Simple Rules Defining the Potential of Compounds for Transdermal Delivery or Toxicity

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Received January 6, 2004; accepted March 10, 2004

Purpose. Simple rules based on readily accessible physicochemical properties enable identification of solutes that penetrate skin very slowly or rapidly.

Methods. Literature *in vitro* maximal flux values (J_{max}) across human skin were collected for 87 penetrants. Penetrants were assigned as "good" ($J_{max} > 10^{-5.52}$ mole·cm^{-2}·h^{-1}), "bad" ($J_{max} < 10^{-8.84}$ mole·cm^{-2}·h^{-1}) or "intermediate" based on mean ± 1SD. The feasibility of using readily available physicochemical properties, such as molecular weight (MW), melting point (MP,°K), octanol-water partition coefficient (K), water solubility (S, molarity), number of atoms available for H-bonding (HB), in assigning solutes was examined.

Results. Good penetrants had MW ≤ 152, log S > −2.3, HB ≤ 5, log K < 2.6, MP ≤ 432. Bad penetrants had MW > 213, log S < −1.6, HB ≥ 4, log K > 1.2, MP ≥ 223. Discriminant analysis using MW, HB, log K correctly assigned 70% of compounds. Individual success rates were good (88%), intermediate (58%), bad (93%). Aqueous J_{max} data for 148 test solutes were used for validation. Discriminant analysis assigned 76% of compounds, with individual rates of good (76%), intermediate (67%), and bad (97%). No good penetrants were misclassified as bad or *vice versa*.

Conclusions. These rules enable rapid screening of potential drug delivery candidates and environmental exposure risks.

KEY WORDS: multivariate data analysis; quantitative structure-activity relationships; transdermal absorption.

INTRODUCTION

Transdermal absorption is a potential route of solute uptake, and it is pertinent to estimate its extent for therapeutic and toxicological purposes. One important goal for the pharmaceutical industry is the identification of solutes with the potential for becoming approved drugs. To reduce the workload, attention has focused on finding relationships between chemical properties and biological activity. One approach is to express the biological activities of a series of compounds as multivariate functions of their physicochemical and/or structural properties. These functions are often referred to as quantitative structure-activity relationships (QSAR). Most QSAR models are based on permeability coefficients (1–7), although some have proposed QSARs for the diffusion component (8,9) or other aspects of interest such as skin corrosivity (10). Many QSARs are based on the solvatochromic parameters (11), which use rather inaccessible estimates of molecular size, polarizability, and H-bonding. Their estimation has been somewhat simplified by the group contribution method of Platts *et al.* (12); however, there is some cause for disquiet with this approach because the intercept terms in their regression analyses are non-zero. A more fundamental drawback from the viewpoint of the formulator or risk assessor is that the least-squares equation is determined mainly by the central data, and the outlying points have less influence on the QSAR. It therefore describes—often quite accurately—the "mediocre" compounds, whereas it is the outliers with exceptionally high or low permeation that might be of interest. An alternative approach is to identify guidelines or "rules of thumb" that identify compounds with high probability of having very high (or low) percutaneous absorption—a simple pass/fail test to enable rapid screening of candidate compounds by mathematically unskilled personnel. This was successfully used by Lipinski *et al.* (13) to identify compounds for intestinal absorption. They gave a set of rules to predict poor absorption or permeation for oral drugs based on readily available parameters globally associated with permeability and solubility.

In topical delivery and in the assessment of dermal toxicity hazard, the parameter of the greatest interest is the maximal flux, J_{max}, as this defines the maximal dermal, toxic, or systemic effect for a particular compound. Furthermore, if the J_{max} for a solute is known, its flux from any vehicle can be estimated using its fractional solubility in the vehicle after accounting for vehicle-induced changes in skin permeability (7). We therefore aimed to find the simplest set of readily accessible physicochemical properties that defined compounds with extreme J_{max} values. These simple properties were: molecular weight (MW), melting point (MP), octanol-water partition coefficient (log K), aqueous solubility (log S), and number of atoms available for H-bonding (HB)—subdivided into H-bond donor (HB-d) and acceptor (HB-a). This method could then be used for rapid screening by predicting whether J_{max} for a novel compound lies outside the acceptably high or low levels set by the investigator. We recognize that this is an empirical approach and that the funda-

Table I. Boundary Values for Good (Group 2) and Bad (Group 0) Penetrants

	Predictors					
Code	MW	MP (K)	HB-d	HB-a	log K	log S (M)
Group 0	> 213	≥ 223	≥ 0	≥ 3	> 1.2	< −1.6
Group 2	≤ 152	≤ 432	≤ 2	≤ 3	< 2.6	≥ −2.3

HB, number of hydrogen bonding atoms, HB-d, number of hydrogen-bond donors; HB-a, number of hydrogen-bond acceptors; K, octanol/water partition coefficient; MP, melting point; MW, molecular weight, S, aqueous solubility at room temperature.

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ABBREVIATIONS: Code Penetrants were assigned as "good" (group 2), "intermediate" (group 1), or "bad" (group 0); HB, number of hydrogen bonding atoms (e.g., HB = 3 for CH_3COOH); HB-d, number of hydrogen-bond donors; HB-a, number of hydrogen-bond acceptors; J, flux (mole·cm^{-2}·h^{-1}); J_{max}, flux from saturated aqueous donor; K, octanol/water partition coefficient; k_p, permeability coefficient (cm/h); MP, melting point (K); MW, molecular weight (Da); PC, principal component; S, aqueous solubility at room temperature (M); SD, standard deviation.

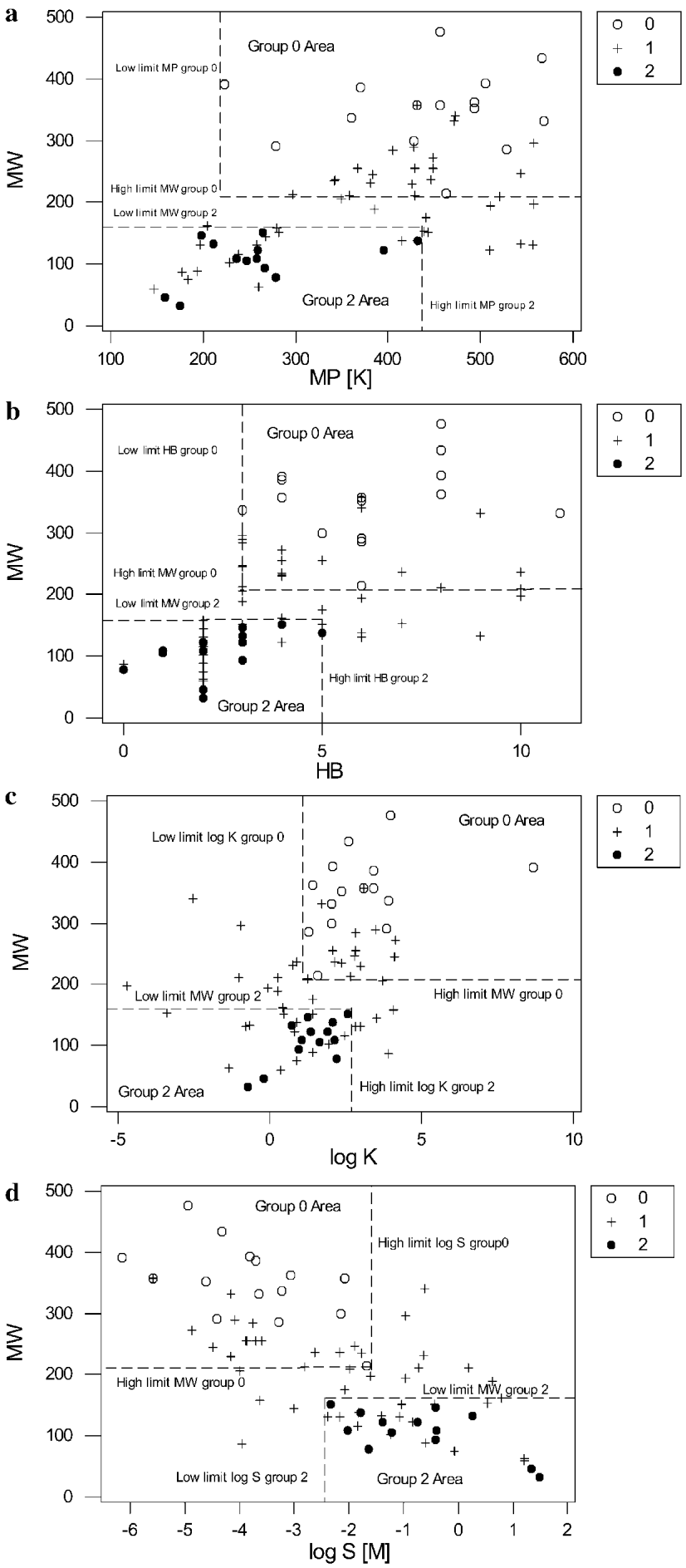

Fig. 1. Limiting values for predictors showed as dashed lines. The areas occupied by group 0 (bad) and group 2 (good) compounds are distinct for MW in combination with (a) MP, (b) HB, (c) log K, and (d) log S.

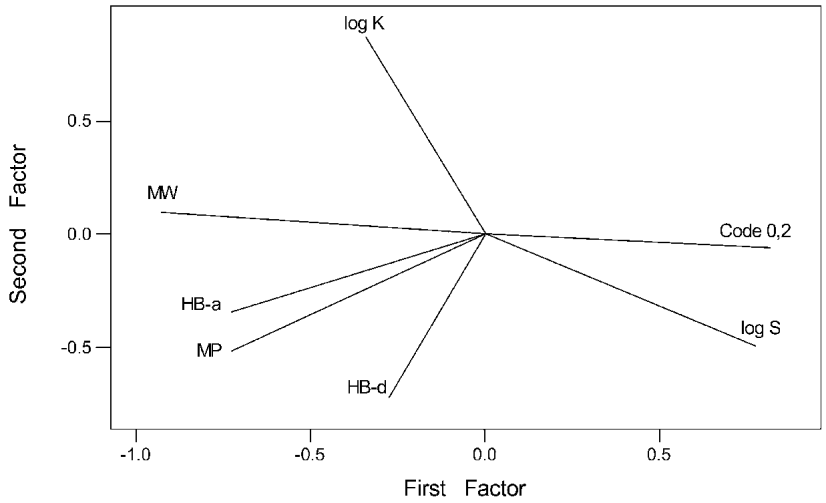

Fig. 2. Loading plot for Factor Analysis of groups 0 and 2 compounds.

mental determinants of flux are likely to be size, polarity, and H-bonding capacity. We hypothesize that these determinants are reflected in the proposed simple properties to an useful extent.

MATERIALS AND METHODS

Permeation and physicochemical data used in this study were collected from the database published by Magnusson *et al.* (14). The developed training set of 87 saturated solutes (both ionized and non-ionized) contained transdermal delivery data of reported J_{max} using mixed vehicles (water-based, propylene glycol, or pure liquid) on human skin (epidermal, full and split thickness skin). A validation set of 148 solutes was developed containing reported and calculated J_{max} values using aqueous solutes on human skin. Penetrants were assigned as good [log J_{max} > −5.52 mole·cm^{-2}·h^{-1} (group 2), based on the cutoff range of more than one standard deviation (1SD) above mean], intermediate [−8.84 < log J_{max} < −5.52 (group 1), mean ± 1SD], or bad [log J_{max} < −8.84 mole·cm^{-2}·h^{-1} (group 0), more than 1SD below mean]. Minitab statistical software (Release 13.32, Minitab Inc, State College, Pennsylvania, USA) was used for data analyses.

RESULTS AND DISCUSSION

Training Dataset

The properties common to members within the good and bad penetrant groups are given in Table I. A compound satisfying some of the criteria could belong to any group. Fifty-five of the 87 compounds were classified as intermediate (group 1). Predictor combinations were used to test whether group 1 (intermediate) compounds would be wrongly assigned as good or bad. For example, octanol is wrongly assigned to group 2 (good) on the basis of size alone (MW = 130), on the combination of MW and HB (2), but correctly excluded on the combination of MW, HB, and log S (−2.4 M) or MW, HB, and log K (3.0). It is evident (Fig. 1, Table I) that MW together with MP, HB, log K, or log S are reliable predictor combinations enabling a rapid screening of good or bad penetrants.

An attempt was then made to improve the accuracy of compound assignment by use of multivariate analysis techniques. The techniques are described in standard statistical texts, and Armstrong and James present the tests used here with worked examples in a form suitable for non-mathema-

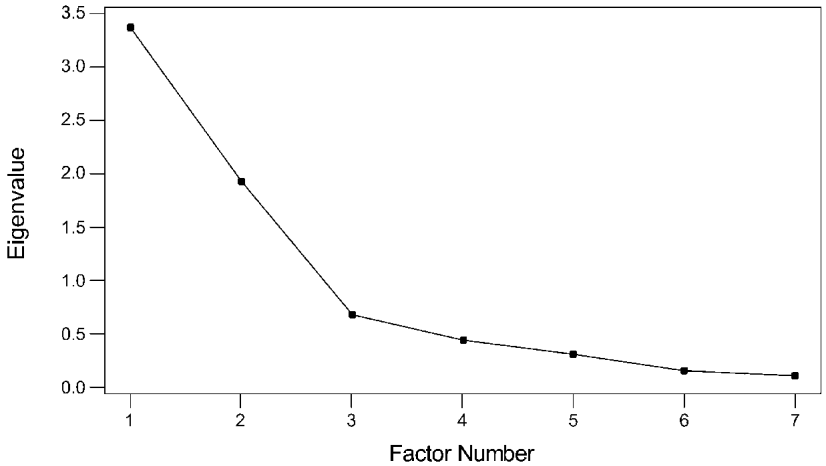

Fig. 3. Scree plot of eigen values from the Factor Analysis. Most of the data variation is contained in the first three Principal Components.

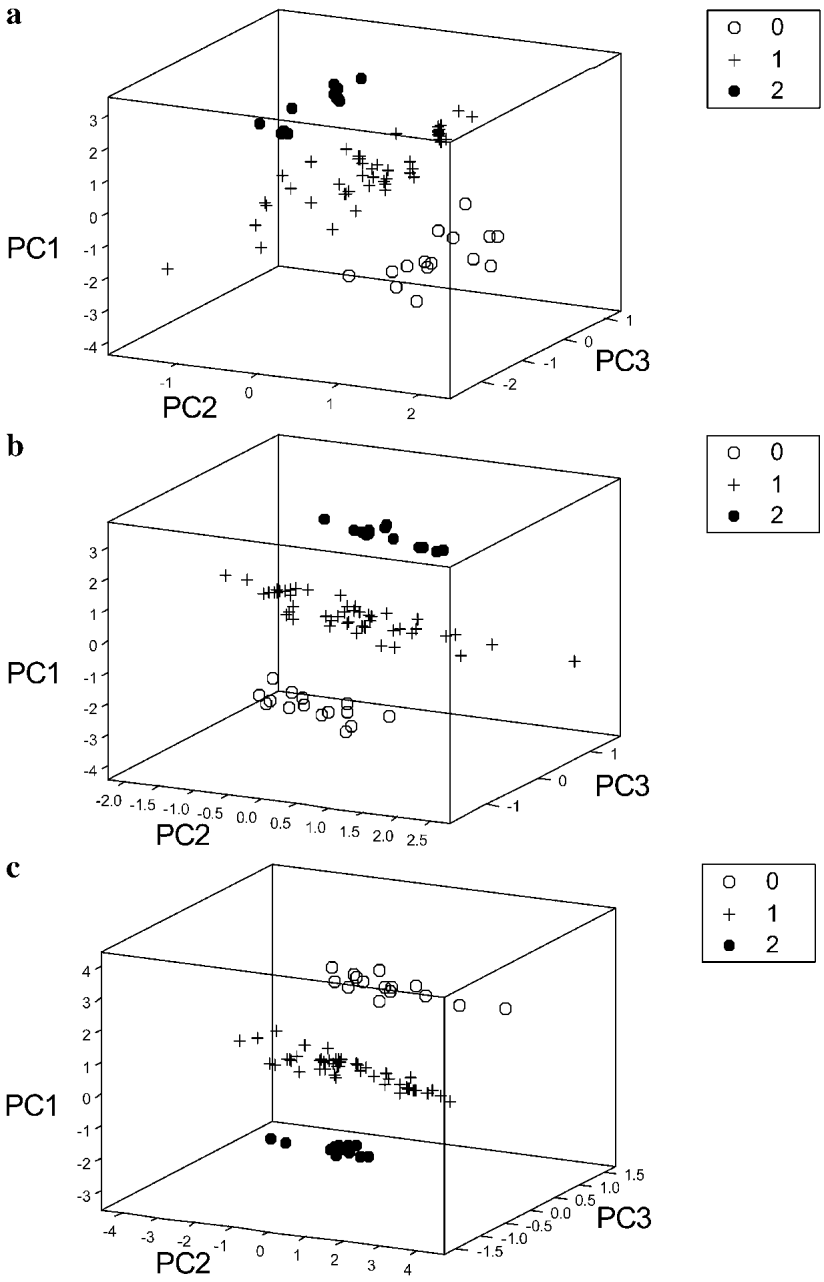

Fig. 4. Three-dimensional plots of the first three PCs of the Principal Component Analysis. (a) MW, MP, HB-a; (b) MW, HB-a, log S; (c) MW, log P, log K.

ticians (15). The simple approach does not quantify the predictors. For example, compounds of MW 214 and 400 are both simply classified as group 0 (bad). Discriminant analysis applies a weighting to these values and would conclude that the MW 400 compound would be more likely to belong to group 0 than the MW 214 compound. This concept is extended to combinations of predictors to refine the assignment. Factorial analysis was used to assess how many predictors would be useful in determining whether a compound should belong to group 0 (bad) or 2 (good). The loadings plot (equimax rotation) in Fig. 2 shows the discriminate power for the different predictors. The scree plot infection of the eigen values (Fig. 3) shows that three principal components (PCs) account for the data variation. Principal Components Analyses were applied to the whole dataset: groups 0, 1, 2. Plots of the scores of the first three PCs when Group Code Number were analyzed with MW/MP/HB-a (Fig. 4a), MW/HB-a/log S (Fig. 4b), and MW/MP/log K (Fig. 4c), shows separation of the three groups. Figs. 5a–5c shows how well the three predictors (MW/MP/HB-a, MW/HB-a/log S, and MW/MP/log K) actually separate the compounds. It can be seen that the intermediate group 1 overlaps more with group 2 (good) than with group 0 (bad). There is no mixing of groups 0 and 2—the important criterion when screening for toxicology or drug delivery.

Discriminant analysis quantifies the success of the predictor combinations in assigning compounds. Each compound in turn was excluded from the dataset and assigned to a group on the basis of its predictor values (cross-validation). Misclassified results are reported, with the probability values for be-

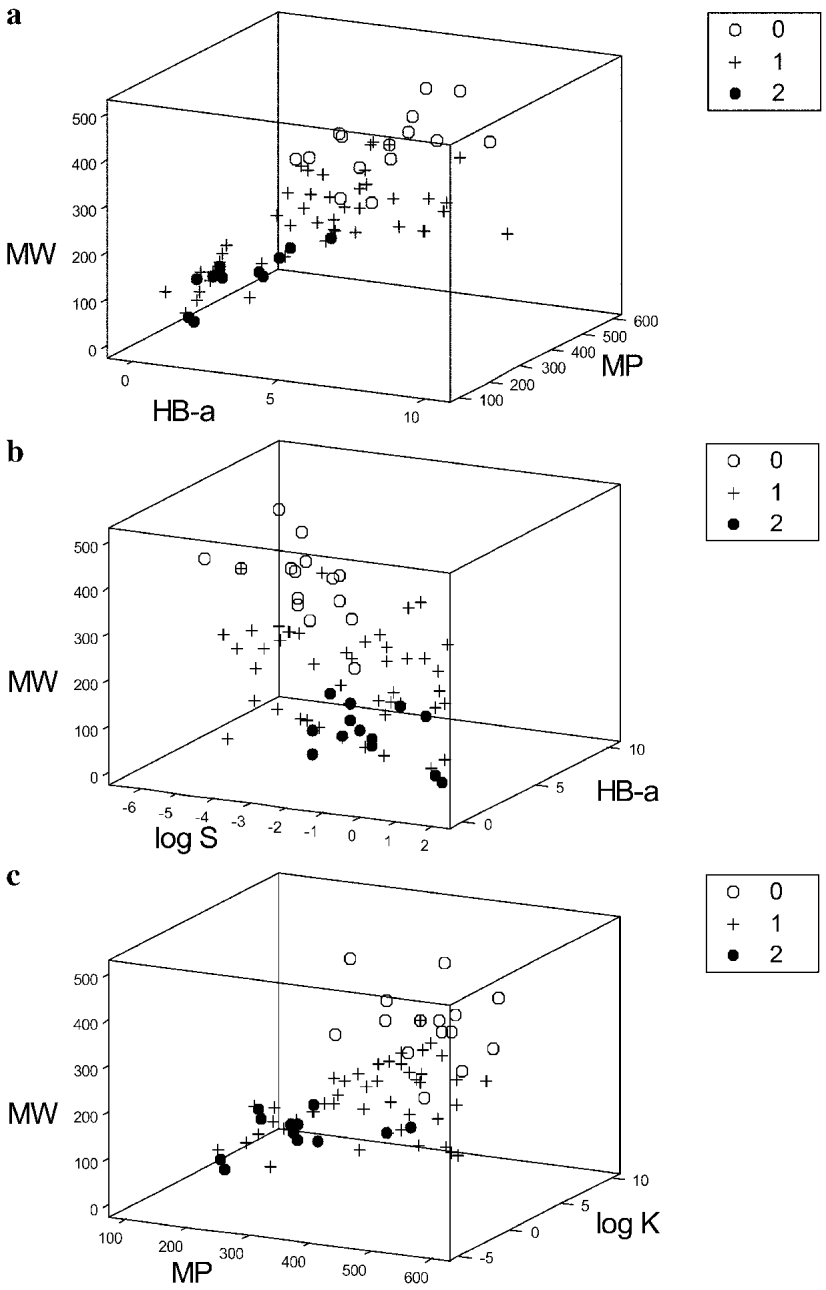

Fig. 5. Separation of compounds on basis of (a) MW, MP, HB-a, (b) MW, HB-a, log S, and (c) MW, MP, log K.

longing to all three groups. This makes it possible to assess whether the assessment is borderline. For example, on the basis of the three predictors, MW, MP, and HB-a, morphine is incorrectly classified as group 1 (intermediate) with a probability of 0.54. There is probability 0.44 that it is group 0 (bad) but only 0.02 that it is group 2 (good), so that it can be judged to be borderline between group 0 and 1.

Table II summarizes the results of the Discriminant Analysis. The final column is the number of potentially serious misclassifications—0 classified as 2 or *vice versa*. At least three predictors should be used to minimize this risk. When a single predictor is used, best results are obtained for MW (66%), followed by HB-a (48%) and log S (48%). Hydrogen bond number, log K, MP, and HB-d assigned compounds incorrectly. By using two predictors, the combinations of MW with HB or MP gives good results with a success rate of 70 and 69%, respectively. From a fail-safe standpoint where the priority is to identify good or bad penetrants correctly and be less concerned about misclassifying group 1, then the combinations of MW together with two of the following predictors MP, HB, log K, or log S are best. The most significant prediction is achieved by the combination of the three properties MW/HB/log K or MW/log S/MP giving a success rate of 70%. The simple rule of thumb given by the criteria for these three predictors in Table I give some guidance whether a compound has low, intermediate, or high flux. Further increasing the number of predictors does not result in a higher success rate.

Table II. Discriminant Analysis Results for Training Set

Predictors			Correct assignments (%)				0↔2Errors (%)
			Overall	Group 0	Group 1	Group 2	
1 Predictor							
MW			66	93	51	88	0
HB-a			48	80	31	77	0
log S			48	80	33	71	0
HB			45	67	26	88	7
MP			44	73	22	88	19
log K			37	60	26	53	13
HB-d			24	47	0	82	71
2 Predictors							
MW	HB		70	93	58	88	0
MW	MP		69	87	58	88	0
MW	log S		64	93	47	94	0
MW	log K		64	93	49	88	0
HB	log K		61	73	51	82	0
MP	log S		58	73	46	82	0
MP	log K		54	93	33	88	6
HB	log S		52	73	35	88	0
log S	log K		52	73	36	82	7
HB	MP		47	60	31	88	13
3 Predictors							
MW	HB	log K	70	93	58	88	0
MW	log S	MP	70	87	62	82	0
MW	log K	MP	69	87	58	88	0
MW	HB	log S	69	93	56	88	0
MW	HB	MP	69	87	58	88	0
MW	log S	log K	68	87	55	94	0
HB	log S	log K	62	67	53	88	0
log S	log K	MP	54	80	38	82	0
HB	log S	MP	51	73	35	82	0

HB, number of hydrogen bonding atoms, HB-d, number of hydrogen-bond donors; HB-a, number of hydrogen-bond acceptors; K, octanol/water partition coefficient; MP, melting point; MW, molecular weight, S, aqueous solubility at room temperature.
Final column shows percentage of group 0 compounds misclassified as group 2 or *vice versa.*

Validation Dataset

The guidelines from the simple approach based on the training set were validated on data for 148 compounds with J_{max} values using aqueous solutes. The simple rule of thumb in Table I is also a guide to J_{max} from aqueous solutions. The loading plot for the validation set shows similar results compared to the training set. Inflection in the scree plot of the eigen values shows that three principal components (PCs) account for the data variation in the validation set. Table III summarizes results for the best combination of the Discriminant Analysis for the validation. The discriminant analysis was more successful, possibly due to higher number of solutes available in this dataset. When a single predictor is used, best results are obtained for MW (78%), followed by log S (70%) and MP (60%). The predictor log S has a higher influence in the validation set due to use of aqueous solutes. Hydrogen bond parameters (HB, HB-a, HB-d) and log K assigned compounds incorrectly. By using two predictors, the combination of MW and MP result in a success rate of 100% for group 0, 67% for group 1, and 91% for group 2. The most successful assignment is on the basis of the three predictors MW, log K, and log S with a success rate of 97% for group 0, 77% for group 1, and 88% for group 2. The use of MW/MP/log K and MW/HB/MP as predictors correctly assigned 80 and 78% of compounds, respectively. No group 0 compounds were misclassified as group 2 or *vice versa.*

Limitations of the Analysis

Rules have been developed for simple screening of potential drug delivery candidates and environmental exposure risk for transdermal delivery. As discussed by Roberts *et al.* (7), vehicles can affect skin permeability by a range of mechanisms including delipidization, dehydration, fluidization, desmosome disruption, and change in polarity. Pure solutes can in some cases enhance the skin permeability by a direct corrosive effect (7,16). Generally substitution of organic vehicle has the potential for enhancing maximal flux (7) so that the estimation of maximal flux from aqueous systems used here enables a baseline decision for drug formulation and risk assessment. It is possible that other methodologies, e.g., fuzzy logic (17), may have a lower error than the discriminant analysis approach used here.

Transdermal vs. Intestinal Delivery

The rules for poor absorption of solutes through oral and transdermal deliveries are given in Table IV. The boundary

Table III. Discriminant Analysis Results for Validation Set

Predictors			Correct assignments (%)				0↔2Errors (%)
			Overall	Group 0	Group 1	Group 2	
1 Predictor							
MW			78	100	67	85	0
MP			60	91	40	79	0
log S			70	88	63	70	0
HB			47	61	22	97	18
HB-a			47	61	21	97	27
HB-d			37	33	23	73	33
log K			42	70	24	58	39
2 Predictors							
log S	log K		83	97	74	91	0
MW	MP		80	100	67	91	0
MW	log S		78	97	73	70	0
MW	HB		78	97	68	85	0
MW	log K		76	97	68	73	0
HB	log S		76	97	67	76	0
MP	log S		76	88	72	73	0
HB	log K		68	91	55	79	0
MP	log K		65	88	55	67	0
HB	MP		60	82	40	85	0
3 Predictors							
log S	log K	MW	84	97	77	88	0
log S	log K	MP	83	97	77	85	0
HB	log S	log K	81	97	72	88	0
MW	MP	log K	80	97	76	73	0
MW	HB	log S	79	97	73	76	0
MW	MP	log S	78	97	72	76	0
MW	MP	HB	78	97	70	79	0
HB	log S	MP	78	97	71	76	0
MW	HB	log K	76	97	67	76	0

HB, number of hydrogen bonding atoms, HB-d, number of hydrogen-bond donors; HB-a, number of hydrogen-bond acceptors; K, octanol/water partition coefficient; MP, melting point; MW, molecular weight, S, aqueous solubility at room temperature.
Final column shows percentage of group 0 compounds misclassified as group 2 or *vice versa.*

value for molecular weight is higher for intestinal delivery compared to transdermal delivery. The human skin has a higher resistance toward larger solutes compared to the more loose composition of the intestinal mucous. Intestinal delivery shows a higher log K value that might result in higher acceptance for lipophilic solutes compared to the lower log K for transdermal delivery. The boundary values for hydrogen-bonding capacity (both HB-a and HB-d) were lower for transdermal delivery. This might be due to the composition of the skin to have a higher affinity to bind solutes compared to the intestinal mucous.

Table IV. Rules for Poor Absorption or Permeation of Solutes Through Oral and Transdermal Delivery

Poor absorption	Predictors			
	MW	log K	HB-a	HB-d
Intestinal[a]	> 500	> 5.0	> 10	> 5
Transdermal	> 213	> 1.2	≥ 3	≥ 0

HB, number of hydrogen bonding atoms, HB-d, number of hydrogen-bond donors; HB-a, number of hydrogen-bond acceptors; K, octanol/water partition coefficient; MP, melting point; MW, molecular weight, S, aqueous solubility at room temperature.
[a] Lipinski et al.[13]

CONCLUSIONS

The assignment of a novel compound as being a good (group 2) or bad (group 0) penetrant can be made on the basis of its physicochemical properties. MW together with MP, HB, log K, or log S values can be used for very rapid screening. If values are unavailable for a particular predictor, then another can be substituted, although this may result in loss of reliability. The most useful predictor property was MW followed by (HB-a, log S) better than (HB, log K, MP, HB-d). Two or three predictors in combination should be used to minimize the risk for misclassification of group 0 as 2 compounds or *vice versa*. Discriminant analysis is a more refined approach that assigns a group together with the probability of being correct. Optimal discrimination for epidermal penetration was obtained using MW in combination with MP, HB, log K, or log S as predictors, which also gave a satisfactory success rate for the validation. No group compounds were ever misclassified. The scheme for percutaneous absorption can be used as a rapid screening of potential drug delivery candidates or environmental exposure risks.

ACKNOWLEDGMENTS

The authors acknowledge the support of Pfizer Pharmaceutical R&D, the National Health and Medical Research Council of Australia, and the Queensland and New South Wales Lions Medical Research Foundation.

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