}) was also a determinant for calculating J_{max} but improvement in the regression by the addition of log S_{oct} was small (r^2 increased to 0.856). Addition of other

physicochemical parameters to MW by forward stepwise regression only marginally improved the regression (with a melting point term $r^2 = 0.879$ and then H-bonding acceptor capability $r^2 = 0.917$). Similar results were obtained for aqueous vehicles with ionizable solutes and solutes from a propylene glycol vehicle [63]. Using J_{max} did not give superior results to the log K_p predictions.

Conclusions

The past decades have given us useful information about how molecules pass across the skin. We have learned a great deal about the mechanisms of skin penetration and effective parameters of skin penetration, the barrier function of the skin and enhancement of skin permeability strategies, and choosing suitable drug candidates for dermal application, among other points. Although there are successful models presented for the dataset studied, we understand that the penetration process through skin is extremely complicated and influenced by several factors. It is not easily possible to point out one particular model as the best model, which might be useful for all drug development studies or strategies. Using more than one model could give more information.

Although the developments mentioned in this review have been made because of our improved understandings and knowledge about penetration properties of compounds through skin, we still know too little about the full impact the physicochemical properties of the penetrant have on the transport (and transport rate) across the skin. More-sophisticated models need to be developed considering more parameters and more possible pathways. This can be achieved in the future by the development of even more-sophisticated experimental and computational techniques.

References

1 Chien, Y.W. (1987) Development of transdermal drug delivery systems. *Drug Dev. Ind. Pharm.* 13, 589–651

2 Katz, M. and Poulsen, B.J. (1971) Absorption of drugs through the skin. In *Handbook of Experimental Pharmacology* (Broie, B.B. and Gillette, J.R., eds), pp. 103–174, Springer–Verlag

3 Barry, B.W. (1983) Structure, function, deseases and topical treatment of human skin. *Dermatological Preparations: Percutaneous Absorbtion* pp. 1–48, Marcel Dekker

4 Bronaugh, R.L. (1990) Metabolism in skin. In *Principles of route-to-routeextrapolation for risk assesment* (Gerrity, T.R. and Henry, C.J., eds), pp. 185–191, Elsevier Science

5 Lynch, D.H. *et al.* (1987) Skin immunology: the Achilles heel to transdermal drug delivery. *J. Cont. Rel.* 6, 39–50

6 Scheuplein, R.J. and Blank, I.H. (1971) Permeability of the skin. *Physiol. Rev.* 51, 702–747

7 Holbrook, K.A. and Odland, G.E. (1974) Regional differences in the thickness (cell layers) of the human stratum corneum: an ultra structural analysis. *J. Invest. Dermatol.* 62, 415–422

8 Menton, D.N. and Eisen, A.Z. (1971) Structure and organisation of mammalian stratum corneum. *J. Ultrastruct. Res.* 35, 247–264

9 Ross, M.H. and Reith, E.J. (1985) The integumentary system: cells of the epidermis. In *Histology: a text and atlas.* Harper & Row, pp. 342–346

10 Amsden, B.G. and Goosen, M.F.A. (1995) Transdermal delivery of peptide and protein drugs: an overview. *AICHE J.* 41, 1972–1997

11 Moffat, C.L.V. (1992) Silicones and transdermal drug delivery. PhD thesis, Welsh School of Pharmacy, University of Wales College of Cardiff, Cardiff, U.K

12 Elias, P.M. *et al.* (1981) Percutaneous transport in relation to stratum corneum structure and lipid composition. *J. Invest. Dermatol.* 76, 297–301

13 Rein, H. (1924) Experimental studies on electroosmosis in surviving human skin. *Z. Biol.* 81, 125–140

14 Michaels, A.S. *et al.* (1975) Drug permeation through human skin: theory and *in vitro* experimental measurement. *AICHE J.* 21, 985–996

15 Degim, I.T. (2005) Understanding skin penetration: computer aided modelling and data interpretation. *Curr. Computer-Aided Drug Design* 1, 11–19

16 Hadgraft, J. *et al.* (1992) Epidermal lipids and topical drug delivery. *Semin. Dermatol.* 11, 139–144

17 Flynn, G.L. *et al.* (1981) Permeation of hairless mouse skin II: membrane sectioning techniques and influence on alkanol permeabilities. *J. Pharm. Sci.* 70, 52–56

18 Roy, S.D. and Flynn, G.H. (1989) Transdermal delivery of narcotic analgesics - comperative permeabilities of narcotic analgesics through the human cadaver skin. *Pharm. Res.* 6, 825–832

19 Rougier, A. and Lotte, C. (1993) Predictive approaches I: the tape stripping technique. *Topical drug Bioavailability, Bioequivalence and Penetration,* pp. 163–181, Plenium Press

20 Knutson, K. *et al.* (1985) Macro and molecular physical-chemical consideration in understanding drug transport in the stratum corneum. *J. Cont. Rel* 2, 67–87

21 Guy, R.H. and Hadgraft, J. (1987) Transdermal drug delivery: a perspective. *J. Cont. Rel.* 4, 237–251

22 Guy, R.H. and Hadgraft, J. (1985) Transdermal drug delivery: the ground rules are emerging. *Pharm. Int.* 6, 112–116

23 Landmann, L. (1986) Epidermal permeability barrier- transformation of lamellar granule-disks into intercellular sheets by a membrane-fusion process-a freeze fracture study. *J. Invest. Dermatol.* 87, 202–209

24 Schurer, N.Y. and Elias, P.M. (1991) The biochemistry and function of stratum corneum: Lipids. *Adv. Lipid Res.* 24, 27–55

25 Kerscher, M. and Korting, H.C. (1991) Skin ceramides: structure and function. *Eur. J. Dermatol.* 1, 39–43

26 Imokawa, G. *et al.* (1989) Importance of intercellular lipids in water retention properties of stratum corneum: Induction and recovery study of surfactant dry skin. *Arch. Dermatol. Res.* 281, 45–51

27 Scheuplein, R.J. (1965) Mechanism of percutaneous absorption I: Routes of penetration and the influence of solubility. *J. Invest. Dermatol.* 45, 334–346

28 Gummer, C.L. (1989) The *in vitro* evaluation of transdermal delivery. In *Transdermal drug delivery – Developmental Issues and Research Initiatives* (Hadgraft, J. and Guy, R.H., eds), pp. 177–196, Marcel Dekker

29 Friend, D.R. (1992) *In vitro* skin permeation techniques. *J. Cont. Rel.* 18, 235–248

30 Degim, I.T. *et al.* (1999) The effects of Azone and capsaicin on the permeation of naproxen through human skin. *Int. J. Pharm.* 179, 21–25

31 Sebastiani, P. *et al.* (2005) Effect of lactic acid and iontophoresis on drug permeation across rabbit ear skin. *Int. J. Pharm.* 292, 119–126

32 Jacobi, U. *et al.* (2005) Comparison of four different *in vitro* systems to study the reservoir capacity of the stratum corneum. *J. Cont. Rel.* 103, 61–71

33 Leveque, N. *et al.* (2004) Comparison of Franz cells and microdialysis for assessing salicylic acid penetration through human skin. *Int. J. Pharm.* 269, 323–328

34 Flynn, G.L. (1990) Physicochemical determinants of skin absorption. In *Principles of route-to-route extrapolation for risk asessment* (Gerrity, T.R. and Henry, C.J., eds), pp. 93–127, Elsevier Science

35 Potts, R.O. and Guy, R.H. (1992) Predicting skin permeability. *Pharm. Res.* 9, 663–669

36 Roberts, M.S. *et al.* (1995) Relationship between H-bonding of penetrants to stratum corneum lipids and diffusion within the stratum corneum. In *Prediction of Percutaneous Penetration*, (vol. 4a) (Brain, K.R. et al. eds), p. 26, STS Publishing

37 Pugh, W.J. and Hadgraft, J. (1994) *Ab initio* prediction of human skin permeability coefficients. *Int. J. Pharm.* 103, 163–178

38 Roberts, M.S. *et al.* (1995) Epidermal permeability-penetrant structure relationships: 1. An analysis of methods of predicting penetration of monofunctional solutes from aqueous solutions. *Int. J. Pharm.* 126, 219–233

39 Politzer, P. and Murray, J.S. (1994) A general interaction properties function (GIPF): an approach to understanding and predicting molecular interactions. In *Quantitative Treatments of Solute/Solvent Interaction,* (Politzer, P. and Murray, J.S., eds), pp. 243–290, Elsevier

40 Pugh, W.J. *et al.* (2000) Epidermal permeability–penetrant structure relationships: 4, QSAR of permeant diffusion across human stratum corneum in terms of molecular weight, H-bonding and electronic charge. *Int. J. Pharm.* 197, 203–211

41 Lien, E.J. and Tong, G.L. (1973) Physicochemical properties and percutaneous absorption of drugs. *J. Soc. Cosmet. Chem.* 24, 371–384

42 Roberts, M.S. *et al.* (1977) Permeability of human epidermis to phenolic compounds. *J. Pharm. Pharmacol.* 29, 677–683
43 Roberts, M.S. (1991) Structure-permeability considerations in percutaneous absorption. In *Prediction of Percutaneous Penetration - Methods, Measurement and Modelling* (Scott, R.C. et al. eds), pp. 210–228, IBC Technical Services
44 el Tayar, N. *et al.* (1991) Percutaneous penetration of drugs: a quantitative structure-permeability relationship study. *J. Pharm. Sci.* 80, 744–749
45 Abraham, M.H. *et al.* (1995) The factors that influence skin penetration of solutes. *J. Pharm. Pharmacol.* 47, 8–16
46 Pugh, W.J. *et al.* (1996) Epidermal permeability-penetrant structure relationships: 3. The effect of hydrogen bonding interactions and molecular size on diffusion across the stratum corneu. *Int. J. Pharm.* 138, 149–165
47 Wilschut, A. *et al.* (1995) Estimating skin permeation. The validation of five mathematical skin permeation models. *Chemosphere* 30, 1275–1296
48 Pugh, W.J. *et al.* (2000) Epidermal permeability-penetrant structure relationships: 4. QSAR of permeant diffusion across human stratum corneum in terms of molecular weight, H-bonding and electronic charge. *Int. J. Pharm.* 197, 203–211
49 Degim, I.T. *et al.* (1998) Skin permeability data: anomalous results. *Int. J. Pharm.* 170, 129–133
50 Mitragotri, S. (2003) Modeling skin permeability to hydrophilic and hydrophobic solutes based on four permeation pathways. *J. Control. Release* 86, 69–92
51 Mitragotri, S. (2002) A theoretical analysis of permeation of small hydrophobic solutes across the stratum corneum based on scaled particle theory. *J. Pharm. Sci.* 91, 744–752
52 Ho, C.K. (2004) Probabilistic modeling of percutaneous absorption for risk-based exposure assessments and transdermal drug delivery. *Statistical Methodology* 1, 47–69
53 Lim, C. *et al.* (2002) Prediction of human skin permeability using a combination of molecular orbital calculations and artificial neural network. *Biol. Pharm. Bull.* 25, 361–366
54 Degim, T. *et al.* (2003) Prediction of skin penetration using artificial neural network (ANN) modeling. *J. Pharm. Sci.* 92, 656–664
55 Pannier, A.K. *et al.* (2003) Fuzzy modeling of skin permeability coefficients. *Pharm. Res.* 20, 143–148
56 Jang, J-S.R. (1993) ANFIS: adaptive-network-based fuzzy inference System. *IEEE Trans. on System Man. and Cybernetics* 23, 665–685
57 Abraham, M.H. *et al.* (1997) Algorithms for skin permeability using hydrogen bond descriptors: the problem of steroids. *J. Pharm. Pharmacol.* 49, 858–865
58 Martinez-Pla, J.J. *et al.* (2003) Biopartitioning micellar chromatography to predict skin permeability. *Biomed. Chromatogr.* 17, 530–537
59 Nasal, A. *et al.* (1995) Hydrophobicity parameter from high-performance liquid chromatography on an immobilized artificial membrane column and its relationship to bioactivity. *J. Chromatogr. A.* 692, 83–89
60 Turowski, M. and Kaliszan, R. (1997) Keratin immobilized on silica as a new stationary phase for chromatographic modeling of skin permeation. *J. Pharm. Biomed. Anal.* 15, 1325–1333
61 Martinez-Pla, J.J. *et al.* (2004) Evaluation of the pH effect of formulations on the skin permeability of drugs by biopartitioning micellar chromatography. *J. Chromatogr. A.* 1047, 255–262
62 Roberts, M. *et al.* (2002) Skin transport. In *Dermatological and Transdermal Formulations* (Walters, K.A., ed.), pp. 89–195, Marcel Dekker
63 Magnusson, B.M. *et al.* (2004) Molecular size as the main determinant of solute maximum flux across the skin. *J. Invest. Dermatol.* 122, 993–999