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Validated Models for Predicting Skin Penetration from Different Vehicles

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Abstract

The permeability of a penetrant though skin is controlled by the properties of the penetrants and the mixture components, which in turn relates to the molecular structures. Despite the well investigated models for compound permeation through skin, the effect of vehicles and mixture components has not received much attention. The aim of this Quantitative Structure Activity Relationship (QSAR) study was to develop a statistically validated model for the prediction of skin permeability coefficients of compounds dissolved in different vehicles. Furthermore, the model can help with the elucidation of the mechanisms involved in the permeation process. With this goal in mind, the skin permeability of four different penetrants each blended in 24 different solvent mixtures were determined from diffusion cell studies using porcine skin. The resulting 96 kp values were combined with a previous dataset of 288 kp data for QSAR analysis. Stepwise regression analysis was used for the selection of the most significant molecular descriptors and development of several regression models. The selected QSAR employed two penetrant descriptors of wiener topological index and total lipole moment, boiling point of the solvent and the difference between the melting point of the penetrant and the melting point of the solvent. The QSAR was validated internally, using a leave-many-out procedure, giving a mean absolute error of 0.454 for the log kp value of the test set.

Keywords: Skin, penetration, QSAR, permeation, formulation, mixture
1. Introduction

Skin is the largest organ of all mammals protecting the underlying muscles, bones, ligaments and internal organs as well as guarding the whole organism from exogenous molecules. Within skin the outermost layer, stratum corneum, is the formidable barrier to the exogenous compounds, limiting penetration of toxicants and drugs alike. Skin penetration of chemicals is an integral part of human health risk assessment of chemicals exposed via the dermal route (Shah, 1993). Skin has also been the focus of research by drug formulators as a site of drug administration, due to the advantages it may offer over other routes of drug delivery (Barry, 2007). Topical delivery affects the tissues under the site of application, while systemic delivery has an effect after distribution to the circulatory system. The rate of drug delivery through skin is influenced by factors including skin health status, age, race, anatomical region, thermodynamic activity of the drug in the formulation and interactions of the drug and formulation with the skin. Drug in the formulation needs to pass the stratum corneum’s intercellular lipids that surround dead keratin-filled corneocytes and also the subcutaneous fat to reach the blood capillaries (Elias, 1983).

Penetration of a compound into skin is controlled by its physicochemical properties and the chemical structure. For example, it has been shown that lipophilicity and hydrogen bonding ability of a compound plays a major role on the skin absorption profile (Pugh et al., 1996; El-Tayar et al. 1991). On the other hand, formulation ingredients can alter the skin penetration of a compound by affecting the barrier properties of the skin or by changing the partitioning of the compound into the SC. Therefore, penetration of the drug depends not only on the nature of the drug but also on the nature of the other ingredients present in the formulation. The vehicle in which a penetrant is dissolved or dispersed is of outmost significance. Vehicles can affect
skin permeability by a range of mechanisms including delipidization, hydration, fluidization and desmosome disruption in the stratum corneum, and also by changing the polarity of the formulation mixture which is followed by a change in the penetrant solubility and partitioning to stratum corneum (Roberts et al., 2002). Solvents are also likely to affect the conformation of stratum corneum in a way that the diffusion and partitioning of the penetrants are modified (Kai et al., 1990; Raykar et al., 1988; Rosado et al., 2003). Pure solutes can in some cases enhance the skin permeability by a direct corrosive effect (Roberts et al. 2002; Zinke et al. 2002). Other common mixture components are surfactants and, in case of drug formulations, penetration enhancers. Surfactants are used in the pharmaceutical/cosmetic preparations, agrochemical products (e.g. herbicides) and industrial solutions. In industry surfactants are added to formulations in order to solubilise lipophilic active ingredients, and in transdermal drug delivery to solubilise lipids within the stratum corneum. Penetration enhancers may increase the diffusion coefficient of drugs in the stratum corneum (i.e. disrupt the barrier nature of the stratum corneum), may act to increase the effective concentration of the drug in the vehicle (for example acting as an anti-solvent), could improve partitioning between the formulation and the stratum corneum (perhaps by altering the solvent nature of the skin membrane to improve partitioning into the tissue) or, less likely, by decreasing the skin thickness (perhaps by providing a permeation ‘shortcut’ as opposed to a tortuous pathway for a permeant) (Williams, Barry, 2004).

The effect of mixture/formulation components on the skin penetration of a compound depends on the nature of the component, i.e. its chemical structure and physicochemical properties. In other words, chemical structure of a formulation component can determine the effect that it will have on the stratum corneum or on the partitioning of the penetrant, leading to the observed changes in the skin penetration profile of the penetrant. The relationship between chemical
structures of the formulation ingredients and the skin penetration modification can be studied quantitatively using Quantitative Structure-Activity Relationship (QSAR) techniques. QSAR has been previously applied to study the effect of structural variation of chemical enhancers on the skin penetration of various drugs (Ghafourian et al., 2004; Moss et al., 2002).

Most mechanistic studies on skin penetration are based on the penetration of individual chemicals (Flynn, 1990), with only few attempts towards a comprehensive investigation on the effect of chemical mixtures. Such a systematic study requires a large volume of tedious experimental measurements involving various penetrant/mixture-component combinations. Riviere and Brooks (2005, 2007) have determined skin permeation coefficient of 12 compounds from a mixture of several solvents, a surfactant and methyl nicotinic acid (288 combinations). A QSAR analysis of the data revealed several penetrant/solvent properties that are significant contributors to the skin permeation coefficients (Ghafourian et al., 2010). The study also revealed several gaps in the chemical space of Riviere’s penetrants in comparison with the well established datasets of Flynn (1990) and Wilschut et al. (1995) which contain skin penetration data of aqueous solutions of over 100 compounds. In this investigation, four chemicals were selected from Flynn and Wilschut et al. datasets for further skin penetration studies using Riviere’s experimental protocol which involves blending of each chemical with 24 mixture combinations. The selections were made from the identified gaps in the chemical space and the compounds are expected to add a high level of diversity to the dataset. These new measurements facilitated the development of statistically validated QSAR models. Statistically validated QSAR models can be used for the estimation of skin penetration of new compounds or the effect of new mixture components on the penetration of a penetrant. The models can aid the understanding of the mechanisms involved in skin penetration of compounds and the effect of mixture components.
2. Material and Methods

*Material:* Caffeine [8-14C] Specific Activity: 50-60 mCi/mmol 1.85-2.22 GBq/mmol, Octanol-n [1-14C] Specific Activity : 2-10 mCi/mmol 74-370 MBq/mmol, Testosterone [4-14C] Specific Activity : 50-60 mCi/mmol 1.85-2.22 GBq/mmol, Codeine [N-methyl-14C], obtained from American Radiolabeled Chemicals, Inc, St. Louis USA. Absolute ethyl alcohol was obtained from Aaper Alcohol and Chemical Co. Shelbyville, KY, USA. Propylene glycol (purity = 99%), Sodium lauryl sulphate (purity = 99%), and Methyl nicotinic acid (purity = 99%) were obtained from Sigma Chemical Co. St. Louis, MO, USA. Water was distilled in our in-house still.

*Skin penetration studies:* Apparent permeability coefficient (kp) of caffeine, codeine, octanol and testosterone each blended in 24 different mixtures, as presented in Table 1, were obtained through flow-through diffusion cell using porcine skin. The flow-through diffusion cell was used to perfuse skin obtained from the dorsal area of weanling female Yorkshire pigs according to protocols approved by the North Carolina State University Institutional Animal Care and Use Committee. Skin was dermatomed to a thickness of 500 µm with a Padgett dermatome. Each circular skin disk was punched to provide a dosing surface area of 0.64 cm² and then placed into a two-compartment Teflon Bronaugh flow-through diffusion cell. Skin was perfused using a Krebs-Ringer bicarbonate buffer spiked with dextrose and bovine serum albumin, and topically dosed nonoccluded with 20 µl of one of the four marker penetrant compounds (10µg/cm²) formulated in one of 24 specified mixtures listed in Table 1. This resulted in a total of 96 treatments with n = 4-5 replicates/treatment designed as a randomized complete factorial experiment.
**QSAR studies:** The kp values measured in this study for caffeine, codeine, octanol and testosterone were merged with the previous dataset of logkp values for 12 other compounds blended with the same mixture components as Table 1 (Riviere, Brooks, 2005). These log kp values are measured using the same experimental procedures as in this study. Therefore, the dataset used for the QSAR studies consisted of a total of 384 unique measurements of kp for the penetrant/ components combinations. Table 2 is the list of the 16 penetrants used in QSAR study.

For the development of QSAR models, properties of the penetrants and the solvent mixtures were assembled. The molecular descriptors (properties) of the penetrants were calculated using two software packages of ACD labs/LogD Suite (7.0.5 release) and TSAR 3D (Accelrys Ltd version 3.3). The molecular descriptors included octanol/ water partition coefficient, molecular connectivity indexes, quantum molecular descriptors, and various atom and group counts. The physicochemical properties of mixture components including boiling point, melting point, solubility, vapour pressure and Henry’s law constant were obtained through ChemBioFinder (CambridgeSoft, 2009) online software and SRC PhysProp database (Syracuse Research Corporation, 2009). Hildebrand solubility parameters (δ) were obtained from Hansen (1967) for the solvents and calculated according to Fedors group contribution method (1974) for the penetrants. As there was a mixture of a number of solvents in the vehicles, averages of physicochemical properties for solvent mixtures were calculated using the fractions of each component.

Stepwise regression analysis was performed between log kp as the dependant variable and the molecular descriptors of the penetrants and the mixture components as the predictors. This enabled the identification of the significant molecular descriptors affecting skin penetration of chemicals. Several stepwise regression analyses using various sets of penetrant molecular
descriptors and solvent properties were performed and several regression models were generated. In order to minimise the risk of chance correlations, the number of descriptors in the regression models were limited to four.

The models were validated for penetrants using a leave-many-out cross validation procedure. To do this, the penetrants were divided into 4 groups with similar ranges of lipophilicity (log P values) in each group. Regression analyses were performed four times, each time leaving one group out. The logkp values of the test sets were estimated using the equations obtained for the training sets and the mean absolute error was calculated from the difference between the observed and the predicted log kp values of the test sets.

3. Results and Discussion

Skin penetration of drugs is controlled by the molecular structures and physicochemical properties of the intended penetrants and the mixture ingredients in the vehicle. In order to rationalize the combined effect of structural characteristics of the penetrants and the physicochemical properties of the mixture components, this investigation focused on the QSAR model development for a dataset of skin permeation of chemicals dissolved into a combination of several solvents, surfactant and methyl nicotinic acid. Permeation coefficients were measured for four compounds that were rationally selected in order to add a high level of diversity to the existing dataset (Ghafourian et al. 2010). Tables 1 and 2 provide the list of the vehicles and the permeants, respectively. The kp data measured in this investigation (n = 96) was merged with the previously obtained dataset of kp (n = 288) and the resulting dataset was used for the QSAR development (Riviere, Brooks, 2010) Stepwise regression analysis of different combinations of solvent properties and molecular descriptors of the penetrants resulted in a number of QSAR models from which 4 were selected based on the goodness of
fit ($R^2$ values). In order to reduce the risk of chance correlations, only four descriptors were allowed in the equations. The selected equations have been listed in Table 3. In equations 1-4, the letter in the brackets indicates if the variable is a descriptor for the penetrant (P) or for the vehicle (V). It can be seen that each equation consists of 2-3 penetrant descriptors and 1-2 vehicle descriptors, with equations 1-3 containing 1 combined vehicle-penetrant descriptor. In equations 1-4, $\Delta m_p$ is the difference between the melting point of the penetrant and that of the solvent, $W$ is the Wiener topological index (the sum of distances between all pairs of vertices in the molecular graph of an alkane (Diudea, Gutman, 1998)), $\delta$ is the Hildebrand solubility parameter, $E_{\text{HOMO}}$ is the energy of the highest occupied molecular orbital, BP is the boiling point, $N_{\text{atoms}}$ is the total number of atoms in the molecules, $BP-\text{MP}$ is the difference between the boiling and melting points of a compound, and Lipole is the total lipole moment of the penetrants.

Considering that $N_{\text{atoms}}$ and $W$ (Diudea, Gutman, 1998) can be regarded as size descriptors, it can be seen from Table 3 that all QSAR models indicate the negative effect of the penetrant’s molecular size on the log $k_p$. Moreover, there is a negative contribution by total lipole of the penetrants in equations 1-4. Total lipole is a measure of lipophilicity distribution calculated as sum of local values of log $P$, like dipole moment (Pedretti et al., 2002). It shows lipophilicity of the molecule in a specific direction. Surfactants are expected to have high total lipole values and they are known enhancers of drug skin penetration (Ma et al., 2007). Thus, according to these equations, the less lipolar penetrants will have higher permeation rates. Chlorpyrifos has the highest total lipole value of 10.0 and caffeine has the lowest value of 0.19.

The other penetrant descriptor, which can be seen in majority of the equations, is $E_{\text{HOMO}}$. This molecular descriptor represents the energy of the highest occupied molecular orbital. $E_{\text{HOMO}}$
measures the nucleophilicity of a molecule. The negative relationship of this descriptor with the logarithm of the permeation rate indicates that the electron rich nucleophilic compounds such as those containing aromatic rings are the least permeable. In equations 2 and 3, the product of the penetrants’ $E_{\text{HOMO}}$ and the vehicles’ solubility parameter is used. $\delta(V).E_{\text{HOMO}}(P)$ is a solvent/penetrant interaction term. This descriptor indicates that a highly nucleophilic penetrant will have a lower penetration from highly associated vehicles, i.e. those vehicles with high intermolecular interaction forces such as hydrogen bonding.

The most persistent vehicle descriptor in the QSARs is the boiling point, with a negative effect on permeation rate of chemicals. Solubility parameter is also present in some equations. Both solubility parameter and boiling point can represent the intermolecular interaction energy of the vehicle which can result from the polarity of the solvents. Therefore the negative relationship indicates a higher skin permeation rate with the less polar vehicles. Similar results have been shown previously where the permeation coefficients of highly lipophilic compounds, nicardipine and nimodipine was increased in the less polar solvent mixtures of ethanol-water (Krishnaiah et al., 2002; Krishnaiah et al., 2004). In the case on nimopidine, 60:40 (v/v) ratio of ethanol: water was an optimum solvent mixture leading to the highest permeation rate of nimopidine, with the $k_p$ dropping slightly at higher ethanol concentrations (Krishnaiah et al., 2004). Solubility parameters of the solvents and the permeants have been implicated as important factors controlling skin penetration of compounds (Dias et al., 2007; Roy, Flynn, 1989). From the parabolic relationship between skin permeation rate and the solubility parameter it has been concluded that the skin has a solubility parameter of around 10 (cal/cm$^3$)$^{1/2}$ (Liron, Cohen, 1984). In equations 2-4, solubility parameter of the vehicle has been selected by stepwise regression analysis as a significant contributor to skin permeation rate. The solvent mixtures in this study have solubility parameters of $>12$. Therefore, it is
expected that the lower the δ value of the vehicle, the closer the value to the skin δ and therefore the higher the permeation constant should be. In equations 2 and 3, \( \delta(V)E_{HOMO}(P) \) has a negative effect on the skin absorption, implying a lower skin absorption of nucleophilic drugs from polar solvents, as explained before.

In equation 1, the difference between melting points of the vehicle and penetrant has been selected as the most significant of all descriptors. The negative coefficient of the descriptor \( \Delta mp \) indicates that the melting point of the penetrants should be close to the melting point of the vehicle for a better skin absorption. Therefore, since the vehicles are all liquids, this implies that penetrants with low melting points are likely to have a higher absorption rates. This finding is in agreement with a previous observation where it has been shown for two optical isomers of ibuprofen that the low melting point S enantiomer has a higher skin permeation rate than the high melting point R enantiomer (Cilurzo et al., 2010). A similar conclusion has been made in a different investigation, when it was observed that among the alkyl analogues of cyclizine the analogue with the lowest melting point, and not the most lipophilic one, showed the highest skin penetration rate (Monene et al. 2005). Melting point has a similar effect on the intestinal absorption of drugs with low melting point drugs generally showing a higher fraction of dose absorbed from GI tract (Chu, Yalkowsky 2009).

In equation 4, the descriptor \( BP-MP(V) \) with negative coefficient indicates that penetration rate is slower from solvents with large boiling and melting point gaps. The difference between these two properties has been attributed to the molecular symmetry, with highly symmetrical molecules having much larger melting points and decreased boiling points (Slovokhotov, 2007). In the solvents used in this study, the biggest difference in melting and boiling points is for propylene glycol. Therefore the vehicles containing higher concentrations of this solvent
will have higher difference between melting and boiling points, leading to lower penetration of the penetrants.

Table 3 shows that equation 1 with the highest $R^2$ and the lowest S value has the best fit to the data. The goodness of fit is reduced from equation 1 to 4. The predictive powers of the equations were tested by an internal validation procedure explained in the methods section. Table 4 shows the statistical parameters obtained from this exercise. It must be noted that during validation tests, each set of four penetrants (dissolved in any solvent mixture) were removed once as the test set and the equation obtained for the remaining 12 penetrants were used for the estimation of the log kp of the test set. Thus, Table 4 represents the results of such log kp estimations for all the four test sets (each set containing 4 penetrants). According to the table, the predictivity of equation 1 is the highest among all the equations with a mean absolute error of 0.454. Equations 2-4 show slightly higher prediction errors for the test sets, with MAE increasing in the order of eq. 4 > eq. 2 > eq3. Therefore, it appears that the QSAR model 1 is robust in terms of prediction of log kp for those new penetrants that fall within the applicability domain of the model. Figure 1 is the plot of observed versus predicted log kp values using Equation 1. The outliers in the graph with underestimated log kp values are testosterone dissolved in different vehicles, most notably in ethanol-water, ethanol-water-methyl nicotinate, water and water-methyl nicotinate. Testosterone is the largest molecule in the dataset in terms of the total number of atoms and the topological index W, where the negative coefficient of W in equation 1 describes the negative effect of molecular size on absorption. Testosterone is also the most affected penetrant by the variation in solvent mixture, as it presents the largest gap between the highest and the lowest kp from different mixture components. Considering that only kp values from certain mixtures components is higher than expected, it is concluded that
the enhancing effects of these vehicles are not fully accounted for by the model and further investigations are required to explain the observed effect.

References


Table 1: Composition of the 24 mixtures

<table>
<thead>
<tr>
<th>Composition</th>
<th>PG</th>
</tr>
</thead>
<tbody>
<tr>
<td>EtOH</td>
<td>PG</td>
</tr>
<tr>
<td>EtOH + MNA</td>
<td>PG + MNA</td>
</tr>
<tr>
<td>EtOH + SLS</td>
<td>PG + SLS</td>
</tr>
<tr>
<td>EtOH + MNA + SLS</td>
<td>PG + MNA + SLS</td>
</tr>
<tr>
<td>EtOH + Water</td>
<td>PG + Water</td>
</tr>
<tr>
<td>EtOH + Water + MNA</td>
<td>PG + Water + MNA</td>
</tr>
<tr>
<td>EtOH + Water + SLS</td>
<td>PG + Water + SLS</td>
</tr>
<tr>
<td>EtOH + Water + MNA + SLS</td>
<td>PG + Water + MNA + SLS</td>
</tr>
<tr>
<td>EtOH + PG + Water</td>
<td>Water</td>
</tr>
<tr>
<td>EtOH + PG + Water + MNA</td>
<td>Water + MNA</td>
</tr>
<tr>
<td>EtOH + PG + Water + SLS</td>
<td>Water + SLS</td>
</tr>
<tr>
<td>EtOH + PG + Water + MNA + SLS</td>
<td>Water + MNA + SLS</td>
</tr>
</tbody>
</table>

EtOH-Ethanol; PG-Propylene glycol; MNA-Methyl nicotinate; SLS-Sodium lauryl sulfate.
Table 2: Penetrants

<table>
<thead>
<tr>
<th>Atrazine</th>
<th>Pentachlorophenol</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chlorpyrifos</td>
<td>Phenol</td>
</tr>
<tr>
<td>Ethylparathion</td>
<td>ρ-Nitrophenol</td>
</tr>
<tr>
<td>Fenthion</td>
<td>Propazine</td>
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<tr>
<td>Methylparathion</td>
<td>Simazine</td>
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<tr>
<td>Nonylphenol</td>
<td>Triazine</td>
</tr>
<tr>
<td>Caffeine</td>
<td>Octanol</td>
</tr>
<tr>
<td>Codeine</td>
<td>Testosterone</td>
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</table>
Table 3. QSAR models obtained from stepwise regression; N is the number of datapoints (penetrant/ vehicle combinations); S, the standard deviation; $R^2$, the squared correlation coefficient

<table>
<thead>
<tr>
<th>EQ</th>
<th>$Logk_p = -0.956 - 0.00322 \Delta m_p - 0.000320 \ W(P) - 0.0121 \ BP(V) - 0.114 \ Lipole(P)$</th>
<th>N</th>
<th>S</th>
<th>$R^2$</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td></td>
<td>384</td>
<td>0.478</td>
<td>0.701</td>
</tr>
<tr>
<td>2</td>
<td>$Logk_p = -310 - 0.000315 \ W(P) - 0.00771 \ \delta(V).E_{HOMO}(P) - 0.0102 \ BP(V) - 0.0750 \ Lipole(P)$</td>
<td>384</td>
<td>0.494</td>
<td>0.681</td>
</tr>
<tr>
<td>3</td>
<td>$Logk_p = -2.48 - 0.0474 \ N_{atoms}(P) - 0.00798 \ \delta(V).E_{HOMO}(P) - 0.0102 \ BP(V) - 0.0723 \ Lipole(P)$</td>
<td>384</td>
<td>0.516</td>
<td>0.653</td>
</tr>
<tr>
<td>4</td>
<td>$Logk_p = -4.29 - 0.0474 \ N_{atoms}(P) - 0.00904 \ BP-MP(V) - 0.345 \ E_{HOMO}(P) - 0.0790 \ Lipole(P)$</td>
<td>384</td>
<td>0.522</td>
<td>0.644</td>
</tr>
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</table>
Table 4. Statistical parameters obtained from internal validation of QSAR equations 1-4; N is the number of datapoints, s is the standard deviation and $R^2$ is the squared correlation coefficient between observed and predicted log kp for the test sets, and MAE is mean absolute error of prediction

<table>
<thead>
<tr>
<th>Equation</th>
<th>N</th>
<th>S</th>
<th>$R^2$</th>
<th>MAE</th>
</tr>
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<tr>
<td>1</td>
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<td>0.454</td>
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<td>0.605</td>
<td>0.517</td>
<td>0.497</td>
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<tr>
<td>4</td>
<td>384</td>
<td>0.618</td>
<td>0.497</td>
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</table>
Figure 1. Scatterplot of observed log kp vs. log kp calculated by equation 1