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A comparison of the fit of flux through hairless mouse skin from water data to three model equations

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ABSTRACT

Data for the delivery of total species containing parent drugs from water through hairless mouse skin by prodrugs, $\log J_{MMAQ}$, has been fitted to the Roberts–Sloan, RS, the Kasting–Smith–Cooper, KSC, and Magnusson–Anissimov–Cross–Roberts, MACR, equations. The RS model which contains a parameter for the dependence of flux on solubility in water, S_{AQ}, as well as solubility in the lipid isopropyl myristate, S_{IPM}, gave the best fit: $\log J_{MMAQ} = -2.30 + 0.575 \log S_{IPM} + 0.425 \log S_{AQ} - 0.0016$ MW, $r^2 = 0.903$. The values for the coefficients to the parameters are quite similar to those obtained when the RS model was fit to flux of solutes from water through human skin, $\log J_{MHAQ}$. There was no trend in predicting the under or over-performance of prodrugs based on their fit to the RS model and whether they were more or less soluble than their parent drugs. There was an inverse dependence of $\log J_{MMAQ}$ on partition coefficients or permeability coefficients similar to that observed for $\log J_{MHAQ}$. The similarities in trends for results for $\log J_{MMAQ}$ and $\log J_{MHAQ}$ suggests that design directives obtained from mouse skin can be extended to design new prodrugs or select new drugs for delivery through human skin.

1. Introduction

The Roberts and Sloan model (RS, Eq. (1)) was initially developed to determine the relationship of maximum flux, J_M, of prodrugs (intact prodrug plus parent drug or total species) through hairless mouse skin from isopropyl myristate (IPM) *in vitro*, J_{MMIPM}, to their solubilities in the lipid IPM, S_{IPM}, and water, S_{AQ}, and to their molecular weights, MW (Roberts and Sloan, 1999). Although the origin of the paradigm that optimized J_M depended on optimized solubilities in both lipid and water was based on observation of trends in J_M for homologous series of prodrugs (Sloan, 1989), Eq. (1) was based directly on Fick's law and a transformation of the partition coefficient between a lipid membrane (skin) and a lipid vehicle (IPM) to S_{IPM} and S_{AQ}. Subsequent variations of Eq. (1) were fitted to data (1) for J_M of solutes through hairless mouse skin from water *in vitro*, J_{MMAQ} (Sloan et al., 2003) where S_{IPM} was the lipid parameter, (2) for J_M of solutes through human skin from water *in vitro*, J_{MHAQ} (Majumdar et al., 2007; Thomas et al., 2007; Juntunen et al., 2008) where solubility in octanol, S_{OCT}, was the lipid parameter, (3) for J_M of non-steroidal anti-inflammatory drugs through human skin from mineral oil *in vivo*, J_{MHMO} (Roberts and Sloan, 2001) where solubility in mineral oil, S_{MO}, was the lipid parameter. Thus, the dependence of J_M on solubility in a lipid and in water obtains regardless of the vehicle, the source of the membrane and whether *in vivo* or *in vitro*. Obviously the dependence of J_M on solubility in water is not based on solubility in an aqueous vehicle. Instead the dependence of J_M on S_{AQ} in Eq. (1) derives from a dependence on solute permeation of the stratum corneum rate limiting barrier by a lipid-aqueous series path in parallel with a lipid-only path instead of by a lipid path alone (Roberts and Sloan, 2000). The almost equal contributions of S_{LIPID} and S_{AQ} to the barrier to permeation derives from the parallel lipid-aqueous series path being the high capacity path.

$$\log J_M = x + y \log S_{LIPID} + (1 - y)\log S_{AQ} - z\,\mathrm{MW} \quad (1)$$

In the most recent report of the fit of the Roberts and Sloan equation to a large database ($n = 184$, which included prodrugs) where maximum flux through human skin from water *in vitro*, J_{MHAQ}, was the dependent variable (Eq. (2)), comparisons were made with other solubility and/or molecular weight, MW, based models (Juntunen et al., 2008). The Kasting, Smith and Cooper (KSC, Kasting et al., 1987) or the Magnusson, Anissimov, Cross and Roberts (MACR, Magnusson et al., 2004) models, Eqs. (3) and (4), respectively, did not give nearly as good a fit as RS did: $r^2 = 0.839$ versus $r^2 = 0.750$ and 0.580, respectively, for KSC and MACR. Surprisingly, the fit of equations where S_{OCT} or S_{AQ} was the only independent variable

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gave as good a fit as Eq. (4): $r^2 = 0.54$ and 0.61, respectively.

$$\log J_{MHAQ} = x + y \log S_{OCT} + (1 - y) \log S_{AQ} - z\,MW \quad (2)$$

$$\log J_{MHAQ} = x + y \log S_{OCT} - z\,MW \quad (3)$$

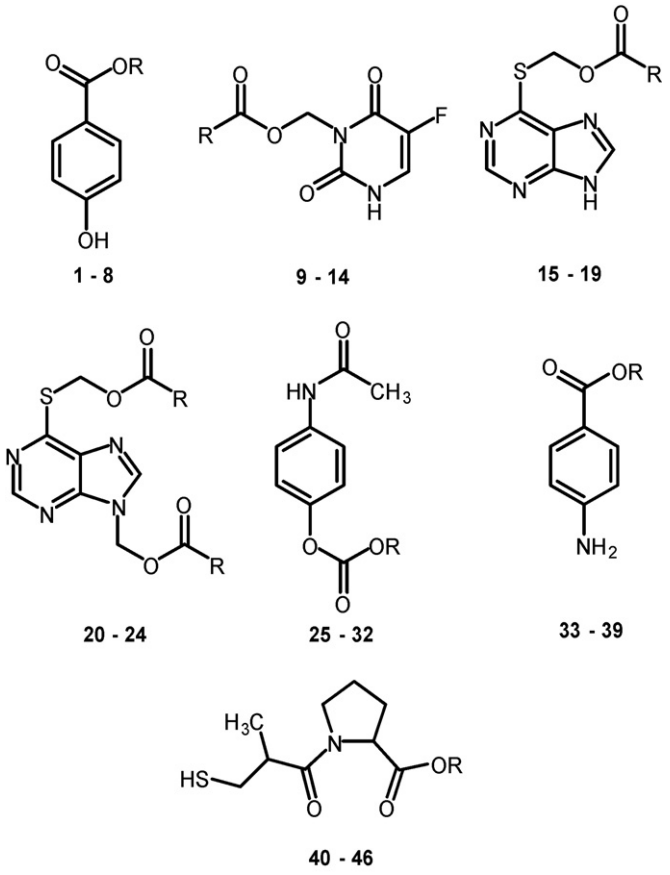
$$\log J_{MHAQ} = x - z\,MW \quad (4)$$

Although the only report of the fit of the RS model to the maximum flux of prodrugs through hairless mouse skin from water *in vitro*, J_{MMAQ}, was reasonably good ($r^2 = 0.765$ and the average of the absolute values for the difference between experimental and calculated log J_M, $\Delta \log J_{MMAQ}$, was 0.193 log units), the fit of maximum flux through hairless mouse skin from IPM, J_{MMIPM}, to the RS model was much better ($r^2 = 0.941$ and $\Delta \log J_{MMIPM} = 0.109$ log units) for the same prodrugs and one parent drug, $n = 17$ (Sloan et al., 2003). In addition, no attempt had been made to compare the fit of the data to RS with the fit of the data to other solubility and/or molecular weight based models (KSC and MACR). Here we report the results of a comparison of the fit of a larger database ($n = 32$) comprised of the $n = 16$ prodrugs in the previous database (Sloan et al., 2003) and the additional prodrugs of acetaminophen (APAP), APAP and parabens, to RS, KSC and MACR. In addition, the effect of the solubility of the prodrugs in water, S_{AQ}, compared to the solubility of its parent drug in water on whether they under- or over-perform their J_{MMAQ} calculated by Eq. (5) (see below), where the lipid parameter is IPM, has been assessed. Finally, each dataset has been sequentially removed from the database and new coefficients to the parameters in Eq. (5) have been determined for the fit of the remaining datasets to Eq. (5). The fit (r^2) of those remaining datasets to Eq. (5) and the residuals ($\Delta \log J_{MMAQ}$) for the entire database calculated from the new coefficients to the parameters in Eq. (5) have been made to determine if the database is homogeneous.

2. Materials and methods

2.1. Materials

Melting points were determined on a Meltemp capillary melting point apparatus and are uncorrected. TLC were run on Brinkman Polygram Sil G UV 254 plates. Ultraviolet, UV, spectra were obtained on a Shimadzu UV-160 or UV-2501 PC spectrophotometer. ^{1}H NMR spectra were recorded on a Varian Unity 400 MHz spectrometer. The vertical Franz diffusion cells (surface area = 4.9 cm^2, receptor phase volume = 20 ml, donor phase volume = 15 ml) were purchased from Crown Glass (Somerville, NJ, USA). A Fisher (Pittsburgh, PA, USA) circulating water bath was used to maintain a constant temperature of 32 °C in the receptor phase. Isopropyl myristate (IPM) was purchased from Givaudan (Clifton, NJ, USA). The female hairless mice (25–30 g, 12–16 weeks old, SKH-hr-1) were from Charles River. The animal research adhered to the "Principles of Animal Care." The C3 and C4 4-hydroxybenzoic acid esters (parabens **3** and **4**), theophylline (Th), 5-fluorouracil (5-FU), 6-mercaptopurine (6-MP) and 4-hydroxyacetanilide (APAP, **25**) were purchased from Eastman Kodak Chemicals; the C6 to C8 parabens (**6–8**) were purchased from Pfaltz and Bauer; the C5 paraben (**5**) was purchased from TCI America Organic Chemicals; the C2 paraben (**2**), chloroformates, acid chloride and all other reagent chemicals for the synthesis of the prodrugs were from Aldrich; all solvents were from Fisher Scientific. The 3-ACOM-5-FU prodrugs (**9–14**, Roberts and Sloan, 2002), the 6-ACOM-6-MP prodrugs (**15–19**, Waranis and Sloan, 1988), the bis-6,9-ACOM-6-MP prodrugs (**20–24**, Waranis and Sloan, 1987), and the 4-AOC-APAP prodrugs (**26–32**, Wasdo and Sloan, 2004) were synthesized as previously reported and were identical with the reported prodrugs by ^{1}H NMR, TLC and melting points. The parabens (**1–8**) were also consistent with their given structures by ^{1}H NMR, TLC and melting points.



2.2. Solubilities and analyses

The S_{IPM} and S_{AQ} values for **9–24** were taken from Sloan et al. (2003). The S_{IPM} and S_{AQ} values for **25–32** were taken from Wasdo and Sloan (2004). The S_{IPM} values for **1–8** were determined in the same way as S_{IPM} for **25–32** (Wasdo and Sloan, 2004). The S_{AQ} values for **1–4** and **6** were taken from Dal Pozzo and Pastori (1996), S_{AQ} values for **7** and **8** were measured in this study in the same way as S_{AQ} for **25–32** (Wasdo and Sloan, 2004), and S_{AQ} for **5** was calculated from the product of $S_{IPM}/SR_{IPM:AQ}$ (the solubility ratio between IPM and water) where the $SR_{IPM:AQ}$ for **5** was interpolated from the experimental $SR_{IPM:AQ}$ values of **4** and **6**.

Intact prodrugs **9–14**, **15–19** and **20–24** and their corresponding parent drugs, 5-FU or 6-MP, in the diffusion cell receptor phases were quantitated by UV as previously described in Sloan et al. (2003). Intact prodrugs **26–32** and APAP in the receptor phases were quantitated by UV as previously described (Wasdo and Sloan, 2004). Intact parabens and 4-hydroxybenzoic acid (HBA) in the receptor phase were quantitated by UV by the method previously described (Wasdo and Sloan, 2004), but using the molar absorptivities of the parabens and HBA at 245.5 (1.082 ± 0.029 and $1.262 \pm 0.044 \times 10^4$ l mol^{-1}, respectively) and 256 nm (1.442 ± 0.028 and $0.899 \pm 0.032 \times 10^4$ l mol^{-1}, respectively). The concentrations of intact prodrug or paraben were combined with the concentrations of parent drug (5-FU, 6-MP or APAP) or HBA, respectively, to give concentrations of total permeants (if hydrolysis occurred) at various times. Plots of total accumulated permeants against time gave slopes in μmol h^{-1} and fluxes were calculated by dividing the slopes by the surface area

of the diffusion cells (4.9 cm^2) to give the flux of total species in $\mu mol\,cm^{-2}\,h^{-1}$.

Theophylline in the diffusion cell receptor phases for the second application studies was quantitated by UV spectroscopy from its absorbance at 270 nm (ε = 10,200 $l\,mol^{-1}$) in pH 7.1 phosphate buffer containing 0.11% formaldehyde as previously described (Sloan et al., 1986). Flux of theophylline was calculated as above.

2.3. Diffusion cell experiments

The diffusion cell experiments were run according to the previously described procedure (Sloan et al., 1986). Briefly, the female hairless mice were sacrificed by CO_2 followed by cervical dislocation. Their skins were removed by blunt dissection and placed epidermal side up in contact with phosphate buffer, pH 7.1, containing 0.11% formaldehyde, 2.7 ml of 36% aqueous formaldehyde per liter of buffer, to prevent microbial growth and to insure the integrity of the mouse skins during the course of the experiment (Sloan et al., 1991). The skins were kept in contact with receptor phase for 24–48 h to condition them and to allow UV absorbing materials to leach out of the skins. The receptor phases were changed at least three times during this conditioning part of the experiment. Suspensions of each permeant in the water donor phase were prepared by stirring an amount that was at least a 10-fold excess of the water solubility of the permeant in water for 1 h before it was applied in the first application period of 48 h. A 1.5 ml aliquot of each suspension was applied to each of three membranes and the donor phase was sealed with parafilm to prevent evaporation of the donor phase. After 24 h a fresh suspension of each permeant was prepared as above and applied. Samples were generally taken at 8, 24, 27, 30, 33, 36, 39 and 48 h and analyzed immediately by UV as described in 2.2. The receptor phase was changed each time a sample was taken. In each case flux values reported were for the last 24 h interval and there was no decrease in flux for the last interval measured: there was no depletion of the donor phase. The donor phases were saved and analyzed by UV (solution) and ^{1}H NMR (solid) spectroscopy.

After this first application period, the membranes were washed 2–3 times with 3–5 ml of methanol. Then the membranes were left in contact with the receptor phases for 24–48 h to allow any permeant residue in the membrane to leach out. Leaching was considered complete when the UV spectrum of the receptor phase showed no measurable absorptions due to the intact prodrug, intact paraben, 5-FU, 6-MP, APAP or HBA.

After leaching was complete, a second application was made to the membranes. The second application consisted of a 0.5 ml aliquot of a 400 mg/6 ml suspension of theophylline (Th) in propylene glycol (PG) that was applied for 24 h. Samples were generally taken at 4, 8, 12 and 24 h during the second application and analyzed by UV as described in 2.2. The flux of Th/PG (J_{JAQ}) was used as an indicator of whether the membrane had been irreversibly changed (made more permeable to a second application) by the first application of prodrug or paraben in water compared to where nothing was applied in a first application as a control. This same procedure has been previously used (Sloan et al., 1986; Sloan et al., 2003) to determine whether hairless mouse skin had been irreversibly changed by applications of various drug/vehicle combinations. The J_{JAQ} value for this database, $0.0279 \pm 0.0132\ \mu mol\,cm^{-2}\,h^{-1}$, was not significantly different from the J_{JAQ} value from the previous database, $0.0246 \pm 0.0063\ \mu mol\,cm^{-2}\,h^{-1}$.

3. Theoretical

The development of the Roberts–Sloan, RS, model for the prediction of maximum flux of solutes through human skin from water *in vitro*, $\log J_{MHAQ}$ (Eq. (2)), where the S_{LIPID} parameter in Eq. (1) is S_{OCT}, has been presented elsewhere (Majumdar et al., 2007). Here, S_{IPM} will be the lipid parameter for prediction of maximum flux of solutes (prodrugs) through hairless mouse skin from water *in vitro*, $\log J_{MMAQ}$, using the RS model (Eq. (5)). Similarly, the development of the Kasting–Smith–Cooper, KSC, model (Kasting et al., 1987), and the modified KSC model (Eq. (3)) has been presented elsewhere (Majumdar et al., 2007). Here, S_{IPM} will be the lipid parameter for predicting $\log J_{MMAQ}$ using the KSC model (Eq. (6)). The development of the Magnusson–Anissimov–Cross–Roberts, MACR, model has been presented elsewhere (Magnusson et al., 2004) and the model in its simplest form is given by Eq. (4).

$$\log J_{MMAQ} = x + y \log S_{IPM} + (1 - y) \log S_{AQ} - z\,MW \quad (5)$$

$$\log J_{MMAQ} = x + y \log S_{IPM} - z\,MW \quad (6)$$

Multiple linear regression analysis was accomplished using the SPSS 14.0 statistical software package.

4. Results and discussion

4.1. Stability of the prodrugs during permeation

In the previous report (Sloan et al., 2003) of the permeation of the alkylcarbonyloxymethyl (ACOM) prodrugs of 6-mercaptopurine (6-MP) and 5-fluorouracil (5-FU) through hairless mouse skin, the conversion of the prodrugs to the parent drugs (6-MP and 5-FU) during permeation had been reported as essentially complete for the 3-ACOM-5-FU (**9–14**) and bis-6,9-ACOM-6-MP (**20–24**) prodrugs, but less complete for the 6-ACOM-6-MP (**15–19**) prodrugs. Here we report the results of the conversions during permeation as % intact prodrugs in the receptor phases during the steady-state portion of the diffusion cell experiments in Table 1. The results for the acetaminophen (APAP) prodrugs (**26–32**) were mixed: some of the prodrugs (**29**, **30** and **32**) were completely hydrolyzed but those were the prodrugs that gave the lowest J_{MMAQ} values in the series, while others (**26**, **27**, **28** and **31**) were less completely hydrolyzed. On the other hand, all of the parabens were found to be substantially intact in the receptor phases. The general trend is that the longer alkyl chain members of each series (except for **19**) tend to be more completely hydrolyzed (Ahmed et al., 1996; Seki et al., 1990). Hydrolysis of a few of the lower alkyl parabens during permeation of full thickness abdominal skin of male Wistar strain rats has been previously reported (Bando et al., 1997). Analysis of their data showed that propylparaben (**3**) gave a higher flux value than butylparaben (**4**) but a lower % conversion to the parent 4-hydroxybenzoic acid (HBA): the opposite of what we have observed. However, the J_M values calculated from their Fig. 1 for **3** and **4** (−0.86 and −1.09, respectively) are of the same order of magnitude as presented here.

As previously reported (Sloan et al., 2003), all of the prodrugs were intact in the donor phases during the first application as assessed by UV described in section 2.2.

4.2. Fit of RS, KSC and MACR equations to J_{MMAQ}

The molecular weights, MW, log solubilities in isopropyl myristate, $\log S_{IPM}$, and water, $\log S_{AQ}$, and the experimental log maximum fluxes for the prodrugs of 5-FU (**9–14**), 6-MP (**15–24**) and APAP (**26–32**) and the parabens through hairless mouse skin from water, Exp $\log J_{MMAQ}$, are given in Table 1. When $\log S_{IPM}$, $\log S_{AQ}$ and MW were the independent variables and Exp $\log J_{MMAQ}$ was the

Table 1
Dataset.

Compound[a]		MW	log S_{AQ}[b]	log S_{IPM}[b]	Exp log J_{MHAQ}[c,d]	Exp log J_{MMAQ}[c]	log $K_{IPM:AQ}$	Exp log P_M[e]	% intact prodrug
	Parabens								
1	R = C1	152	1.2	2.310	−1.60	−0.649	1.110	−1.849	76
2	C2	166	0.74	2.375	−1.49	−0.753	1.635	−1.493	62
3	C3	180	0.31	2.530	−1.58	−0.983	2.220	−1.293	42
4	C4	194	0.09	2.938	−1.62	−0.906	2.848	−0.996	65
5	C5	208	−0.42	3.120		−0.991	3.540	−0.571	76
6	C6	222	−0.985	3.240	−2.46	−1.419	4.225	−0.434	29
7	C7	236	−1.57	3.230		−1.620	4.800	−0.050	15
8	C8	250	−2.28	3.180		−1.887	5.460	0.393	36
	3-ACOM-5-FU								
9	R = C1	202	1.8	0.090		−1.770	−1.710	−3.570	0
10	C2	216	2.25	1.200		−1.410	−1.050	−3.660	0
11	C3	230	1.93	1.420		−1.130	−0.510	−3.060	0
12	C4	244	1.32	1.470		−1.430	0.150	−2.750	0
13	C5	258	0.92	1.630		−1.410	0.710	−2.330	0
14	C7	286	−0.25	1.600		−1.850	1.850	−1.600	0
	6-ACOM-6-MP								
15	R = C1	224	0.86	0.020		−2.550	−0.840	−3.410	42
16	C2	238	0.61	0.360		−2.190	−0.250	−2.800	18
17	C3	252	0.31	0.520		−2.000	0.210	−2.310	6
18	C4	266	−0.1	0.620		−2.180	0.720	−2.080	8
19	C5	280	−0.63	0.570		−2.370	1.200	−1.740	24
	6,9-ACOM-6-MP								
20	R = C1	296	0.46	0.720		−1.980	0.260	−2.440	0
21	C2	324	0.22	1.530		−1.890	1.310	−2.110	0
22	C3	352	−0.71	1.960		−2.270	2.670	−1.560	0
23	C4	380	−1.33	2.240		−2.480	3.570	−1.150	0
24	C5	408	−2.98	1.700		−3.070	4.680	−0.090	0
	APAP Prodrugs								
25	APAP	151	2	0.280		−1.730	−1.720	−3.730	–
26	R = C1	209	1.31	1.080		−1.500	−0.230	−2.810	18
27	C2	223	0.58	0.970		−1.690	0.390	−2.270	42
28	C3	237	0.43	1.370		−1.660	0.940	−2.090	39
29	C4	251	−0.37	1.140		−2.150	1.510	−1.780	0
30	C6	279	−1.32	1.220		−2.280	2.540	−0.960	0
31	MeO-C2[f]	253	1.54	1.010		−1.450	−0.530	−2.990	24
32	MeO-C3i[g]	267	0.52	0.530		−2.380	0.010	−2.900	0

[a] C1, C2, ... refer to the number of carbons in alkyl group.
[b] Units of mM.
[c] Units of μmol cm^{-2} h^{-1}.
[d] Dal Pozzo and Pastori (1996).
[e] Units of cm h^{-1}.
[f] 2-Methoxyethyl.
[g] 2-Methoxy-1-methylethyl.

dependent variable fitted to RS Eq. (5), the result was as follows:

$$\log J_{MMAQ} = -2.30 + 0.575 \log S_{IPM} + 0.425 \log S_{AQ} - 0.00160\,MW$$

where $r^2 = 0.903$ and the average of the absolute values for the differences between experimental logJ_{MMAQ} and logJ_{MMAQ} calculated from the fit to Eq. (5), $\Delta \log J_{MMAQ}$, was 0.141 log units. When log S_{IPM} and MW were the independent variables and Exp logJ_{MMAQ} was the dependent variable fitted to KSC Eq. (6), the result was as follows:

$$\log J_{MMAQ} = -0.326 + 0.248 \log S_{IPM} - 0.00722\,MW$$

where $r^2 = 0.764$ and $\Delta \log J_{MMAQ} = 0.218$ log units. When MW was the only independent variable and Exp logJ_{MMAQ} was the dependent variable fitted to MACR Eq. (4) the result was as follows:

$$\log J_{MMAQ} = 0.0396 - 0.00722\,MW$$

where $r^2 = 0.56$ and $\Delta \log J_{MMAQ} = 0.313$ log units. The logJ_{MMAQ} calculated from Eqs. (5), (6) and (4) and the resulting individual $\Delta \log J_{MMAQ}$ are given in Table 2. For the RS model the difference between the Exp logJ_{MMAQ} and logJ_{MMAQ} calculated by Eq. (5) (Δ' logJ_{MMAQ}), which has a positive or negative value, is given in Table 2. The much better fit of this data to RS is consistent with previous results where RS gave better fit to data for the delivery of solutes through human skin from water *in vitro* (logJ_{MHAQ}) than KSC or MACR (Majumdar et al., 2007; Thomas et al., 2007; Juntunen et al., 2008). The plots of Exp logJ_{MMAQ} versus logJ_{MMAQ} calculated from Eqs. (5), (6) or (4) are given in Figs. 1–3, respectively. The figures are all on the same scale so the better fit of RS to the data is qualitatively obvious from inspection of the figures.

The present fit of logJ_{MMAQ} data to RS is much better than the fit previously obtained for the ACOM prodrugs (Sloan et al., 2003) where $r^2 = 0.765$ and $\Delta \log J_{MMAQ} = 0.193$ log units. When the $\Delta \log J_{MMAQ}$ for the individual datasets were calculated the parabens gave a better fit to RS, ($\Delta \log J_{MMAQ} = 0.080$ log units) than the other datasets. On the other hand the APAP prodrugs (minus APAP itself) gave a better fit to KSC ($\Delta \log J_{MMAQ} = 0.194$ log units) and MACR ($\Delta \log J_{MMAQ} = 0.239$ log units) than the other datasets, and the APAP prodrugs also gave the second best fit to RS ($\Delta \log J_{MMAQ} = 0.118$ log units). Obviously the two additional

Table 2
Comparison of fit to three model equations[a].

Compound[b]		RS Cal	RS Δ' log J_{MMAQ}	Predicted (±) (y) or (n)	RS Δ log J_{MMAQ}	KSC Cal	KSC Δ log J_{MMAQ}	MACR Cal	MACR Δ log J_{MMAQ}
	Parabens								
1	*R* = C1	−0.703	+0.054	+(y)	0.054	−0.851	0.202	−1.058	0.409
2	C2	−0.884	+0.131	+(y)	0.131	−0.936	0.183	−1.159	0.406
3	C3	−1.000	+0.017	+(y)	0.017	−0.999	0.016	−1.260	0.277
4	C4	−0.881	−0.025	+(y)	0.025	−0.999	0.093	−1.361	0.455
5	C5	−1.016	+0.024	+(y)	0.024	−1.055	0.063	−1.462	0.471
6	C6	−1.209	−0.210	+(n)	0.210	−1.126	0.293	−1.563	0.144
7	C7	−1.486	−0.134	+(n)	0.134	−1.230	0.390	−1.664	0.045
8	C8	−1.839	−0.048	+(n)	0.048	−1.343	0.544	−1.765	0.121
	3-ACOM-5-FU								
9	*R* = C1	−1.805	+0.035	+(y)	0.035	−1.763	0.007	−1.419	0.351
10	C2	−0.998	−0.412	−(y)	0.412	−1.589	0.179	−1.520	0.110
11	C3	−1.030	−0.100	+(n)	0.100	−1.635	0.505	−1.621	0.491
12	C4	−1.282	−0.148	+(n)	0.148	−1.724	0.294	−1.722	0.292
13	C5	−1.383	−0.027	+(n)	0.027	−1.785	0.375	−1.823	0.413
14	C7	−1.942	+0.092	+(y)	0.092	−1.995	0.145	−2.025	0.175
	6-ACOM-6-MP								
15	*R* = C1	−2.280	−0.270	−(y)	0.270	−1.939	0.611	−1.578	0.972
16	C2	−2.213	+0.023	−(n)	0.023	−1.956	0.234	−1.679	0.511
17	C3	−2.271	+0.271	−(n)	0.271	−2.017	0.017	−1.780	0.220
18	C4	−2.410	+0.230	+(y)	0.230	−2.094	0.086	−1.881	0.299
19	C5	−2.686	+0.316	+(y)	0.316	−2.207	0.163	−1.982	0.388
	6,9-ACOM-6-MP								
20	*R* = C1	−2.162	+0.182	−(n)	0.182	−2.285	0.305	−2.098	0.118
21	C2	−1.843	−0.047	−(y)	0.047	−2.287	0.397	−2.300	0.410
22	C3	−2.036	−0.234	+(n)	0.234	−2.382	0.112	−2.502	0.232
23	C4	−2.183	−0.297	+(n)	0.297	−2.515	0.035	−2.704	0.224
24	C5	−3.240	−0.170	+(n)	0.170	−2.851	0.219	−2.906	0.164
	APAP Prodrugs								
25	APAP	−1.529	−0.201		0.201	−1.347	0.383	−1.051	0.679
26	*R* = C1	−1.455	−0.045	+(n)	0.045	−1.568	0.068	−1.469	0.031
27	C2	−1.851	+0.161	+(y)	0.161	−1.696	0.006	−1.570	0.120
28	C3	−1.707	+0.047	+(y)	0.047	−1.698	0.038	−1.672	0.012
29	C4	−2.202	+0.052	+(y)	0.052	−1.856	0.294	−1.773	0.377
30	C6	−2.604	+0.324	+(y)	0.324	−2.039	0.241	−1.975	0.305
31	MeO-C2[c]	−1.468	+0.018	+(y)	0.018	−1.903	0.453	−1.787	0.337
32	MeO-C3i[d]	−2.200	−0.180	+(n)	0.180	−2.123	0.257	−1.888	0.492
	x=	−2.299				−0.326		0.0396	
	y=	0.575				0.248			
	z=	0.00160				0.00722		0.00722	
	r^2=	0.903				0.764		0.56	
	Δ log J_{MMAQ}				0.141 ± 0.11		0.218 ± 0.17		0.314± 0.20

[a] All log *J* values are in units of μmol cm^{-2} h^{-1}.
[b] C1,C2, . . . refer to number of carbons in alkyl group.
[c] 2-Methoxyethyl.
[d] 2-Methoxy-1-methylethyl.

datasets were especially responsible for the better fit to RS. To determine which dataset was primarily responsible for the greatly improved fit, one dataset at a time was deleted from the database and the data for the remaining datasets were fit to RS. The new coefficients to *x*, *y* and *z* for the 5 leave-one-out databases are given in Table 3. New log J_{MMAQ} were then calculated for all 5 of the datasets using the new coefficients to the parameters for each of the 5 leave-one-out databases as well as Δ log J_{MMAQ} for all 5 of the datasets which included the dataset left out in the calculations of the new coefficients. The biggest difference in r^2 and Δ log J_{MMAQ} occurred when the parabens (**1–8**) were left out: r^2 decreased to 0.832 and Δ log J_{MMAQ} increased to 0.211 log units. However, even when the parabens were left out, the fit was better than that previously reported, r^2 = 0.765 (Sloan et al., 2003). The coefficients *x*, *y* and *z* also changed substantially when the parabens were left out, but especially *z* which almost doubled in value. Thus the big improvement in the fit of this *n* = 32 database compared to the previous *n* = 17 database to RS is due to the inclusion of the parabens.

4.3. The relationship of partition coefficients and permeabililty coefficients to flux

The log partition coefficients between IPM and AQ, log $K_{IPM:AQ}$, and the log permeability coefficients, Exp log P_M, derived from Exp log J_{MMAQ} − log S_{AQ} are also given in Table 1. Plots of log S_{AQ}, log S_{IPM}, Exp log J_{MMAQ}, log $K_{IPM:AQ}$ and Exp log P_M versus increasing alkyl chain length of the promoiety (and increasing compound number) are given in Fig. 4. In all cases the Exp log P_M and log $K_{IPM:AQ}$ values trend in the opposite direction as Exp log J_{MMAQ}. Thus, log P_M is directly related to log $K_{IPM:AQ}$ as would be predicted by the direct relationship between log P_M and log $K_{OCT:AQ}$ reported previously (Potts and Guy, 1992). On the other hand, log S_{AQ} trends in the same direction as log J_{MMAQ} and log S_{IPM} increases initially and then tends to decreases with chain lengths longer than C_5 to C_7. Thus, Fig. 4 shows that maximum flux for a homologous series of prodrugs tends to be exhibited by those members of the series that exhibit the greater solubilities in water (Sloan et al., 1984), or by

Table 3
Leave one out experiment[a].

Compound	Cal $\log J_{MMAQ}$ excluding **1**–**8**	$\Delta \log J_{MMAQ}$	Cal $\log J_{MMAQ}$ excluding **9**–**14**	$\Delta \log J_{MMAQ}$	Cal $\log J_{MMAQ}$ excluding **15**–**19**	$\Delta \log J_{MMAQ}$	Cal $\log J_{MMAQ}$ excluding **20**–**24**	$\Delta \log J_{MMAQ}$	Cal $\log J_{MMAQ}$ excluding **25**–**32**	$\Delta \log J_{MMAQ}$
1	−0.445	0.204	−0.697	0.048	−0.727	0.078	−0.810	0.161	−0.722	0.073
2	−0.603	0.151	−2.482	0.127	−2.342	0.151	−2.672	0.213	−2.371	0.151
3	−0.690	0.293	−2.439	0.017	−2.484	0.034	−2.782	0.074	−2.487	0.039
4	−0.538	0.368	−2.331	0.021	−2.457	0.011	−2.742	0.006	−2.448	0.001
5	−0.633	0.358	−2.378	0.033	−2.499	0.034	−2.784	0.029	−2.491	0.051
6	−0.788	0.631	−2.292	0.197	−2.763	0.204	−2.990	0.232	−2.706	0.181
7	−1.037	0.583	−2.427	0.118	−2.857	0.131	−3.091	0.181	−2.806	0.103
8	−1.353	0.533	−2.592	0.028	−2.986	0.049	−3.225	0.121	−2.939	0.015
9	−1.914	0.144	−3.051	0.003	−2.841	0.086	−3.182	0.100	−2.879	0.040
10	−1.071	0.339	−2.577	0.446	−2.710	0.365	−2.993	0.373	−2.700	0.405
11	−1.078	0.052	−2.743	0.131	−2.519	0.056	−2.862	0.086	−2.559	0.091
12	−1.295	0.135	−2.790	0.174	−2.686	0.107	−3.009	0.159	−2.709	0.136
13	−1.369	0.041	−2.875	0.051	−2.660	0.010	−3.002	0.064	−2.699	0.014
14	−1.873	0.023	−3.104	0.075	−2.878	0.124	−3.222	0.004	−2.918	0.109
15	−2.349	0.201	−3.207	0.303	−3.270	0.224	−3.565	0.244	−3.269	0.262
16	−2.252	0.062	−3.245	0.007	−3.053	0.066	−3.391	0.023	−3.088	0.033
17	−2.293	0.293	−3.132	0.244	−2.962	0.312	−3.296	0.247	−2.994	0.282
18	−2.411	0.231	−3.219	0.206	−3.052	0.268	−3.386	0.182	−3.083	0.243
19	−2.668	0.298	−3.292	0.294	−3.151	0.352	−3.480	0.244	−3.178	0.331
20	−2.265	0.285	−3.125	0.150	−2.951	0.228	−3.286	0.097	−2.984	0.193
21	−1.900	0.010	−3.123	0.073	−2.898	0.007	−3.242	0.182	−2.938	0.033
22	−2.017	0.253	−3.037	0.252	−3.135	0.202	−3.424	0.422	−3.129	0.216
23	−2.132	0.348	−3.033	0.310	−3.258	0.269	−3.526	0.533	−3.235	0.276
24	−3.136	0.066	−3.628	0.163	−3.503	0.191	−3.830	0.116	−3.528	0.194
25	−1.541	0.031	−2.954	0.233	−2.705	0.155	−3.053	0.064	−2.749	0.194
26	−1.436	0.064	−2.891	0.071	−2.710	0.005	−3.046	0.004	−2.744	0.034
27	−1.800	0.110	−3.022	0.139	−2.799	0.197	−3.142	0.176	−2.839	0.174
28	−1.630	0.030	−2.995	0.028	−2.786	0.081	−3.127	0.037	−2.824	0.062
29	−2.097	0.053	−3.184	0.036	−3.040	0.082	−3.370	0.016	−3.068	0.068
30	−2.455	0.175	−3.270	0.314	−3.102	0.349	−3.436	0.239	−3.133	0.344
31	−1.563	0.113	−2.918	0.016	−2.676	0.065	−3.023	0.000	−2.719	0.026
32	−2.270	0.110	−3.226	0.048	−3.167	0.136	−3.483	0.223	−3.184	0.170
x=	−1.852		−2.302		−2.315		−2.629		−2.318	
y=	0.671		0.566		0.583		0.585		0.571	
z=	0.00354		0.00147		0.00170		0.000201		0.00156	
r^2=	0.832		0.914		0.918		0.898		0.912	
$\Delta \log J_{MMAQ}$		0.211 ± 0.163		0.141 ± 0.115		0.145 ± 0.110		0.152 ± 0.127		0.142 ± 0.11

[a] All $\log J$ values are in units of μmol cm^{-2} h^{-1}.

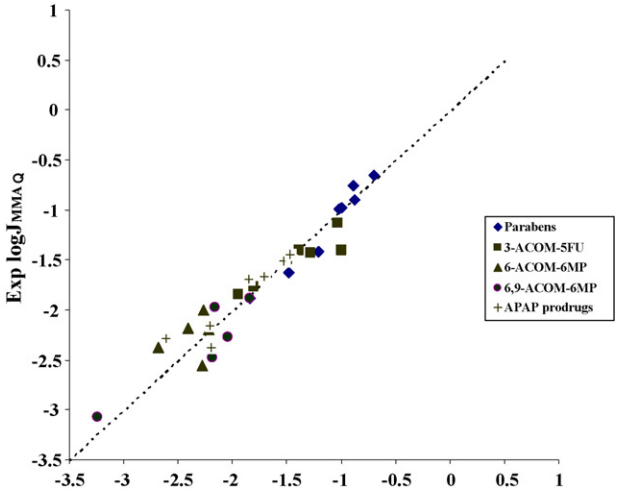


Fig. 1. Calculated (Cal) vs experimental (Exp) $\log J_{MMAQ}$; RS equation. Cal $\log J_{MMAQ} = -2.299 + 0.575^{*}\log S_{IPM} + (1 - 0.575)^{*}\log S_{AQ} - 0.001597^{*}MW$; $r^2 = 0.903$.

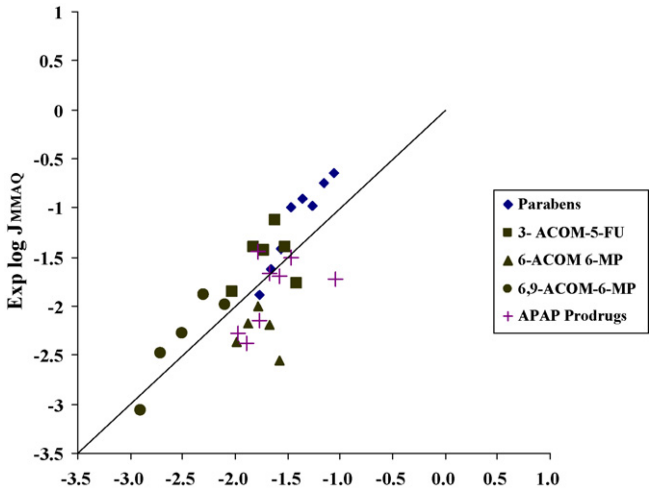


Fig. 3. Calculated (Cal) vs experimental (Exp) $\log J_{MMAQ}$; MACR equation. Cal $\log J_{MMAQ} = 0.0396 - 0.00722^{*}MW$, $r^2 = 0.56$.

that member that exhibits the best balance between S_{AQ} and S_{LIPID} (in this case IPM) as previously reported (Sloan, 1989). On the other hand, $\log P_M$ is a poor predictor of $\log J_{MMAQ}$ so that $\log P_M$ values are not useful for constructing paradigms that can be used to design better prodrugs or new drugs exhibiting optimized topical delivery, i.e. flux, the only useful measurement of clinical efficacy (Sloan et al., 2006).

4.4. The effect of hydrolysis of prodrugs on predictions of flux

Previously, two hypotheses about the effect of hydrolysis of prodrugs on their abilities to deliver total species containing the parent drug (prodrug plus parent drug) had been presented (Sloan et al., 2006; Juntunen et al., 2008). The first hypothesis was that, if the prodrug hydrolyzed upon permeation of the skin, whether the prodrug was more or less water soluble than the parent would make no difference in whether the experimentally measured flux, Exp $\log J_{MMAQ}$, was greater or lesser than that calculated by RS Eqs. (2) or (5): the prodrugs over- or under-performed, respectively. Although the sum of the differences between the Exp $\log J_{MMAQ}$ and $\log J_{MMAQ}$ calculated by Eq. (5) ($\Delta' \log J_{MMAQ}$) for each individual member in the database should be zero and the average of the absolute values of the differences between Exp $\log J_{MMAQ}$ and $\log J_{MMAQ}$ calculated by Eq. (5) ($\Delta \log J_{MMAQ}$) should be a positive value, the individual $\Delta' \log J_{MMAQ}$ values for the members would be (+) or (−). A (+) value would mean that the $\log J_{MMAQ}$ of the individual prodrug calculated by Eq. (5) was less than the Exp $\log J_{MMAQ}$: the prodrug over-performed. A (−) value would mean that the prodrug under-performed. There should be no correlation between (+) and (−) values and whether the prodrug was more or less soluble than its parent drug. The basis for the first hypothesis was that $\log J_M$ in the RS model only depends on the solubility of the permeant in the first few layers of the membrane, S_{M1}, as stated in Fick's law.

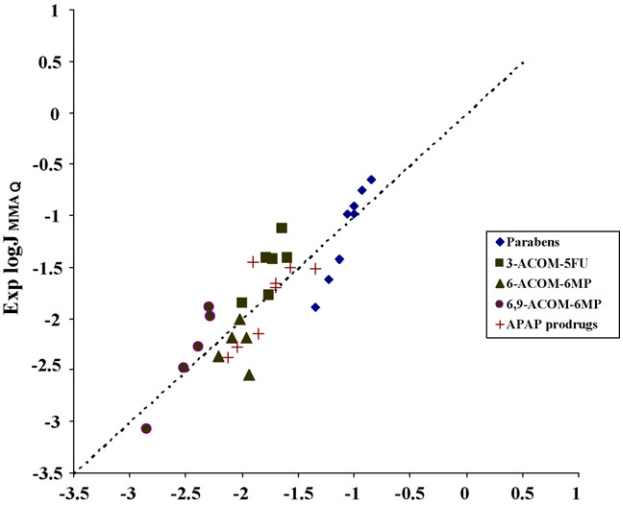


Fig. 2. Calculated (Cal) vs experimental (Exp) $\log J_{MMAQ}$; KSC equation. Cal $\log J_{MMAQ} = -0.326 + 0.248^{*}\log S_{IPM} - 0.007223^{*}MW$; $r^2 = 0.764$.

On the other hand, the second hypothesis (Ahmed et al., 1996; Stinchcomb et al., 2002) was that, if the prodrug hydrolyzed during permeation and it was more soluble in water than its parent, it would under-perform (−): Exp $\log J_{MMAQ}$ would be less than that calculated by Eq. (5). If the prodrug hydrolyzed during permeation and it was less soluble in water than its parent, it would over-perform (+) the Exp $\log J_{MMAQ}$. Thus if the second hypothesis were correct, $\Delta' \log J_{MMAQ}$ should be negative (−) when the prodrug was more soluble than its parent drug and (+) when the prodrug was less soluble. Then, if the sign of the actual $\Delta' \log J_{MMAQ}$ agreed with the sign, (+) or (−), predicted by the second hypothesis, a yes (y) was assigned, and if not, a no (n) was assigned: the (y) and (n) values are presented in Table 2 with the (+) or (−) values predicted by the second hypothesis. The basis for the second hypothesis was that $\log J_M$ of total species depended not only on the S_{M1} of the prodrug, but also on its solubility and that of its parent drug in the more aqueous environment of the viable epidermis/dermis layer. For instance if the prodrug was more soluble in water than its parent drug, the first hypothesis and Eq. (5) would predict a $\log J_M$ based only on the greater contribution of the S_{AQ} of the prodrug to the S_{AQ} parameter. The second hypothesis would predict a lower flux of total species than that predicted by the first hypothesis and Eq. (5) because the parent drug, formed during permeation from the prodrug, would be less soluble than the prodrug in the more aqueous-like viable epidermis/dermis layer that comprised a solubility based resistance to permeation in addition to the solubility based resistance in the more lipid-like first few layers of the membrane, M1. On the other hand, if the prodrug was less soluble than its parent drug in water and hydrolyzed during permeation, its flux of total species would be greater because the parent drug would

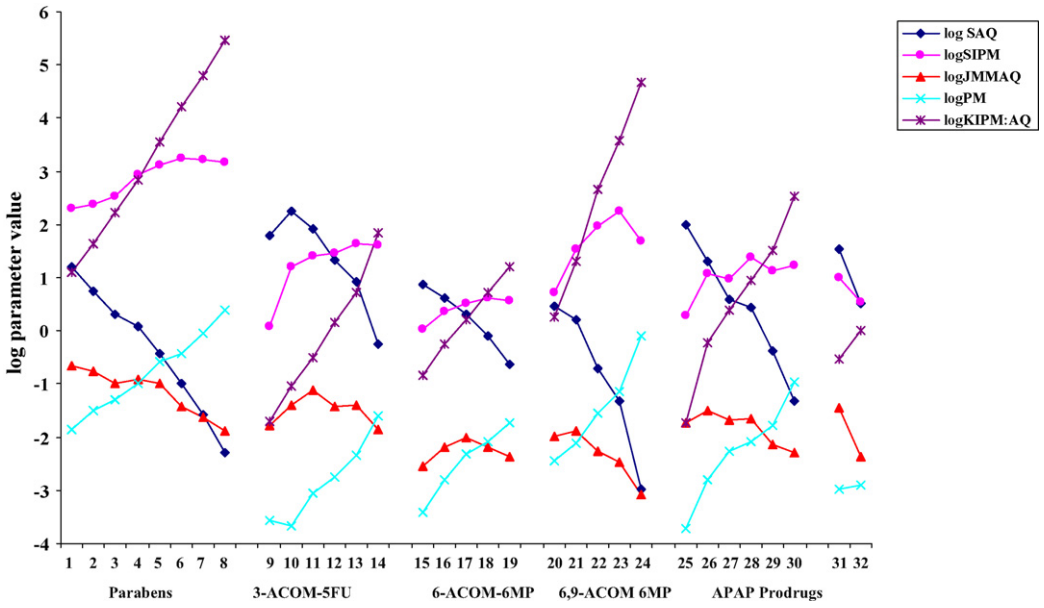


Fig. 4. Log S_{AQ}, log S_{IPM}, log J_{MMAQ}, log P_M, log $K_{IPM:AQ}$ versus alkyl chain length in compounds.

be more soluble in the more aqueous-like viable epidermis/dermis layer and that layer would present less resistance to permeation of the parent drug than the prodrug.

When a yes (y) or a no (n) was assigned based on whether the prodrug was more or less soluble in water than its parent and whether the prodrug gave a higher or lower flux of total species than that calculated by Eq. (5) (Table 2), there were 16 yes and 15 no. Thus, there was no trend towards yes assignments that would suggest that the second hypothesis was correct. There was no substantial effect of hydrolysis of the prodrugs on the accuracy of the calculation of their fluxes by Eq. (5). Solubility in the first few layers of the membrane – log S_{M1}, predicted by y log S_{LIPID} + (1 − y) log S_{AQ} + log c (Sloan et al., 2006); diffusivity – log D, predicted by log $D_0 - z$MW; and thickness of the membrane – log L, assumed to be a constant, are the only parameters needed to calculate log J_{MMAQ} based on Fick's law. A second, aqueous-like resistance in the viable epidermis/dermis layer is not necessary to explain the data.

5. Conclusions

The Roberts–Sloan (RS) model, where flux depends directly on solubility in both a lipid and in water and inversely on molecular weight, has been shown to be the best model to predict flux from water through human skin, J_{MHAQ} (Juntunen et al., 2008; Thomas et al., 2007; Majumdar et al., 2007). Now the RS model has been shown to be the best model to predict flux from water through hairless mouse skin, J_{MMAQ}. When comparing the coefficients x, y and z to the parameters in the two RS models, it is obvious that x and y are quite similar: for J_{MHAQ} $x = -2.506$ and $y = 0.538$, for J_{MMAQ} $x = -2.299$ and $y = 0.575$. The z values are quite different 0.00402 and 0.00160, respectively. Taken together this suggests that hairless mouse skin is very similar to human skin except for the greater inverse dependence on molecular weight which should lead to lower flux values through human skin. The only overlap between the log J_{MHAQ} and J_{MMAQ} databases is the parabens. The log J_{MHAQ} data (Dal Pozzo and Pastori, 1996) has been inserted in Table 1 next to the log J_{MMAQ} data where it can be seen that log J_{MHAQ} is 0.806 ± 0.183 log units less than log J_{MMAQ}. Thus J_{MMAQ} is about 6.4 times greater than J_{MHAQ} which is of the same order of difference previously reported (Sloan et al., 1997). The result is that trends in the design of new drugs or prodrugs that result from studying permeation through hairless mouse skin can be extended to expected trends in permeation of human skin except that the fluxes will be less.

In addition, no trend in the effect of the solubility in water of the prodrugs compared to their parent drugs on their delivery of total species containing the parent drug has been observed, which was also observed for the flux of prodrugs through human skin (Juntunen et al., 2008). No direct correlation between flux, log J_{MMAQ}, and the respective permeability coefficients, log P_M, was observed, which was also observed for the flux of prodrugs and solutes through human skin (Sloan et al., 2006; Juntunen et al., 2008). Since flux is the only clinically relevant measure of permeation, changes in flux are the only rational basis for designing prodrugs or selecting new drugs for topical delivery. And, since the Roberts–Sloan model provides the best fit to the flux data, it should be used as the basis for design and selection.

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