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Epidermal permeability–penetrant structure relationships: 4, QSAR of permeant diffusion across human stratum corneum in terms of molecular weight, H-bonding and electronic charge

W. John Pugh *, I. Tuncer Degim [1], Jonathan Hadgraft

The Welsh School of Pharmacy, Cardiff University, King Edward VII Avenue, Cardiff, CF1 3XF, UK

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Abstract

Principal components analysis (PCA) and multivariate regression analysis (MRA) are used to assess the predictors of permeant diffusion across human stratum corneum. Log (D/h), was estimated from $\log k_p + 0.024 - 0.59 \log K_{oct}$, where D = diffusion coefficient (cm^2/h), h = path length (cm), k_p permeability coefficient (cm/h), K_{oct} = partition coefficient (octanol/water). Molecular weight (MW) with (1) scaled H-bonding parameters α and β, or (2) summed modulus of partial charge from molecular modelling were tested as predictors of (D/h). Charge may be computed for any molecule, whilst α and β values are generally unavailable for molecules of biological interest. PCA suggests a dominant permeation pathway since 93% of data variation is in PC1 of log (D/h), MW and charge and 82% in PC1 of log (D/h), MW, α and β. MRA using MW, α and β is unsatisfactory because of collinearity amongst predictors. The best predictor was the product MW*charge. Similarity of the eigenvectors in PCA and normalised coefficients in MRA indicates that charge and MW are equally important predictors of diffusion. © 2000 Elsevier Science B.V. All rights reserved.

Keywords: Principal component analysis; Multivariate regression analysis; Human stratum corneum; Partial charge; Molecular modelling; Diffusion

1. Introduction

Quantitative structure activity relationships (QSARs) are useful in predicting behaviour of novel compounds and providing insights into mechanisms of activity. In transdermal studies the technique is often based on multivariate regression analysis 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 (Lien and Tong, 1973; Roberts et al., 1977; Roberts, 1991; El Tayar et al., 1991) were limited to small data

* Corresponding author.

E-mail address: pugh@cardiff.ac.uk (W.J. Pugh)

[1] Present address: Gazi University, Faculty of Pharmacy, Department of Pharmaceutical Technology, 06330, Etiler, Ankara, Turkey.

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sets until Flynn (1990) published a collection of over 90 k_p and $\log K_{oct}$ values which formed the basis of prediction of $\log k_p$ from MW and $\log K_{oct}$ (Potts and Guy, 1992), functional group contributions (Pugh and Hadgraft, 1994), solvatochromic parameters (Abraham et al., 1995; Roberts et al., 1995), and Hildebrand solubility parameters (Roberts et al., 1995). Pugh et al. (1996) criticised the use of the composite term, k_p, and reported the dependency of diffusion across the SC on MW and the scaled H-bonding values α and β. Wilschut et al. (1995) questioned the reliability of some of Flynn's data and applied inclusion criteria for k_p values. Disquiet about data reliability and MRA without consideration of collinearities and interactions amongst predictors led us to conduct fresh analyses using PCA and MRA.

2. Methods

2.1. Partial charge calculation

k_p values were obtained from Wilschut et al. (1995) except atropine, naproxen and nicotine (Degim et al., 1998), $\log K_{oct}$ values were from Medchem (Biobyte, Claremont, CA). Scaled H-bonding values, α and β were from Abraham (1993) and Abraham et al. (1995). Molecular modelling was performed using the NEMESIS V1.0 package (Oxford Molecular, Oxford, UK). Conformation analysis using a step size of 30° was used to find the approximate energy minimum conformation, followed by optimisation 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 π systems. The partial charges of the atoms (H, C, O, N and halogen) constituting the molecule were noted. Various combinations of these charges were tried in the statistical analyses, but none gave superior results to the simple sum of the modulus of the charges. These summed charges are given in Table 1. $\text{Log}\,(D/h)$, was estimated from $\log k_p + 0.024 - 0.59 \log K_{oct}$ (Pugh et al., 1996).

2.2. Statistical analyses

Statistical analyses were performed with Minitab release 10.5 (Minitab, State College, PA, USA). The tests are described in detail in standard texts to which the interested reader should refer for authoritative accounts-see for example Cureton and D'Agostino (1983), Everitt and Dunn (1991), Hair et al. (1995).

3. Results

Permeants and associated properties are in Table 1. Principal components analysis (PCA) results are in Table 2. Regression analysis results (MRA) are presented in Section 4 as required. Probabilities that the predictor coefficients are not significant are shown in italics. Coefficients of determination (R^2) are adjusted for degrees of freedom (Minitab Reference Manual, Minitab).

4. Discussion

4.1. Principal components analysis (PCA)

This detects relationships called principal components (PCs) amongst the variables in a table (matrix) that account for the data variation. Consider the PCA relating $\log (D/h)$, MW, α and β as an example (Table 2a).

The sum of eigenvalues is the number of PCs (4). The eigenvalue of a PC shows the proportion of the total variation in the matrix attributable to that PC. Thus if $\log (D/h)$ were completely determined by a single process involving MW, α and β then PC1 would have an eigenvalue of 4 and PCs 2, 3, 4 would all be zero. This proportion for PC1 is 0.82 (i.e. 3.27/4). Within PC1 the eigenvector of a variable indicates how much of the variation in data is attributable to that variable. The communality,

Table 1
Data used in the analysis[a]

	MW	Log k_p	Log K_{oct}	Log D/h	α	β	Charge
1 3-nitrophenol	139.1	−2.25	2.00	−3.40	0.79	0.23	1.227
2 4-nitrophenol	139.1	−2.25	1.91	−3.36	0.82	0.26	1.235
3 aldosterone	360.4	−4.24	1.08	−4.85	0.40	1.90	3.826
4 amobarbital	226.3	−2.64	1.96	−3.77	*	*	2.680
5 atropine	289.4	−3.25	1.83	−4.31	*	*	2.939
6 barbital	184.2	−3.95	0.65	−4.31	*	*	2.413
7 benzyl alcohol	108.1	−1.77	1.10	−2.39	0.35	0.50	1.362
8 4-bromophenol	170.3	−1.44	2.59	−2.95	0.67	0.20	1.181
9 butanol	74.12	−2.52	0.80	−2.97	0.37	0.48	1.226
10 butanol	74.12	−2.60	0.80	−3.05	0.37	0.48	1.226
11 2-chlorophenol	128.6	−1.48	2.15	−2.73	*	*	1.180
12 chlorpheniramine	274.8	−2.66	3.39	−4.63	*	*	2.030
13 codeine	299.4	−4.31	1.14	−3.96	*	*	2.716
14 corticosterone	345.5	−3.53	1.94	−4.64	0.40	1.63	3.689
15 decanol	158.3	−1.10	4.57	−3.77	0.37	0.48	1.778
16 2,4-dichlorophenol	163.0	−1.22	3.06	−3.00	*	*	1.205
17 diethylcarbamazine	218.2	−3.89	1.75	−4.89	*	*	2.016
18 ephedrine	165.2	−2.22	0.93	−2.75	*	*	2.350
19 estradiol	272.4	−2.49	4.01	−4.84	0.88	0.95	2.680
20 estradiol	272.4	−2.47	4.01	−4.81	0.88	0.95	2.680
21 estradiol	272.4	−2.46	4.01	−4.80	0.88	0.95	2.680
22 estradiol	272.4	−2.40	4.01	−4.74	0.88	0.95	2.680
23 estradiol	272.4	−2.39	4.01	−4.73	0.88	0.95	2.680
24 estradiol	272.4	−2.28	4.01	−4.63	0.88	0.95	2.680
25 estradiol	272.4	−2.27	4.01	−4.61	0.88	0.95	2.680
26 estradiol	272.4	−2.21	4.01	−4.56	0.88	0.95	2.680
27 ethanol	46.07	−3.00	−0.31	−2.79	0.37	0.48	1.044
28 ethanol	46.07	−3.10	−0.31	−2.89	0.37	0.48	1.044
29 2-ethoxy ethanol	91.10	−3.60	−0.32	−3.39	0.30	0.83	1.556
30 ethyl ether	74.10	−2.80	0.93	−3.32	0.00	0.45	0.787
31 heptanol	116.2	−1.42	2.72	−3.01	0.37	0.48	1.502
32 heptanol	116.2	−1.49	2.72	−3.08	0.37	0.48	1.502
33 hexanol	102.2	−1.56	2.03	−2.73	0.37	0.48	1.410
34 hexanol	102.2	−1.89	2.03	−3.06	0.37	0.48	1.410
35 isoquinoline	129.2	−1.78	2.03	−2.95	*	*	1.007
36 m-cresol	108.1	−1.82	1.95	−2.94	0.57	0.34	1.222
37 meperidine (pethidine)	247.4	−2.43	2.45	−3.85	*	*	1.866
38 methanol	32.40	−3.00	−0.77	−2.52	0.43	0.47	0.970
39 methanol	32.40	−3.30	−0.77	−2.82	0.43	0.47	0.970
40 methyl 4-OH benzoate	152.1	−2.04	1.90	−3.14	0.69	0.45	1.812
41 naproxen	230.3	−2.54	3.34	−4.48	*	*	2.161
42 nicotine	162.2	−2.48	1.17	−3.15	*	*	1.510
43 nonanol	144.3	−1.22	4.26	−3.71	0.37	0.48	1.686
44 *o*−cresol	108.1	−1.80	1.95	−2.93	0.52	0.30	1.220
45 octanol	130.2	−1.28	3.00	−3.03	0.37	0.48	1.594
46 *p*-cresol	108.0	−1.76	1.94	−2.88	0.57	0.31	1.221
47 phenol	94.10	−1.71	1.46	−2.55	0.60	0.30	1.171
48 phenol	94.10	−2.09	1.46	−2.92	0.60	0.30	1.171
49 propanol	60.00	−2.85	0.25	−2.98	0.37	0.48	1.134
50 propanol	60.00	−2.77	0.25	−2.89	0.37	0.48	1.134
51 propanol	60.00	−2.92	0.25	−3.04	0.37	0.48	1.134
52 salicylic acid	138.1	−3.48	2.26	−4.79	*	*	2.116

Table 1 (*Continued*)

	MW	Log k_p	Log K_{oct}	Log D/h	α	β	Charge
53 salicylic acid	138.1	−2.20	2.26	−3.51	*	*	2.116
54 scopolamine	303.4	−4.30	1.24	−5.01	*	*	3.124
55 testosterone	287.4	−2.66	3.32	−4.59	0.32	1.19	2.478
56 thymol	150.1	−1.28	3.30	−3.20	0.52	0.44	1.444
57 2,4,6-trichlorophenol	197.4	−1.23	3.69	−3.38	*	*	1.214

[a] Log k_p values (cm/h) are from Wilschut et al. (1995) and Degim et al. (1998), log K_{oct} from the Medchem database, log (D/h) calculated from $\log k_p + 0.024 - 0.59 \log K_{oct}$ (Pugh et al., 1996), α and β are the scaled H-bonding donor and acceptor values of Abraham (1993) and Abraham et al. (1995) and the charge is the sum of the modulus of partial charges calculated as described in the text.

defined as the sum of the squares of the eigenvectors $(0.54^2) + (-0.54^2) + (-0.44^2) + (-0.48^2) = 1$. The contribution of log (D/h) is thus 0.29 (i.e. 0.54^2) which means it plays an important role in the PC (or mechanism). Eigenvector sign is significant, so that PC1 suggests a mechanism involving log (D/h) inversely with MW and H-bonding. PCA thus enables us: (1) to identify relationships between groups of variables; (2) estimate the importance of each relationship in determining the overall process and (3) estimate the importance of each variable within a relationship.

4.1.1. Multivariate regression regression analysis (MRA)

This calculates a least squares fit between an outcome such as log (D/h) and a number of predictors of the outcome. It has been used extensively to identify and quantify the effect of predictors on skin permeability parameters (Kamlet et al., 1988; Potts and Guy, 1992; Pugh and Hadgraft, 1994; Abraham et al., 1995; Roberts et al., 1995; Wilschut et al., 1995; Pugh et al., 1996). Two important criteria are negligible error associated with the predictors and absence of collinearity (correlations) amongst the predictors.

4.2. Examination of the mechanisms that determine k_p

We have previously (Pugh et al., 1996) criticised the use of the composite term, log k_p (i.e. $\log K_{sc} + \log D/h$) as the dependent variable in MRA. PCA using log k_p instead of log D/h supports these doubts. Consider the PCA results (Table 2c) for the relationship between log k_p, MW and log K_{oct} suggested by Potts and Guy (1992).

PC1 accounts for 55% of the data variation and PC2 for 43%, suggesting that two mechanisms-presumably partition and diffusion-are involved. As expected there is a direct relationship between log k_p and log K_{oct} (a measure of lipophilicity), but the relationship between log k_p and MW is also direct (not inverse as in the Potts and Guy regression). This is difficult to explain in mechanistic terms, suggesting that the interaction between log K_{oct} and MW is a confounding effect. PC2 shows an inverse relationship between log k_p and MW. We therefore decided to eliminate log K_{oct} from the predictor variables by using it to calculate log (D/h) from log k_p. This has the additional bonus of using a more fundamental quantity in QSAR analysis.

4.3. Elimination of outliers

Meta analysis involving data from a multiplicity of sources, coupled with the high experimental variability associated with permeability studies (Southwell et al., 1984) makes it likely that some data will be unreliable. Although Wilschut et al. (1995) applied strict exclusion criteria to their collection we applied two extra checks for outliers. The first involved plotting PC2 against PC1 (Fig. 1a,b). This gives a non-quantifiable indication of unusual data. The second measured the deviation of experimental from regression values as DFIT values. DFIT combines leverage (measurement of how unusual a predictor set is) and Studentized residual (residual for a compound

Table 2
Principal components analysis[a]

	PC1	PC2	PC3	PC4
(a) Matrix of log (D/h), MW, α and β; 36 data values				
Eigenvectors				
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	0.49	0.05
Proportion of variation	0.82	0.14	0.02	0.01
Cumulative proportion	0.82	0.96	0.98	1.00
(b) Matrix of log (D/h), MW, charge; 36 data values				
Eigenvectors				
Log (D/h)	0.58	−0.40	0.71	
MW	−0.58	0.39	0.71	
Charge	−0.57	−0.82	−0.01	
Eigenvalue	2.78	0.17	0.06	
Proportion	0.93	0.06	0.02	
Cumulative	0.93	0.99	1.00	
(c) Matrix of log k_p, MW, log K_{oct}; 57 data values				
Eigenvectors				
Log k_p	0.35	0.77	−0.52	
MW	0.54	−0.63	0.57	
Log K_{oct}	0.77	0.08	−0.64	
Eigenvalue	1.65	1.28	0.07	
Proportion	0.55	0.43	0.02	
Cumulative	0.55	0.98	1.00	
(d) Matrix of log (D/h), MW, charge. 52 data values				
Eigenvectors				
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	0.88	0.08	0.04	
Cumulative	0.88	0.96	1.00	
(e) Matrix of log (D/h), MW, charge; Subset 1: 26 data values				
Eigenvectors				
Log (D/h)	0.59	−0.34	0.72	
MW	−0.58	0.46	0.67	
Charge	−0.56	−0.82	0.07	
Eigenvalue	2.64	0.24	0.13	
Proportion	0.88	0.08	0.04	
Cumulative	0.88	0.96	1.00	
(f) Matrix of log (D/h), MW, charge; Subset 2: 26 data values				
Eigenvectors				
Log (D/h)	0.59	−0.27	0.76	
MW	−0.55	0.51	0.63	
Charge	−0.56	−0.82	0.15	
Eigenvalue	2.63	0.27	0.10	
Proportion	0.88	0.09	0.03	
Cumulative	0.88	0.97	1.00	

[a] Eigenanalysis of the correlation matrix.

when its predictors are omitted from the regression). Data are regarded with suspicion if the DFIT value exceeds $2\sqrt{[(k+1)/n]}$ where k is the number of predictors and n the number of data points (Minitab Reference Manual, Minitab).

This procedure was applied to the 40 compounds for which α and β values were available. PCA (Fig. 1a, b) suggests that aldosterone, corticosterone, estradiol and ethyl ether are unusual when examined on the basis of both charge and H-bonding properties. DFIT plots (Fig. 2) suggest that aldosterone, corticosterone, 4-bromophenol and ethyl ether were very unusual and they were excluded from the analysis in Section 4.4.

4.4. Comparison of H-bonding and charge as predictors of diffusion, data set of 36 compounds

4.4.1. PCA comparison

A PC describes a relationship between log (D/h) and the other variables, so that the existence of non polar and polar pathways would be expected to give rise to individual PCs. Jolliffe (1986) recommends that PCs with eigenvalues <0.75 should be ignored, so that PCA on the basis of both H-bonding and charge (Table 2a,b) indicates a single, dominant mechanism. PC1 of log (D/h), MW, α and β (Table 2a) accounts for 82% of data variation, and the eigenvector signs show inverse relationships between log (D/h) and both size and H-bonding. This is consistent with diffusion along a non polar pathway hindered by interaction with immobilised polar head groups in the SC lipids (Pugh et al., 1996).

A similar conclusion is reached when charge is used instead of H-bonding (Table 2b), where PC1 accounts for 93% of data variation.

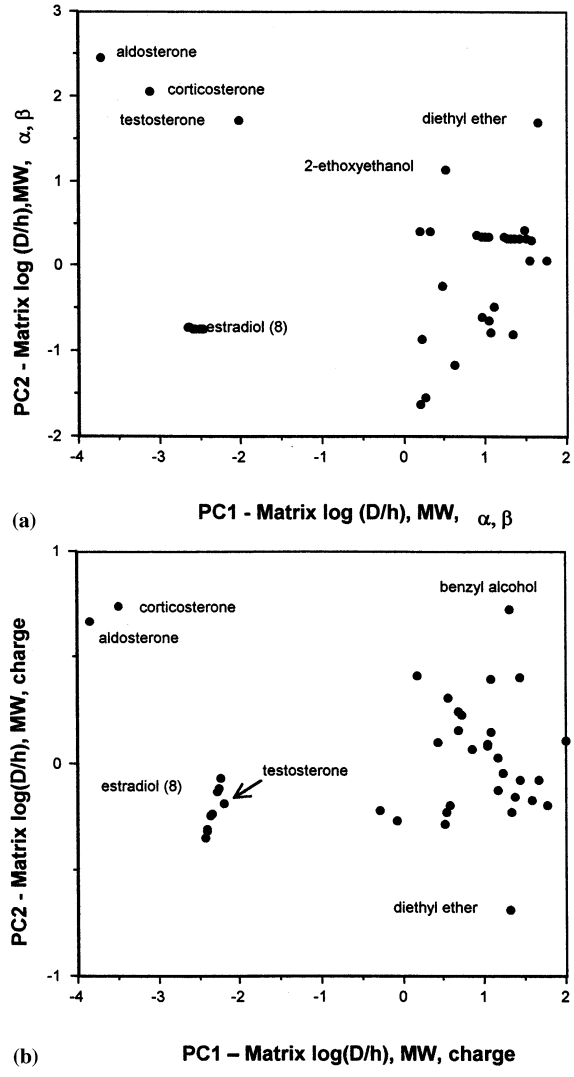

Fig. 1. Plots of the first and second principal components for 40 compounds. (a) Correlation matrix of log (D/h), MW, α, β. (b) Correlation matrix of log (D/h), MW, charge.

4.4.2. MRA comparison

Interpretation is complicated by correlation between MW and the other predictors and the analysis should check for predictor interactions by including their product terms.

The regression of log (D/h) against MW α and β is satisfactory:

$$\log(D/h) = 1.76 - 0.00490\ \mathrm{MW} - 0.597\ \alpha - 1.14\ \beta \quad (1)$$

$P < 0.001$, $P < 0.001$, 0.046, $P < 0.001$; $N = 36$; S.D. $= 0.223$; $R^2 = 0.92$.

Although collinearity between MW and α and β may be significant (R values 0.75 and 0.81, respectively).

Detailed analyses of all possible interaction terms showed significant interaction between MW and both α and β. Better regressions were obtained using MW*α and MW*β although collinearity was still high ($R = 0.87$).

$$\log(D/h) = -2.65 - 0.00326\ \mathrm{MW}^*\alpha - 0.00501\ \mathrm{MW}^*\beta \quad (2)$$

$P < 0.001$, 0.002, $P < 0.001$; $N = 36$; S.D. $= 0.230$; $R^2 = 0.92$.

The regression of log(D/h) against MW and charge was less satisfactory:

$$\log(D/h) = -2.03 - 0.00738\ \mathrm{MW} - 0.212\ \mathrm{charge} \quad (3)$$

$P < 0.001$, $P < 0.001$, 0.095; $N = 36$; S.D. $=$ 0.261; $R^2 = 0.89$.

Because of the high value of P for charge and high collinearity ($R = 0.86$) of MW and charge.

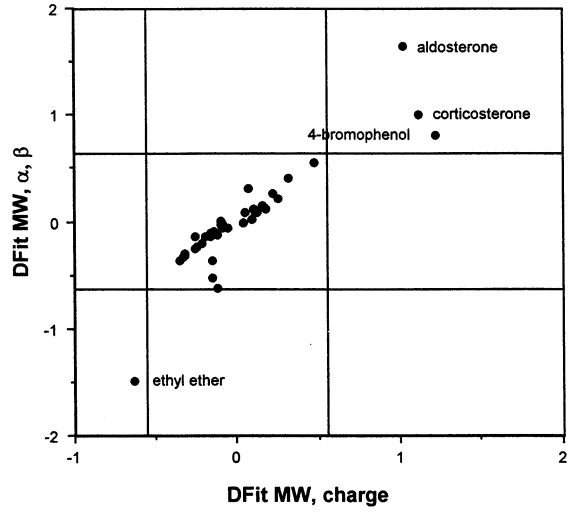

Fig. 2. Plots of the DFITs values for the regressions of log (D/h) against MW, α, β and MW, charge. Forty compounds. Grid lines define outliers.

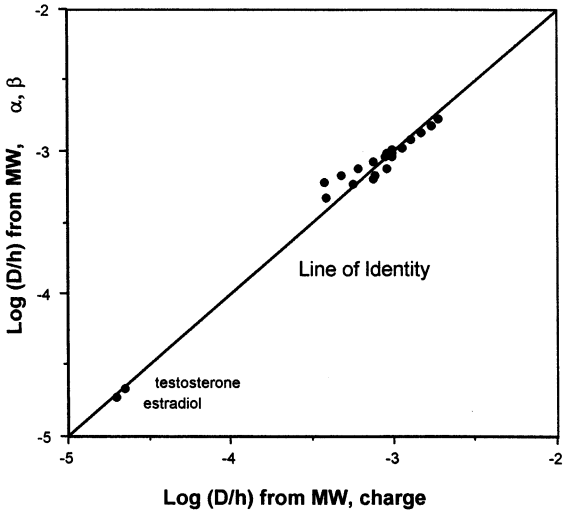

Fig. 3. Comparison of log (D/h) values predicted by regression against MW, α, β and MW, charge. Thirty-six compounds.

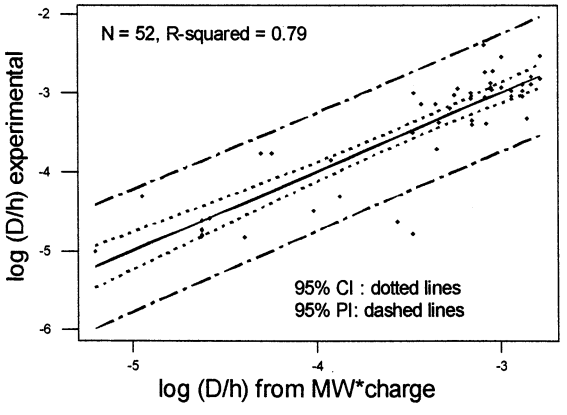

Fig. 4. Log (D/h) experimental values versus values predicted fitted from the regression against MW*charge. Ninty-five percent prediction and confidence intervals shown. Fifty-two compounds.

The cross product term MW*charge is the only predictor necessary and removes the collinearity problem.

$$\log(D/h) = -2.62 - 0.00283\ \text{MW*charge} \quad (4)$$

$P < 0.001$, $P < 0.001$; $N = 36$; S.D. $= 0.235$; $R^2 = 0.91$.

Log (D/h) predicted from either H-bonding or charge are very similar (Fig. 3). This is useful since charge may be easily calculated from molecular modelling while α and β values are generally unavailable for multifunctional compounds of interest in permeability applications. However there is an obvious absence of data for log (D/h) from -3.5 to -4.5 and the only molecules of pharmacological interest are testosterone and estradiol.

4.5. Examination of an extended dataset using MW and charge as predictors

Since molecular modelling enables calculation of charge, the gap around log $(D/h) - 4$ was filled using an extended dataset. Diethylcarbamazine, codeine, aldosterone, corticosterone and ephedrine were excluded after screening (Section 4.3) and analyses performed on the remaining 52 compounds.

PCA (Table 2d) shows a dominant mechanism with log (D/h) inversely related to MW and charge. The eigenvectors in PC1 indicate equal importance of log (D/h), MW and charge.

Although regression analysis appears satisfactory:

$$\log(D/h) = -1.99 - 0.00617\ \text{MW} - 0.332\ \text{charge} \quad (5)$$

$P < 0.001$, $P < 0.001$, 0.006; $N = 52$; S.D. $= 0.360$; $R^2 = 0.79$.

The high collinearity of charge and MW ($R = 0.78$), suggests that the interaction term MW*charge is a better predictor of log (D/h):

$$\log(D/h) = -2.70 - 0.00264\ \text{MW*charge} \quad (6)$$

$P < 0.001$, $P < 0.001$; $N = 52$; S.D. $= 0.366$; $R^2 = 0.78$.

Fig. 4 plots experimental against predicted values. The 95% confidence interval (CI) gives limits for the value of log (D/h), while the 95% prediction interval gives limits for its experimental determination (Bolton, 1984).

4.6. Relative importance of size and charge

The similarity of eigenvectors for MW (0.58) and charge (0.56) in PC1 (Table 2d) indicates equal importance of these factors.

In regression analysis direct comparison of the coefficients is meaningless because charge varies

from 0.8 to 3.8 and MW from 46 to 360. When predictor values were normalised by subtracting means and dividing by standard deviations the similarity of coefficients suggests approximately equal importance of size MW and charge.

$$\log(D/h) = -3.54 - 0.501\ \text{MW}\ \# - 0.234\ \text{charge}\ \# \quad (7)$$

$P < 0.001$, $P < 0.001$, 0.006; $N = 52$; S.D. = 0.360; $R^2 = 0.79$.

4.7. Check for idiosyncraticity of the data set

A pitfall in data analysis is that conclusions might be idiosyncratic for the particular data set and not of general application. The data were divided into two subsets. The PCA results are in Table 2e and f, and regression analyses are:

Subset 1:

$$\log(D/h) = -2.76 - 0.00260\ \text{MW*charge} \quad (8)$$

$P < 0.001$, $P < 0.001$; $N = 26$; S.D. = 0.421; $R^2 = 0.73$.

Subset 2:

$$\log(D/h) = -2.65 - 0.00268\ \text{MW*charge} \quad (9)$$

$P < 0.001$, $P < 0.001$; $N = 26$; S.D. = 0.313; $R^2 = 0.84$.

The similarity between results for the subsets indicates that the conclusions are of general significance.

Appendix A. Abbreviations and symbols

α, β	scaled values of H-bond donor and receptor potentials
D	diffusion coefficient (cm^2/h)
h	Diffusional path length across stratum corneum (cm)
k_p	permeability coefficient (cm^2/h)
K_{oct}	octanol/water partition coefficient
MRA	multivariate regression analysis
MW	molecular weight
N	number of data points
P	probability of error
PCn	nth principal component
PCA	principal component analysis
R	correlation coefficient
R^2	coefficient of determination adjusted for degrees of freedom
SC	stratum corneum
S.D.	standard deviation

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