Available online at www.sciencedirect.com
ScienceDirect
International Journal of Pharmaceutics 351 (2008) 92–103
INTERNATIONAL JOURNAL OF PHARMACEUTICS
www.elsevier.com/locate/ijpharm

The effect of water solubility of solutes on their flux through human skin *in vitro*: A prodrug database integrated into the extended Flynn database

Juha Juntunen [a,b], Susruta Majumdar [c], Kenneth B. Sloan [a,*]

[a] *Department of Medicinal Chemistry, University of Florida, P.O. Box 100485, Gainesville, FL 32610, United States*
[b] *Department of Pharmaceutical Chemistry, University of Kuopio, P.O. Box 1627, Fin-70211 Kuopio, Finland*
[c] *Laboratory of Molecular Pharmacology and Chemistry, Memorial Sloan Kettering Cancer Center, 1275 York Avenue, New York, NY 10021, United States*

Received 19 July 2007; received in revised form 13 September 2007; accepted 17 September 2007
Available online 29 September 2007

Abstract

A database ($n = 50$) consisting of values of solubility in water, S_{AQ}, solubility in octanol, S_{OCT}, molecular weight, MW, and maximum flux based on the delivery of total species containing a parent drug by its prodrugs through human skin *in vitro* from water has been integrated into the extended Flynn database ($n = 114$). In addition, data for two more recent contributions ($n = 8$) and one ($n = 7$) contribution that was overlooked for inclusion in the extended Flynn database were added to the prodrug database, as well as the data for the parent drugs ($n = 6$), to give $n = 71$ and $n = 185$ for the total integrated database. This integrated database was fit to five equations where the independent variable was S_{AQ}, S_{OCT} or MW alone or were combinations of S_{OCT} and MW (Kasting–Smith–Cooper, KSC model) or S_{OCT}, S_{AQ} and MW (Roberts–Sloan, RS model). The RS equation gave the best fit: $\log J_{MAQ} = -2.506 + 0.538 \log S_{OCT} + 0.462 \log S_{AQ} - 0.00402\text{MW}$, $r^2 = 0.839$, S.D. = 0.423 and the residual ($\Delta \log J_{MAQ}$) was 0.474 log units. Integration of a substantial number of prodrugs into the extended Flynn database did not change the dependence of J_{MAQ} on a balance of S_{AQ} and S_{OCT}. No trend in the effect of the prodrug being more or less water soluble than its parent drug on over- or underpredicting flux ($\pm\Delta' \log J_{MAQ}$) by the RS model was found. Thus optimization of the S_{AQ} of a prodrug in its design, as well as the design of new drugs, is indicated.

Keywords: Prodrugs; Water solubility; Octanol solubility; Roberts–Sloan equation; Hydrolysis

1. Introduction

It is clear for the delivery of total species containing a parent drug by its prodrugs from their saturated solutions in the lipid vehicle, isopropyl myristate (IPM), through hairless mouse skin *in vitro* that the water solubility of the prodrugs, S_{AQ}, is an important predictor of delivery (Roberts and Sloan, 1999). The database for the most recent analysis of the direct dependence of maximum flux (J_{MIPM}) on S_{IPM} and S_{AQ}, in addition to an inverse dependence on molecular weight, MW, is $n = 63$ where the coefficients for the parameters in the Roberts–Sloan (RS, Eq. (1)) are $x = -0.50$; $y = 0.517$; $z = 0.00266$; $r^2 = 0.91$; the average absolute difference between experimental $\log J_{MIPM}$ and calculated $\log J_{MIPM}$ ($\Delta \log J_{MIPM}$) is 0.16 log units (Sloan and Wasdo, 2007):

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

In addition, it has been shown for a much smaller subset ($n = 17$) of the $n = 63$ prodrug database, that the delivery of total species containing the parent drug by its prodrugs from their saturated solutions in water through hairless mouse skin *in*

Abbreviations: KSC, Kasting–Smith–Cooper equation; MACR, Magnusson–Anissimov–Cross–Roberts equation; RS, Roberts–Sloan equation; S_{OCT}, solubility in octanol; MW, molecular weight; S_{AQ}, solubility in water or in water at different pH values as indicated by subscript; J_{MAQ}, maximum flux values using water as the vehicle or water at different pH values as indicated by subscript; $\Delta \log J_{MAQ}$, residual or average absolute difference between experimental $\log J_{MAQ}$ and calculated $\log J_{MAQ}$; $\Delta' \log J_{MAQ}$, difference between experimental $\log J_{MAQ}$ and calculated $\log J_{MAQ}$; FULL, full thickness skin; EPI, heat separated epidermis; (n), over- or underprediction of $\log J_{MAQ}$ not predicted by second hypothesis; (y), over- or underprediction of $\log J_{MAQ}$ predicted by second hypothesis.

* Corresponding author. Tel.: +1 352 273 7745; fax: +1 352 392 9455.
E-mail address: sloan@cop.ufl.edu (K.B. Sloan).

0378-5173/$ – see front matter © 2007 Elsevier B.V. All rights reserved.
doi:10.1016/j.ijpharm.2007.09.034

Table 1
Esters of nalidixic acid (NAD)[a]

RCO_2R^1 compound	MW	log S_{AQ}[b]	log $K_{OCT:AQ}$	log S_{OCT}[b,c]	log J_{MAQ}[d]	Δ'log J_{MAQ}[d,e]	Δ'log J_{MAQ}[d,f]
RCO_2 = NAD, R^1 = H	232	−0.98	1.51	0.53	−2.49	–	1.111
RCO_2 = NAD, R^1 = a[g]	246	0.30	1.23	1.53	−2.01	0.114 (n)	0.523
RCO_2 = NAD, R^1 = b[g,h]	345	1.57	1.43	3.00	−3.27	–	–
RCO_2 = NAD, R^1 = c[g]	304	0.51	1.21	1.72	−2.64	−0.341 (y)	−0.069
RCO_2 = NAD, R^1 = d[g]	318	0.17	1.70	1.87	−2.51	−0.089 (y)	0.194
RCO_2 = NAD, R^1 = e[g]	332	0.057	2.20	2.26	−1.80	0.507 (n)	0.802
RCO_2 = NAD, R^1 = f[g]	332	−0.017	2.17	2.16	−2.19	0.208 (n)	0.500
						0.252[i]	0.533[i]

[a] Bundgaard et al. (1989).
[b] Units of mM.
[c] Calculated from log $K_{OCT:AQ}$ + log S_{AQ}.
[d] Units of μmol cm^{-2} h^{-1}.
[e] Calculated from experimental log J_{MAQ} − calculated log J_{MAQ} from Eq. (1) and Table 14 (n = 50). See Section 5.1 for definitions of (n) and (y).
[f] Calculated from experimental log J_{MAQ} − calculated log J_{MAQ} from Eq. (1) and Table 14 (n = 185).
[g] (a) —CH_3; (b) —CH_2—(C=O)—$N(C_2H_5)_2$; (c) —CH_2O—(C=O)—CH_3; (d) —CH_2O—(C=O)—C_2H_5; (e) —CH_2O—(C=O)—C_3H_7; (f) —CH_2O—(C=O)—$CH(CH_3)_2$.
[h] Deleted from final database since log $K_{OCT:AQ}$ is too high compared to its $SR_{IPM:AQ}$ and so is the S_{OCT} calculated from log $K_{OCT:AQ}$.
[i] Δlog J_{MAQ} for prodrugs.

Table 2
Alkylcarbonyl esters of morphine[a]

	MW	log $S_{7.0}$[b]	log $K_{OCT:7.4}$	log S_{OCT}[b,c]	log $J_{M7.0}$[d]	Δ'log $J_{M7.0}$[d,e]	Δ'log $J_{M7.0}$[d,f]
Morphine	285	0.80	−0.06	0.72	<−4.45	–	–
3,6-Dipropionate	397	0.95	1.66	2.61	−2.06	0.099 (n)	0.199
3,6-Dihexanoate	481	−1.38	4.66	3.28	−2.22	0.922 (y)	1.093
3-Hexanoate	384	0.83	2.04	2.87	−1.05	0.909 (n)	1.072
						0.643[g]	0.787[g]

[a] Drustrup et al. (1991).
[b] Units of mM.
[c] Calculated from log $K_{OCT:7.4}$ + log $S_{7.0}$.
[d] Units of μmol cm^{-2} h^{-1}.
[e] Calculated from experimental log $J_{M7.0}$ − calculated log $J_{M7.0}$ from Eq. (1) and Table 14 (n = 50). See Section 5.1 for definitions of (n) and (y).
[f] Calculated from experimental log $J_{M7.0}$ − calculated log $J_{M7.0}$ from Eq. (1) and Table 14 (n = 185).
[g] Δlog $J_{M7.0}$ for prodrugs.

Table 3
Alkylcarbonyloxyalkyl esters of naproxen (NAP)[a]

RCO_2R^1 compound	MW	log $S_{7.4}$[b]	log $K_{OCT:7.4}$	log S_{OCT}[b,c]	log $J_{M7.4}$[d]	Δ'log $J_{M7.4}$[d,e]	Δ'log $J_{M7.4}$[d,f]
RCO_2 = NAP, R^1 = H	230	2.01	0.30	2.31	−2.18[g]	–	–
RCO_2 = NAP, R^1 = a[h]	316	−1.22	3.50	2.28	−3.36	−0.690 (n)	−0.242
RCO_2 = NAP, R^1 = b[h]	330	−2.40	3.30	0.90	−3.66	0.399 (y)	0.797
RCO_2 = NAP, R^1 = c[h]	344	−3.40	2.80	−0.60	−4.70	0.759 (y)	1.082
						0.616[i]	0.707[i]

[a] Rautio et al. (1998).
[b] Units of mM.
[c] Calculated from log $K_{OCT:7.4}$ + log $S_{7.4}$.
[d] Units of μmol cm^{-2} h^{-1}.
[e] Calculated from experimental log $J_{M7.4}$ − calculated log $J_{M7.4}$ from Eq. (1) and Table 14 (n = 50). See Section 5.1 for definitions of (n) and (y).
[f] Calculated from experimental log $J_{M7.4}$ − calculated log $J_{M7.4}$ from Eq. (1) and Table 14 (n = 185).
[g] Value of −2.18 from Rautio et al. (2000a,b).
[h] (a) —$(CH_2)_2O$—(C=O)—CH_3; (b) —$(CH_2)_3O$—(C=O)—CH_3; (c) —CH_2O—(C=O)—$C(CH_3)_3$.
[i] Δlog $J_{M7.4}$ for prodrugs.

vitro, J_{MAQ}, also depends directly on their S_{IPM} and S_{AQ}, and inversely on MW (x = −1.497; y = 0.66; z = 0.0047; r^2 = 0.77; Δlog J_{MAQ} = 0.19 log units) based on the fit of the data to Eq. (1) where J_{MIPM} has been replaced by J_{MAQ} (Sloan et al., 2003). Interestingly, the rank order of maximum flux, J_M, in each series was the same regardless of whether the vehicle was IPM or AQ. Also, there was no apparent effect of the composition of the total species containing parent drug that had permeated on the rank

Table 4
Aminoalkylcarbonyloxyalkyl esters of naproxen (NAP)[a]

RCO_2R^1 compound	MW	log $S_{5.0}$[b]	log $K_{OCT:5.0}$	log S_{OCT}[b,c]	log $J_{M5.0}$[d]	Δ'log $J_{M5.0}$[d,e]	Δ'log $J_{M5.0}$[d,f]
RCO_2 = NAP, R^1 = H	230	−0.40	2.38	1.98	−2.75	–	−0.195
RCO_2 = NAP, R^1 = a[g]	331	1.02	0.67	1.69	−2.40	−0.104 (y)	0.061
RCO_2 = NAP, R^1 = b[g]	387	−0.51	2.16	1.65	−2.80	0.433 (y)	0.605
RCO_2 = NAP, R^1 = c[g]	387	0.23	2.13	2.36	−2.29	0.219 (n)	0.388
RCO_2 = NAP, R^1 = d[g]	345	0.77	0.99	1.76	−2.82	−0.393 (y)	−0.233
RCO_2 = NAP, R^1 = e[g]	415	0.03	2.72	2.75	−2.74	−0.228 (y)	−0.071
						0.275[h]	0.259[h]

[a] Rautio et al. (1999).
[b] Units of mM.
[c] Calculated from log $K_{OCT:5.0}$ + log $S_{5.0}$.
[d] Units of μmol cm^{-2} h^{-1}.
[e] Calculated from experimental log $J_{M5.0}$ − calculated log $J_{M5.0}$ from Eq. (1) and Table 14 (n = 50). See Section 5.1 for definitions of (n) and (y).
[f] Calculated from experimental log $J_{M5.0}$ − calculated log $J_{M5.0}$ from Eq. (1) and Table 14 (n = 185).
[g] (a) −$(CH_2)_2$O−(C=O)−CH_2−NH_2; (b) −$(CH_2)_2$O−(C=O)−CH(−NH_2)−$CH_2CH(CH_3)_2$; (c) −$(CH_2)_2$O−(C=O)−CH(−NH_2)−CH(−CH_3)−CH_2CH_3; (d) −$(CH_2)_3$O−(C=O)−CH_2−NH_2; (e) −$(CH_2)_4$O−(C=O)−CH(−NH_2)−CH(−CH_3)−CH_2CH_3.
[h] Δlog $J_{M5.0}$ for prodrugs.

Table 5
Morpholinyl- and methylpiperazinylalkylcarbonyloxyalkyl esters of naproxen (NAP)[a]

RCO_2R^1 compound	MW	log $S_{7.4}$[b]	log $K_{OCT:7.4}$	log S_{OCT}[b,c]	log $J_{M7.4}$[d]	Δ'log $J_{M7.4}$[d,e]	Δ'log $J_{M7.4}$[d,f]
RCO_2 = NAP, R^1 = H	230	2.00	0.30	2.30	−2.18	–	0.915
RCO_2 = NAP, R^1 = a[g]	401	−1.15	2.14	0.99	−3.15	0.834 (y)	0.967
RCO_2 = NAP, R^1 = b[g]	414	0.61	1.16	1.77	−1.61	1.309 (y)	1.326
RCO_2 = NAP, R^1 = c[g]	442	0.94	1.30	2.24	−2.14	0.539 (y)	0.503
RCO_2 = NAP, R^1 = d[g]	456	1.64[h]	3.04	4.68	−2.11	–	–
RCO_2 = NAP, R^1 = e[g]	470	1.69	2.69	4.38	−1.88	−0.645 (n)	−0.622
						0.832[i]	0.867[i]

[a] Rautio et al. (2000a).
[b] Units of mM.
[c] Calculated from log $K_{OCT:7.4}$ + log $S_{7.4}$.
[d] Units of μmol cm^{-2} h^{-1}.
[e] Calculated from experimental log $J_{M7.4}$ − calculated log $J_{M7.4}$ from Eq. (1) and Table 14 (n = 50). See Section 5.1 for definitions of (n) and (y).
[f] Calculated from experimental log $J_{M7.4}$ − calculated log $J_{M7.4}$ from Eq. (1) and Table 14 (n = 185).
[g] (a) −$(CH_2)_2$O−(C=O)−CH_2−N($CH_2CH_2)_2$O; (b) −$(CH_2)_2$O−(C=O)−CH_2−N($CH_2CH_2)_2$−NCH_3; (c) −$(CH_2)_4$O−(C=O)−CH_2−N($CH_2CH_2)_2$−NCH_3; (d) −$(CH_2)_4$O−(C=O)−$(CH_2)_2$−N($CH_2CH_2)_2$−NCH_3; (e) −$(CH_2)_4$O−(C=O)−$(CH_2)_3$−N($CH_2CH_2)_2$−NCH_3.
[h] Assumed a decimal point error: log $S_{7.4}$ = 1.64 and not 2.64. However NAP, R^1 = d was not used as part of the database in the analysis of fit to Eqs. (1)–(5).
[i] Δlog $J_{M7.4}$ for prodrugs.

Table 6
Morpholinylalkyl and piperazinylalkyl esters of naproxen (NAP)[a]

RCO_2R^1 compound	MW	log $S_{7.4}$[b]	log $K_{OCT:7.4}$	log S_{OCT}[b,c]	log $J_{M7.4}$[d]	Δ'log $J_{M7.4}$[d,e]	Δ'log $J_{M7.4}$[d,f]
RCO_2 = NAP, R^1 = H	230	2.01	0.3	2.31	−2.19	–	−0.915
RCO_2 = NAP, R^1 = a[g]	371	−0.70	2.6	1.90	−2.82	0.231 (y)	0.474
RCO_2 = NAP, R^1 = b[g]	342	1.48	0.74	2.22	−2.29	−0.436 (n)	−0.290
RCO_2 = NAP, R^1 = c[g]	356	1.51	2.29	3.80	−1.23	−0.291 (n)	−0.041
RCO_2 = NAP, R^1 = d[g]	384	1.71	2.44	4.15	−1.56	−0.734 (n)	−0.535
RCO_2 = NAP, R^1 = e[g]	412	−2.22	3.92	1.70	−3.40	0.613 (y)	0.877
						0.461[h]	0.443[h]

[a] Rautio et al. (2000b).
[b] Units of mM.
[c] Calculated from log $K_{OCT:7.4}$ + log $S_{7.4}$.
[d] Units of μmol cm^{-2} h^{-1}.
[e] Calculated from experimental log $J_{M7.4}$ − calculated log $J_{M7.4}$ from Eq. (1) and Table 14 (n = 50). See Section 5.1 for definitions of (n) and (y).
[f] Calculated from experimental log $J_{M7.4}$ − calculated log $J_{M7.4}$ from Eq. (1) and Table 14 (n = 185).
[g] (a) −$(CH_2)_4$−N($CH_2CH_2)_2$O; (b) −$(CH_2)_2$−N($CH_2CH_2)_2$−NH; (c) −$(CH_2)_2$−N($CH_2CH_2)_2$−NCH_3; (d) −$(CH_2)_4$−N($CH_2CH_2)_2$−NCH_3; (e) −$(CH_2)_6$−N($CH_2CH_2)_2$−NCH_3.
[h] Δlog $J_{M7.4}$ for prodrugs.

Table 7
Alkylaminocarbonyloxymethyl esters of naproxen (NAP) and benzoic acid (BA)[a]

RCO_2R^1 compound	MW	$\log S_{5.0}$[b]	$\log K_{OCT:5.0}$	$\log S_{OCT}$[b,c]	$\log J_{M5.0}$[d]	$\Delta'\log J_{M5.0}$[d,e]	$\Delta'\log J_{M5.0}$[d,f]
RCO_2 = NAP, R^1 = H	230	−0.34	2.38[g]	2.04	−2.80	–	−0.307
RCO_2 = NAP, R^1 = a[h]	403	−2.70	3.35	0.65	−4.22	0.566	0.801
RCO_2 = BA, R^1 = H	122	1.59	1.34[i]	2.93[j]	−1.29	–	0.604
RCO_2 = BA, R^1 = b[h]	295	0.11	2.26	2.37	−2.45	−0.476	−0.089
RCO_2 = BA, R^1 = c[h]	323	−0.78	3.02	2.24	−3.30	−0.706	−0.325
RCO_2 = BA, R^1 = d[h]	337	−1.47	3.63	2.16	−3.04	−0.076	−0.335
						0.456[k]	0.410[k]

[a] Mendes et al. (2002).
[b] Units of mM.
[c] Calculated from $\log K_{OCT:5.0} + \log S_{5.0}$.
[d] Units of $\mu mol\,cm^{-2}\,h^{-1}$.
[e] Calculated from experimental $\log J_{M5.0}$ − calculated $\log J_{M5.0}$ from Eq. (1) and Table 14 ($n = 50$).
[f] Calculated from experimental $\log J_{M5.0}$ − calculated $\log J_{M5.0}$ from Eq. (1) and Table 14 ($n = 185$).
[g] Rautio et al. (1999).
[h] (a) $-CH_2O-(C{=}O)-N(-CH_3)-CH_2CO_2C_2H_5$; (b) $-CH_2O-(C{=}O)-NH-CH(-CH_3)-CO_2C_2H_5$; (c) $-CH_2O-(C{=}O)-NH-CH[CH(CH_3)_2]-CO_2C_2H_5$; (d) $-CH_2O-(C{=}O)-NH-CH[CH_2CH(CH_3)_2]-CO_2C_2H_5$.
[i] Calculated from $\log S_{OCT} - \log S_{5.0}$.
[j] Yalkowsky et al. (1983).
[k] $\Delta\log J_{M5.0}$ for prodrugs.

order of flux for either J_{MIPM} or J_{MAQ} data bases: J_M, solubility and MW data fit the RS model (Eq. (1)) well regardless of whether the prodrug delivered mainly intact prodrug or mainly the parent drug.

More recently, we have shown that the solubilities and MW data for the delivery of non-prodrugs from their saturated aqueous solutions through human skin *in vitro* can also be fitted to the RS Eq. (1) with excellent results based on two studies. The first study was based on an edited Flynn database (Majumdar et al., 2007) where only those permeants in the original Flynn (1990) database for which S_{AQ} values were known or could be estimated were included: $n = 62$. The second study was based on *in vitro* diffusion cell experiments using human skin published since the Flynn database was published up to 2004, an extended Flynn database (Thomas et al., 2007), where only those non-prodrug permeants for which S_{AQ} values were known or could be estimated were included: $n = 52$. In those databases the lipid solubility parameter was solubility in octanol, S_{OCT}, instead of S_{IPM} and $x = -2.574$, $y = 0.586$, $z = 0.00440$, $r^2 = 0.887$ and the standard deviation = 0.40 for the combined edited and extended databases ($n = 114$). However, neither the fit of this $n = 114$ database to the RS model nor the fit of other extended databases

Table 8
Glycoside ester of flurbiprofen (FLB), ibuprofen (IBP), ketoprofen (KP) and naproxen (NAP)[a]

RCO_2R^1 compound	MW	$\log S_{7.0}$[b]	$\log K_{OCT:7.0}$	$\log S_{OCT}$[b,c]	$\log J_{M7.0}$[d]	$\Delta'\log J_{M7.0}$[d,e]	$\Delta'\log J_{M7.0}$[d,f]
RCO_2 = FLB, R^1 = H	244	0.84	–[g]	–[g]	−0.93	–	–
RCO_2 = FLB, R^1 = a[h]	406	0.40	1.92	2.32	−2.52	0.082	0.184
RCO_2 = FLB, R^1 = b[h]	406	0.45	1.56	2.01	−2.92	−0.152	−0.081
RCO_2 = IBP, R^1 = H	206	1.11	–[g]	–[g]	−0.84	–	–
RCO_2 = IBP, R^1 = a[h]	368	1.09	1.57	2.66	−2.30	−0.408	−0.249
RCO_2 = IBP, R^1 = b[h]	368	0.90	1.79	2.69	−2.10	−0.156	0.022
RCO_2 = KP, R^1 = H	254	1.14	–[g]	–[g]	−1.45	–	–
RCO_2 = KP, R^1 = a[h]	416	1.09	0.98	2.07	−3.08	−0.520	−0.524
RCO_2 = KP, R^1 = b[h]	416	0.92	1.11	2.03	−3.10	−0.449	−0.441
RCO_2 = NAP, R^1 = H	230	1.91	–[g]	–[g]	−1.75	–	–
RCO_2 = NAP, R^1 = a[h]	382	0.86	1.32	2.18	−3.45	−1.041	−0.959
RCO_2 = NAP, R^1 = b[h]	382	0.81	1.31	2.12	−3.32	−0.848	−0.767
						0.457[i]	0.403[i]

[a] Swart et al. (2005).
[b] Units of mM.
[c] Calculated from $\log K_{OCT:7.0} + \log S_{7.0}$.
[d] Units of $\mu mol\,cm^{-2}\,h^{-1}$.
[e] Calculated from experimental $\log J_{M7.0}$ − calculated $\log J_{M7.0}$ from Eq. (1) and Table 14 ($n = 50$).
[f] Calculated from experimental $\log J_{M7.0}$ − calculated $\log J_{M7.0}$ from Eq. (1) and Table 14 ($n = 185$).
[g] Experimental $\log K_{OCT:7.0}$ reported is about 2 log units different from $\log K_{OCT:7.0}$ calculated by ACD/log *P* software version 4.56 so cannot estimate $\log S_{OCT}$.
[h] (a) Glucoside; (b) mannoside.
[i] $\Delta\log J_{M7.4}$ for prodrugs.

Table 9
Alkylamino acid amides of naproxen (NAP)[a]

RCO_2R^1 compound	MW	log S_{AQ}[b]	log $K_{OCT:AQ}$[c]	log S_{OCT}[b,d]	log J_{MAQ}[e]	Δ'log J_{MAQ}[e,f]	Δ'log J_{MAQ}[e,g]
RCO_2 = NAP, R^1 = H	230	−0.84	3.00	2.16	−3.49	–	–
RCO_2 = NAP, R^1 = a[h]	329	−0.64	3.17	2.53	−2.94	−0.566	−0.177
RCO_2 = NAP, R^1 = b[h]	343	−1.596	3.71	2.11	−3.62	0.537	−0.133
RCO_2 = NAP, R^1 = c[h]	357	−1.929	4.24	2.31	−3.59	0.419	−0.000
						0.508[i]	0.103[i]

[a] Pignatello et al. (2005).
[b] Units of mM.
[c] *C* log *P* from ACD/log *P* 5.15 software version 4.56.
[d] Calculated from log S_{AQ} + log $K_{OCT:AQ}$.
[e] Units of μmol cm^{-2} h^{-1} using Q_{22}/12 h.
[f] Calculated from experimental log J_{MAQ} − calculated log J_{MAQ} from Eq. (1) and Table 14 (n = 50).
[g] Calculated from experimental log J_{MAQ} − calculated log J_{MAQ} from Eq. (1) and Table 14 (n = 185).
[h] (a) —NHCH(CO_2CH_3)—CH_2CH_3; (b) —NHCH(CO_2CH_3)—$CH_2CH_2CH_3$; (c) —NHCH(CO_2CH_3)—$CH_2CH_2CH_2CH_3$.
[i] Δlog J_{MAQ} for prodrugs.

based on delivery through human skin *in vitro* to other model equations was evaluated for the effect of including prodrugs in the database. For instance, although the parabens have been reported to hydrolyze during their permeation of rat skin (Bando et al., 1997), the publication from which the dataset for the permeation of the parabens was obtained for the n = 114 database did not mention the extent of hydrolysis of the parabens during their permeation of human skin (Dal Pozzo and Pastori, 1996). It is possible that the process by which skin samples are obtained by one lab is quite different than by another lab and esterase activity is lost in one or the other lab.

In the rational design of new prodrugs to deliver their parent drugs through human skin *in vivo*, it is reasonable to first determine the contributions of various physicochemical predictors to the delivery of parent drugs by their prodrugs through human skin *in vitro* using a standard vehicle and use the resulting model to predict delivery *in vivo*. Since we have developed a substantial database for the delivery of non-prodrugs from water through human skin *in vitro* (Majumdar et al., 2007; Thomas et al., 2007), and most published data for the delivery of parent drugs by prodrugs through human skin *in vitro* is from water, we have selected a database for the delivery of parent drugs by prodrugs from saturated aqueous vehicles and analyzed its fit to various model equations. As in the previous analyses of the edited and extended Flynn databases using Eq. (1), each independent variable in Eq. (1) (Eqs. (3)–(5)) and the combination of MW and S_{OCT} (Eq. (2)) has been fitted individually to this prodrug database (Majumdar et al., 2007; Thomas et al., 2007). The effect of the composition of the diffusing species (intact prodrug and parent drug) based solely on the composition of species in the receptor phase, and their quite different physicochemical properties, has been evaluated based on the

Table 10
Alkyl esters of aspirin (AS)[a]

RCO_2R^1 compound	MW	log $S_{4.5}$[b]	log $K_{OCT:4.5}$	log S_{OCT}[b,c]	log $J_{M4.5}$[d]	Δ'log $J_{M4.5}$[d,e]	Δ'log $J_{M4.5}$[d,f]
RCO_2 = AS, R^1 = H	180	1.56	−0.85	0.71	−0.58	–	1.547
RCO_2 = AS, R^1 = a[g]	194	1.225	−0.25	0.975	−1.285	0.512	0.910
RCO_2 = AS, R^1 = b[g]	208	1.203	0.30	1.503	−0.866	0.697	1.112
RCO_2 = AS, R^1 = c[g]	222	0.858	0.86	1.718	−2.065	−0.419	0.013
RCO_2 = AS, R^1 = d[g]	222	0.370	0.75	1.120	−1.975	0.228	0.650
RCO_2 = AS, R^1 = e[g]	236	−0.528	1.32	0.792	−2.172	0.660	1.101
RCO_2 = AS, R^1 = f[g]	236	−0.072	1.23	1.158	−2.145	0.288	0.720
RCO_2 = AS, R^1 = g[g]	236	0.508	1.09	1.598	−1.510	0.430	0.850
RCO_2 = AS, R^1 = h[g]	250	2.152	1.95	−0.202	−3.921	0.229	0.693
RCO_2 = AS, R^1 = i[g]	250	−0.699	1.64	0.94	−3.699	−0.808	−0.371
RCO_2 = AS, R^1 = j[g]	250	−0.351	1.65	1.293	−2.866	−0.325	0.112
						0.460[h]	0.734[h]

[a] Gerber et al. (2006).
[b] Units of mM.
[c] Calculated from log $K_{OCT:4.5}$ + log $S_{4.5}$.
[d] Units of μmol cm^{-2} h^{-1}.
[e] Calculated from experimental log $J_{M4.5}$ − calculated log $J_{M4.5}$ from Eq. (1) and Table 14 (n = 50).
[f] Calculated from experimental log $J_{M4.5}$ − calculated log $J_{M4.5}$ from Eq. (1) and Table 14 (n = 185).
[g] (a) —CH_3; (b) —C_2H_5; (c) —C_3H_5; (d) —$CH(CH_3)_2$; (e) —C_4H_9; (f) —$CH(CH_3)C_2H_5$; (g) —$C(CH_3)_3$; (h) —C_5H_{11}; (i) —$CH(CH_3)C_3H_7$; (j) —$CH(C_2H_5)_2$.
[h] Δlog $J_{M4.5}$ for prodrugs.

Table 11
Mono-, di- and trihydroxybenzenes[a]

Compound	MW	log S_{AQ}[b,c]	log $K_{OCT:AQ}$[d,e]	log S_{OCT}[b,d]	log J_{MAQ}[f,g]	Δ'log J_{MAQ}[f,h]	Δlog P_{MAQ}[i]
Phenol	94	2.42	1.48	3.90	0.39	0.058	−2.03
Catechol	110	2.38	0.88	3.26	−0.13	−0.035	−2.51
Resorcinol	110	2.56	0.76	3.32	−0.51	−0.531	−3.07
Hydroquinone	110	2.18	0.64	2.82	−0.77	−0.346	−2.95
Pyrogallol	126	2.84	0.29	3.13	−1.06	−1.044	−3.90
Benzenetriol	126	2.76	0.06	2.82	−0.05	0.170	−2.81
Phloroglucinol	126	2.45	0.06	2.51	−0.06	0.470	−2.51
						0.379[j]	–

[a] du Plessis et al. (2002).
[b] Units of mM.
[c] Calculated from log S_{OCT} − log $K_{OCT:AQ}$.
[d] du Plessis et al. (2001).
[e] Calculated from ACD software.
[f] Units of μmol cm^{-2} h^{-1}.
[g] Calculated from log P_{MAQ} + log S_{AQ}.
[h] Calculated from experimental log J_{MAQ} − calculated log J_{MAQ} from Eq. (1) and Table 14 (n = 185).
[i] Units of cm h^{-1}.
[j] Δlog J_{MAQ}.

Table 12
N-Alkyl derivatives of carbamazepine (CAB)[a]

CAB-R compound	MW	log $S_{7.4}$[b]	log $K_{OCT:7.4}$	log S_{OCT}[b,c]	log $J_{M7.4}$[d]	Δ'log $J_{M7.4}$[d,e]
R = H	236	0.271	2.7	2.97	−1.856	−0.125
R = CH_3	250	0.291	3.5	3.79	−0.295	0.042
R = C_2H_5	264	0.235	4.0	4.24	−1.497	−0.318
R = C_2H_4OH	280	0.445	2.8	3.25	−1.332	0.348
						0.208[f]

[a] Fourie et al. (2004).
[b] Units of mM.
[c] Calculated from log $K_{OCT:7.4}$ + log $S_{7.4}$.
[d] Units of μmol cm^{-2} h^{-1}.
[e] Calculated from experimental log $J_{M7.4}$ − calculated log $J_{M7.4}$ from Eq. (1) and Table 14 (n = 185).
[f] Δlog $J_{M7.4}$.

Table 13
N-Alkyl derivatives of cyclizine (CYC)[a]

CYC-R compound	MW	log S_{AQ}[b]	log $K_{OCT:7.4}$	log S_{OCT}[b,c]	log $J_{M7.4}$[d,e]	Δ'log $J_{M7.4}$[d,f]	log $P_{M7.4}$[g]
R = CH_3	266	−0.157	3.11	2.95	−3.304 (−2.68)	−1.246	−2.52
R = C_2H_5	280	0.308	3.64	3.95	−1.605 (−1.05)	−0.241	−1.36
R = C_3H_7	294	−0.189	4.18	3.99	−3.070 (−2.55)	−1.442	−2.36
R = C_4H_9	308	0.194	4.71	4.90	−2.642 (−2.10)	−1.637	−2.30
						1.141[h]	

[a] Monene et al. (2005).
[b] Units of mM.
[c] Calculated from log $K_{OCT:AQ}$ + log S_{AQ}.
[d] Units of μmol cm^{-2} h^{-1}.
[e] Value in parenthesis calculated from log $P_{M7.4}$ + log S_{AQ}.
[f] Calculated from experimental log $J_{M7.4}$ − calculated log $J_{M7.4}$ from Eq. (1) and Table 14 (n = 185).
[g] Units of cm h^{-1}.
[h] Δlog $J_{M7.4}$.

fit of each dataset to Eq. (1). In particular, the extent of over- or underprediction of flux of total species has been determined and the consequences in support or contradiction of previous published hypotheses on the effect of hydrolysis of prodrugs on delivery of total species containing the parent drug (see below) has been assessed. Then this prodrug database n = 50, together with data for 6 parent drugs and for 3 other datasets of non-prodrugs (n = 15) was added on to the n = 114 database to give

Table 14
Parameter coefficients, residuals ($\Delta\log J_{MAQ}$) standard deviations (S.D.), r^2 and F values for the fit of databases to equations (1)–(5)

Equation	N	x	y	z	r^2	$\Delta\log J_{MAQ}$	S.D.	F-value
RS(1)	50	−1.639	0.626	0.00632	0.642	0.473	0.296	29.59
KSC(2)	50	−1.667	0.691	0.00666	0.457	0.467	0.397	19.78
MACR(3)	50	−1.900	–	0.00204	0.030	0.652	0.478	1.5
OCT(4)	50	−3.423	0.423	–	0.219	0.578	0.364	13.47
AQ(5)	50	−2.574	0.500	–	0.554	0.417	0.367	59.63
RS(1)	185	−2.506	0.538	0.00402	0.839	0.474	0.423	137.57
KSC(2)	185	−1.619	0.610	0.00699	0.750	0.607	0.486	272.82
MACR(3)	185	0.776	–	0.01012	0.580	0.788	0.628	252.82
OCT(4)	185	−4.374	0.945	–	0.542	0.838	0.632	216.19
AQ(5)	185	−2.439	0.819	–	0.608	0.771	0.589	283.47
RS(1)	114	−2.574	0.586	0.0044	0.887	0.446	0.399	139.03
KSC(2)	114	−2.090	0.727	0.0066	0.802	0.602	0.571	224.89
MACR(3)	114	1.118	–	0.0115	0.653	0.803	0.670	210.71
OCT(4)	114	−4.952	1.161	–	0.681	0.784	0.623	239.50
AQ(5)	114	−2.468	0.901	–	0.608	0.901	0.648	174.10

(1) $\log J_{MAQ} = x + y\log S_{OCT} + (1-y)\log S_{AQ} - z$MW; (2) $\log J_{MAQ} = x + y\log S_{OCT} - z$MW; (3) $\log J_{MAQ} = x - z$MW; (4) $\log J_{MAQ} = x + y\log S_{OCT}$; (5) $\log J_{MAQ} = x + y\log S_{AQ}$.

the $n = 185$ database which has also been fitted to Eqs. (1)–(5) (Table 14).

2. Methods

As in the selection of previous *in vitro* human skin based databases, no entries were excluded because of the degree of ionization of the permeant at the pH of the applied phase, nor was any attempt made to estimate intrinsic (non-ionized) aqueous solubilities to be fitted to the two Eqs. (1) and (5) containing $\log S_{AQ}$ as an independent variable. Also no entries were excluded because the thickness of the skin used in the diffusion cells were different from that of heat separated epidermis. However, only entries from contributions which gave S_{AQ}, J_{MAQ} and $K_{OCT:AQ}$ values or values which could be used to calculate S_{AQ}, J_{MAQ} and S_{OCT} were included in this database. Alternatively, solubility and $K_{OCT:AQ}$ values from other published sources were used if those values were consistent from source to source. Some entries from datasets designed as prodrugs permeated intact: no parent drug was found in the receptor phase although they were found to have hydrolyzed in plasma. Those stable prodrugs have been included in the prodrug database ($n = 50$). Data for the parent drugs ($n = 6$) were not included in the $n = 50$ database, but were in the $n = 185$ database. Similarly, data for a previously overlooked contribution (du Plessis et al., 2002; $n = 7$ entries) and for two contributions published since the collection of the extended Flynn database in 2004 (Fourie et al., 2004; Monene et al., 2005; $n = 8$ entries) were included in the final $n = 185$ database.

For each prodrug dataset the average of the absolute values for experimental $\log J_{MAQ}$ − calculated $\log J_{MAQ}$ ($\Delta\log J_{MAQ}$) was calculated for their fit to RS Eq. (1) using the $n = 50$ and $n = 185$ coefficients for the parameters. For each individual prodrug entry the value for experimental $\log J_{MAQ}$ − calculated $\log J_{MAQ}$ ($\Delta'\log J_{MAQ}$) was calculated for its fit to RS Eq. (1) using the $n = 50$ and $n = 185$ coefficients for the parameters. The $\Delta\log J_{MAQ}$ and $\Delta'\log J_{MAQ}$ values for each dataset and individual prodrug entry, respectively, is presented in the corresponding table for each dataset. The $\Delta'\log J_{MAQ}$ values are either positive (the prodrug delivered more total species containing the parent drug than fit to Eq. (1) would predict – overperformed) or negative (the prodrug delivered less total species containing the parent drug than fit to Eq. (1) would predict – underperformed). Subsequently, for those prodrug datasets (Tables 1–6, $n = 25$) where hydrolysis was reported, the effect of prodrug hydrolysis to parent drug during its permeation and consequently the effect of the different physicochemical properties presented by prodrug and parent drug on over- and underprediction of flux was assessed based on two hypotheses.

The first hypothesis is that there will be no substantial trend in positive or negative $\Delta'\log J_{MAQ}$ values based on whether the prodrug is more soluble in water and hydrolyzes to the parent drug during permeation or is less soluble in water and hydrolyzes to the parent drug during permeation. This hypothesis is based on the design paradigm that it is only the solubility of the prodrug in the first few layers of the membrane, which in turn is based on contributions from both S_{AQ} and S_{OCT} of the prodrug, that predicts flux, in addition to an inverse dependence on MW (Sloan et al., 2006).

The second hypothesis is that if the prodrug is more soluble in water than the parent drug and hydrolyzes partially to the parent drug during permeation, it will give a negative $\Delta'\log J_{MAQ}$: it will underperform the flux predicted by Eq. (1) because the less water soluble parent drug will not permeate the viable epidermis/dermis as well as the prodrug. Conversely, if the prodrug is less soluble in water than the parent drug and hydrolyzes partially to the parent drug during permeation it will give a positive $\Delta'\log J_{MAQ}$: it will overperform the flux predicted by Eq. (1) because the more water soluble parent drug will permeate the viable epidermis/dermis better than the prodrug (Ahmed et al., 1996; Stinchcomb et al., 2002). The magnitude of the positive and negative values will depend on the degree of hydrolysis which cannot be determined with the data in hand for this database. However, it is assumed that even partial hydrolysis of

the prodrug should lead to an obvious trend. This hypothesis is based on a paradigm that flux of total species (prodrug and parent drug) will depend not only on solubility in the first few layers of the membrane (stratum corneum, SC) but also on solubility in the viable epidermis/dermis as a rate limiting barrier to permeation. Since the SC and viable epidermis/dermis have such different solubilizing properties (lipid versus aqueous), the much different solubility properties of prodrug and parent drug and the degree of hydrolysis will substantially affect flux. Data from the last four series of prodrugs (Tables 7–10), which apparently permeated intact, were not included in this analysis.

All five Eqs. (Eqs. (1)–(5) that will be used) fit into the general linear model framework so that linear regression was used to fit the data to them. Model inferences were obtained by Proc Reg of SAS 9.0 version. The ordinary least squares estimates of parameters x, y and z for the RS Eq. (1) were calculated under the restriction that the coefficient of the independent variable $\log S_{AQ}$ was linearly restricted by the coefficient of the independent variable $\log S_{OCT}$.

3. Theoretical

The development of the Roberts–Sloan, RS model, Eq. (1) has been presented elsewhere (Roberts and Sloan, 1999). The development of the Kasting–Smith–Cooper, KSC model (Kasting et al., 1987), and the modified KSC model Eq. (2) have been presented elsewhere (Majumdar et al., 2007). The development of the Magnusson–Anissimova–Cross–Roberts, MACR model, has been presented elsewhere (Magnusson et al., 2004), and the model in its simplest form is given as Eq. (3). Eq. (4) and (5) are merely the basis for fitting the independent variables, $\log S_{OCT}$ and $\log S_{AQ}$, separately to the dependent variable, $\log J_{MAQ}$:

$$\log J_{MAQ} = x + y \log S_{OCT} - z\mathrm{MW} \quad (2)$$

$$\log J_{MAQ} = x - z\mathrm{MW} \quad (3)$$

$$\log J_{MAQ} = x + y \log S_{OCT} \quad (4)$$

$$\log J_{MAQ} = x + y \log S_{AQ} \quad (5)$$

4. Database

The edited ($n = 62$) (Majumdar et al., 2007) and extended databases ($n = 114$) (Thomas et al., 2007) have been presented previously and will not be presented here. The first 10 tables contain $n = 50$ prodrugs and $n = 6$ parent drugs which are not part of the $n = 50$ prodrug database but are included in the $n = 185$ integrated database. Tables 11–13 include new non-prodrug entries published since 2004 and one contribution which had previously been overlooked (du Plessis et al., 2002) because some solubility data was not published with the permeability coefficient data from which flux could be calculated.

4.1. Nalidixic acid (NAD) esters, Table 1 (n = 5 prodrugs)

Full thickness human skin, which was frozen then thawed and from which subcutaneous fat had been removed, was used as the membrane. The receptor phase was pH 7.2 isotonic phosphate buffer kept at 37 °C. The prodrugs "c–f" hydrolyzed completely to the parent drug during permeation. Prodrug "a" was 35% hydrolyzed and prodrug "b" was 50% hydrolyzed to nalidixic acid during permeation. All of the prodrugs were more soluble in water than the parent drug and all except prodrugs "c" and "d" overperformed (Bundgaard et al., 1989).

4.2. Alkylcarbonyl esters of morphine, Table 2 (n = 3 prodrugs)

Full thickness human skin, which was frozen then thawed and from which subcutaneous fat had been removed, was used as the membrane. The receptor phase was pH 7.2 isotonic phosphate buffer kept at 37 °C. Both the 3-hexanoate and the 3,6-dihexanoate hydrolyzed completely during permeation while the 3,6-dipropionate was 50% hydrolyzed. The 3,6-dipropionate and 3-hexanoate were both more soluble in water than the parent drug and they both overperformed. The 3,6-dihexanoate was less soluble in pH 7.4 buffer than morphine and it also overperformed (Drustrup et al., 1991).

4.3. Alkylcarbonyloxyalkyl esters of naproxen (NAP), Table 3 (n = 3 prodrugs)

Heat separated epidermis was frozen and dried, then thawed and rehydrated immediately before it was used as the membrane. The receptor phase was pH 7.4 isotonic phosphate buffer containing 0.02% sodium azide as a preservative and kept at 37 °C. Prodrug "c" was completely hydrolyzed to NAP during permeation while prodrugs "a" and "b" permeated mainly (>50%) as intact prodrug or the corresponding hydroxyalkyl ester of NAP. The half-lives of the prodrug esters in human serum was 25–137 min. Using the S_{OCT} and $S_{7.4}$ values for NAP in Table 3, which were from a different publication by the same authors (Rautio et al., 2000a,b), the prodrugs were all less soluble in pH 7.4 buffer than NAP (also less soluble in OCT) and prodrugs "b" and "c" overperformed, while prodrug "a" underperformed (Rautio et al., 1998).

4.4. Aminoalkylcarbonylalkyl esters of naproxen (NAP), Table 4 (n = 5 prodrugs)

Heat separated epidermis was frozen and dried, then thawed and rehydrated immediately before it was used as the membrane. The receptor phase was 0.05 M isotonic phosphate buffer pH 5.0 containing 0.02% sodium azide and kept at 37 °C. Although the esters were designed as prodrugs and exhibited half-lives of 5–19 min in 80% human serum, all five prodrugs permeated as mixtures of NAP, intact prodrug and the corresponding hydroxyalkyl ester of NAP. No indication of the extent of hydrolysis was given. Most of the prodrugs (4/5) were more soluble in pH 5.0 buffer than NAP, and prodrug "b" was only 25% less soluble than NAP. The prodrugs "a", "d" and "e" were more water soluble than NAP and underperformed while prodrug "c" was

also more soluble in pH 5.0 buffer but overperformed (Rautio et al., 1999).

4.5. Morpholinyl- and methylpiperazinylalkyicarbonyloxymethyl esters of naproxen (NAP), Table 5 (n = 4 prodrugs)

Heat separated epidermis, which was frozen and dried then thawed and rehydrated immediately before use, was used as the membrane. The receptor phase was pH 7.4 isotonic phosphate buffer that was kept at 37 °C. Although the esters were designed as prodrugs and exhibited half-lives of 1–26 min in 80% human serum, all four prodrugs permeated as mixtures of NAP, intact prodrug and the intermediate hydroxyalkyl ester of NAP. No extent of hydrolysis to NAP in the mixture was given. Three of the four prodrugs ("a"–"c") overperformed. Prodrug "e" was also more soluble in water than NAP, hydrolyzed partially to NAP and underperformed (Rautio et al., 2000a).

4.6. Morpholinyl- and piperazinylalkyl esters of naproxen (NAP), Table 6 (n = 5 prodrugs)

Heat separated epidermis, which was frozen and dried then thawed and rehydrated immediately before use, was used as the membrane. The receptor phase was pH 7.4 isotonic phosphate buffer that was kept at 37 °C. Although the esters were designed as prodrugs and exhibited half-lives of 0.4–77 min in 80% human serum, all five of the prodrugs permeated as mixtures of NAP and intact prodrug. No indication of the extent of hydrolysis was given. All five prodrugs were less soluble in water than NAP and they all partially hydrolyze to NAP. Three of the five prodrugs underperformed (Rautio et al., 2000b).

4.7. Alkylaminocarbonyloxymethyl esters of naproxen (NAP) and benzoic acid (BA), Table 7 (n = 4 prodrugs)

Heat separated epidermis, which was frozen and dried then thawed and rehydrated immediately before use, was used as the membrane. The receptor phase was pH 7.4 isotonic buffer and was kept at 37 °C. Although the esters were designed as prodrugs and they exhibited half-lives of 0.5–22 min in 80% human plasma, they all permeated the skin intact. No assessment of the effect of water solubility and hydrolysis of the prodrugs during permeation was possible (Mendes et al., 2002).

4.8. Glycoside esters of flurbiprofen (FLB), ibuprofen (IBP), ketoprofen (KP) and naproxen (NAP), Table 8 (n = 8 prodrugs)

Heat separated epidermis, which had been frozen then thawed before it was heated at 60 °C for 1 min immediately before use, was used as the membrane. The receptor phase was pH 7.4 isotonic phosphate buffer and was kept at 37 °C. All the prodrugs permeated intact and, since no hydrolysis data was given, it is not sure that the glycoside esters function as prodrugs. No assessment of the effect of water solubility and hydrolysis of the prodrugs during permeation was possible (Swart et al., 2005).

4.9. Alkylamino acid amides of naproxen (NAP), Table 9 (n = 3 prodrugs)

Heat separated epidermis, which had been dried than rehydrated immediately before use, was used as the membrane. The receptor phase was pH 7.4 phosphate buffer and was kept at 35 °C. Although the amides had been designed as prodrugs and exhibited half-lives of 2.6–3.5 h in 80% human plasma, all the prodrugs permeated intact (Pignatello et al., 2005).

4.10. Alkyl esters of aspirin (AS), Table 10 (n = 10 prodrugs)

Heat separated epidermis, which was air dried then stored at −20 °C before being thawed within 6 months of its preparation, was used as the membrane. The receptor phase was pH 7.4 isotonic TRIS buffer kept at 37 °C. All of the prodrugs permeated intact with no aspirin or salicylic acid observed in HPLC chromatograms of the receptor phase. However an extra, unidentified peak was observed in the receptor phase of each prodrug (Gerber et al., 2006).

4.11. Mono-, di- and trihydroxybenzenes, Table 10 (n = 7)

Full thickness skin was frozen at −17 °C and thawed immediately before it was heated at 60 °C for 1 min to give the epidermis used as the membrane. The receptor phase was pH 7.4 phosphate buffer and was kept at 37 °C (du Plessis et al., 2002).

4.12. N-Alkyl derivatives of carbamazepine (CAB), Table 11 (n = 4)

Full thickness skin was frozen at −20 °C and thawed immediately before it was heated at 60 °C for 1 min to give the epidermis used as the membrane. The receptor phase was pH 7.4 phosphate buffer and was kept at 37 °C (Fourie et al., 2004).

4.13. N-Alkyl derivatives of cyclizine (CYC), Table 13 (n = 4)

Heat separated epidermis was frozen at −20 °C then thawed and used immediately as the membrane. The full thickness skin was heat separated within 1 month of receipt. The receptor phase was pH 7.4 phosphate buffer and was kept at 37 °C (Monene et al., 2005).

There is some inconsistency between the reported values for $J_{M7.4}$ and $P_{M7.4}$. If the reported $\log P_{M7.4}$ values are added to $\log S_{AQ}$, much larger values of $\log J_{M7.4}$ are obtained compared to the reported $\log J_{M7.4}$ values. These calculated $\log J_{M7.4}$ are given in parenthesis. If the calculated $\log J_{M7.4}$ values are used to calculate $\Delta \log J_{M7.4}$, a much lower value (0.72 log units) for the dataset is obtained. However, the reported $\log J_{M7.4}$ values have been used in all these calculations.

5. Results and discussions

5.1. The effect of hydrolysis of prodrugs during permeation on predictions of flux

In each of Tables 1–6 where the prodrugs were identified as having at least partially hydrolyzed during their permeation, the $\Delta'\log J_{MAQ}$ and solubility in water ($\log S_{AQ}$, $\log S_{5.0}$, $\log S_{7.0}$ or $\log S_{7.4}$) values are given. Based on the second hypothesis, if the prodrug partially hydrolyzes and is more water soluble than the parent drug, then the permeation of total species containing the parent should be less than the value predicted by Eq. (1) which is based on the properties of only the prodrug: a negative value (−). Conversely, if the prodrug partially hydrolyzes to the parent drug and is less water soluble than the parent drug, then the permeation of total species containing the parent should be greater than the value predicted by Eq. (1) which is based on the properties of only the prodrug: a positive value (+). A yes (y) or no (n) assessment is noted after each $\Delta'\log J_{MAQ}$ value in each Tables 1–6 to show whether the second hypothesis (see above) predicts under- or overperformance ((−) or (+), respectively) of each prodrug entry. When those (y) and (n) assessments were totaled there were 14 (y) and 11 (n). In other words, there was no apparent trend in the effect of hydrolysis on under- or overperformance based on the fit of the data to Eq. (1). There was also no apparent trend in the effect of hydrolysis on under- or overperformance by members of the database based on the fit of Eq. (1) and whether full thickness (FULL) or heat separated epidermis (EPI) was used: FULL, 5 (n) and 3 (y); EPI, 6 (n) and 11 (y).

For each prodrug entry in Tables 1–6 the $\Delta'\log J_{MAQ}$ value for the fit of the data to the $n = 185$ coefficients for Eq. (1) is also given. Although there is a shift to more positive $\Delta'\log J_{MAQ}$ values for the fit to the $n = 185$ coefficients than for the fit to the $n = 50$ coefficients, there is no change in whether $\Delta'\log J_{MAQ}$ is a positive or negative value for each entry except for Table 1, R^1 = d; Table 4, R^1 = a: in each case (y) became a (n). Thus, the assessment of whether the second hypothesis predicts under- or overperformance by the prodrug is substantially unchanged for the fit of the prodrug database to the $n = 185$ coefficient to Eq. (1): (y) = 12 and (*n*) = 13.

Obviously, whether the prodrug hydrolyzes during permeation and is initially more or less water soluble than its parent has no substantial effect on the trends in the fit of the $n = 50$ database to Eq. (1), regardless of which coefficients the data is fitted to, if the first hypothesis obtains. Permeation of total species containing the parent drug depends on the solubility of the prodrug in the first few layers of the membrane to provide the driving force for permeation according to Fick's law. In turn, solubility of the prodrug in those first few layers of the membrane depends on a balance of lipid and aqueous solubilities of the prodrug which mirrors the properties of lipid and aqueous phases in those layers.

The results from the fit of the $n = 50$ prodrugs to Eq. (1) and analysis of the trend in the effect of hydrolysis of the $n = 25$ prodrugs within that $n = 50$ prodrugs on the delivery by the prodrugs of total species containing the parent drug from water through human skin *in vitro* gives the same conclusion as analysis of the trend in the effect of hydrolysis of $n = 60$ prodrugs on the delivery by the prodrugs of total species containing the parent drug from isopropyl myristate (IPM) through hairless mouse skin *in vitro*. The hairless mouse skin database ($n = 63$), which included three parent drugs, had been fitted to Eq. (1) to give $x = -0.502$, $y = 0.517$, $z = 0.00266$, $\Delta\log J_{MAQ} = 0.155$ log units and $r^2 = 0.911$ (Sloan and Wasdo, 2007). The $\Delta'\log J_{MAQ}$ values were not given, but when the (+) or (−) $\Delta'\log J_{MAQ}$ values for the prodrugs (data not shown) were combined with whether the prodrugs were more or less soluble than the parent drug, a total of 35 (n) and 25 (y) values were obtained for whether the second hypothesis correctly predicted the (+) or (−) $\Delta'\log J_{MAQ}$ values for the fit of the data to Eq. (1). Thus the effect of an aqueous viable epidermis/dermis barrier to permeation, on delivery of total species containing the parent drug by its prodrug does not have a predictable effect on the trend in the difference between experimental flux and flux calculated from the coefficients derived from the fit of the data to Eq. (1). This lack of a predictable effect obtains whether human or mouse skin is used and whether the vehicle is water or IPM.

5.2. Comparison of the fit of the n = 50 with the fit of the n = 185 database to Eqs. (1)–(5)

The prodrugs that comprise the $n = 50$ database have a different distribution of MW than the $n = 185$ database. The mean MW is 342 ± 71 for the $n = 50$ prodrug database and the mean MW is 272 ± 117 for the $n = 185$ integrated database. The histograms for the two databases are shown in Figs. 1 and 2. Further, Johnson et al. (1997) showed that the effect of molecular weight on lateral diffusion coefficient was weak, if not nonexistent for solutes larger than 300 Da. Thus, it is not surprising that the dependence of flux on molecular weight alone in Eq. (3) for the $n = 50$ database is also essentially nonexistent ($r^2 = 0.03$). On the other hand, the dependence on molecular weight in Eq. (2) is about the same for the $n = 50$ and $n = 185$ database and there is

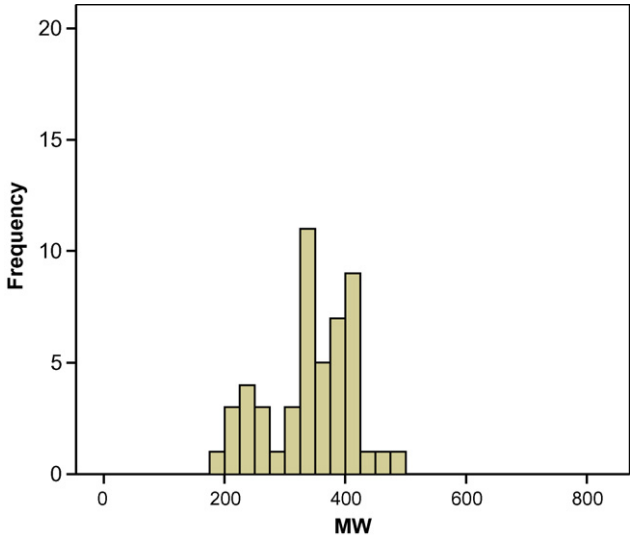

Fig. 1. Molecular weight histogram, $n = 50$.

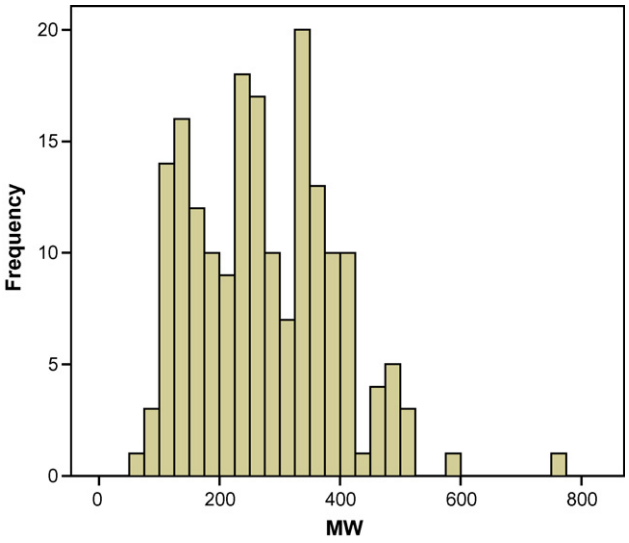

Fig. 2. Molecular weight histogram, $n = 185$.

actually a greater dependence on molecular weight in Eq. (1) for the $n = 50$ database than the $n = 185$ database. Thus molecular weight remains as an important consideration in the design of prodrugs taken together with S_{OCT} and S_{AQ} or taken with S_{OCT} but not alone.

It is also interesting that the dependence of flux on S_{OCT} alone is also weak for $n = 50$ in Eq. (4) ($r^2 = 0.219$). On the other hand, the dependence of flux on S_{OCT} is somewhat greater in the fit of the $n = 50$ database to Eqs. (1) and (2) than in the fit of the $n = 185$ database. It should also be noted that the dependence of flux on S_{OCT} in Eqs. (1), (2) and (4) is somewhat less in the fit of the $n = 185$ database than in the fit of the $n = 114$ database to the same equation s. The result is that the dependence on S_{OCT} decreases and the dependence on S_{AQ} increases slightly upon adding the higher molecular weight prodrug dataset, but not significantly.

5.3. The effect of skin thickness and ionization of the permeant

Similar results to those found in the analysis of the extended Flynn database (Thomas et al., 2007) were obtained here. Both of the datasets using FULL give higher J_{MAQ} values than predicted by Eq. (1) while seven of the eleven datasets using EPI gave lower J_{MAQ} values than predicted by Eq. (1). Both trends were the opposite of that expected based on the assumed increase in the number of barriers to permeation in FULL versus EPI.

Ionization of the permeants in the database should have no effect on the delivery of total species containing the parent drug. In the derivation of Eq. (1) (Sloan et al., 2006), the product of $(K_{OCT:AQ})^y c$, where c is a constant, and S_{AQ} used to calculate the solubility in the membrane, S_M is a constant because S_M should be a constant unless the pH of the aqueous vehicle causes a change in the solubilizing capacity of the skin. As S_{AQ} goes up, for instance as pH goes up with acidic permeants, $K_{OCT:AQ}$ goes down and the product S_{OCT} remains a constant (Ni et al., 2002).

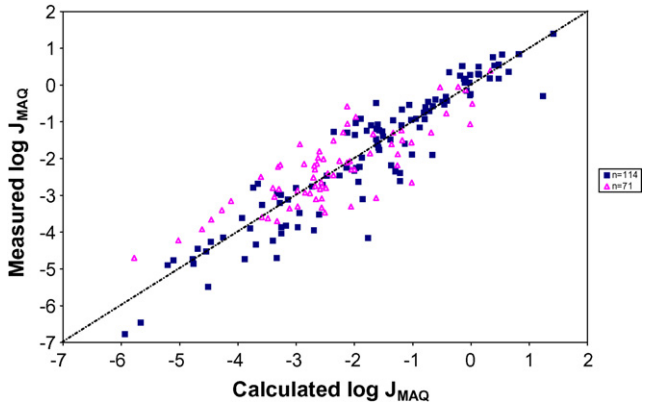

Fig. 3. Calculated vs. experimental flux using the Roberts–Sloan equation ($n = 185$). $\log J_{MAQ} = -2.506 + 0.538 \log S_{OCT} + (1 - 0.538) \log S_{AQ} - 0.0040\text{-}2MW$.

6. Conclusions

The integrated $n = 185$ database fitted to Eqs. (1)–(5) gives coefficients to the independent variables that show the same strong dependence on solubility in water ($\log S_{AQ}$, $\log S_{5.0}$, $\log S_{7.0}$ or $\log S_{7.4}$) as had the $n = 62$ edited Flynn database (Majumdar et al., 2007) and $n = 114$ extended Flynn database (Thomas et al., 2007) as well as a strong dependence on S_{OCT} and an inverse dependence on MW. The Roberts–Sloan, RS, Eq. (1) gave the best fit to the database (Fig. 3) and the Kasting–Smith–Cooper, KSC Eq. (2) gave the next best fit, which was expected based on previous results. Differences between the experimental and calculated $\log J_{MAQ}$ values ($\Delta \log J_{MAQ}$) were comparable for the fits of Eqs. (3) and (4) to the $n = 114$ database and the $n = 185$ database, however r^2 decrease substantially. On the other hand, the $\Delta \log J_{MAQ}$ values for the fit of Eq. (5) to the $n = 185$ database decreased compared to the $\Delta \log J_{MAQ}$ values for the fit of Eq. (5) to the $n = 114$ database, while r^2 remained the same. Compared to the other independent variables ($\log S_{OCT}$ and MW), the dependence of flux on S_{AQ} has become somewhat larger, although not significantly, with the increased database comprised of higher MW entries.

The fact that prodrugs, that are more or less water soluble than their parent drug, hydrolyze to their parent drugs with no significant effect on trends in under- or overperformance of the prodrugs based on their fit to Eq. (1) supports the hypothesis that permeation of all species containing the parent drugs depends substantially only on the solubility of the prodrug in the first few layers of the skin. That solubility in those first few layers depends on a balance of solubility in a lipid and in water, regardless of the vehicle. Thus, in the design of new prodrugs or analogs of existing drugs, or the design of new drugs, consideration needs to be given to optimize both lipid and water solubility while minimizing molecular weight (Sloan et al., 2006).

Acknowledgements

The authors would like to acknowledge Antara Majumdar of Global Biometric Sciences, Bristol Myers Squibb, Wallingford,

CT 06492 for assisting with the statistics in this manuscript. This was supported in part (JJ) by the Finnish Cultural Foundation, the Helsingin Sanomat Foundation, the Finnish Pharmaceutical Society and the University of Kuopio.

References

Ahmed, S., Imai, T., Otagiri, M., 1996. Evaluation of stereoselective transdermal transport and concurrent cutaneous hydrolysis of several ester prodrugs of propranolol: mechanism of stereoselective permeation. Pharm. Res. 13, 1524–1529.

Bando, H., Mohri, S., Yamashita, F., Takakura, Y., Hashida, M., 1997. Effects of skin metabolism on percutaneous penetration of lipophilic drug. J. Pharm. Sci. 86, 759–761.

Bundgaard, H., Mork, N., Hoelgaard, A., 1989. Enhanced delivery of nalidixic acid through human skin via acyloxymethyl ester prodrugs. Int. J. Pharm. 55, 91–97.

Dal Pozzo, A., Pastori, N., 1996. Percutaneous absorption of parabens from cosmetic formulations. Int. J. Cosmet. Sci. 18, 57–66.

Drustrup, J., Fullerton, A., Christrup, L., Bundgaard, H., 1991. Utilization of prodrugs to enhance the transdermal absorption of morphine. Int. J. Pharm. 71, 105–116.

du Plessis, J., Pugh, W.J., Judefeind, A., Hadgraft, J., 2001. The effect of hydrogen bonding on diffusion across model membranes: consideration of the number of H-bonding groups. Eur. J. Pharm. Sci. 13, 135–141.

du Plessis, J., Pugh, W.J., Judefeind, A., Hadgraft, J., 2002. Physico-chemical determinants of dermal drug delivery: effects of the number and substitution pattern of polar groups. Eur. J. Pharm. Sci. 16, 107–112.

Flynn, G.L., 1990. Physicochemical determinates of skin absorption. In: Gerrity, J.R., Henry, C.J. (Eds.), Principles of Route-to-Route Extrapolation for Risk Assessment. Elsevier, Amsterdam, pp. 93–127.

Fourie, L., Breytenbach, J.C., du Plessis, J., Goosen, C., Swart, H., Hadgraft, J., 2004. Percutaneous delivery of carbamazepine and selected *N*-alkyl and *N*-hydroxyalkyl analogues. Int. J. Pharm. 279, 59–66.

Gerber, M., Breytenbach, J.C., Hadgraft, J., du Plessis, J., 2006. Synthesis and transdermal properties of acetylsalicylic acid and selected esters. Int. J. Pharm. 310, 31–36.

Johnson, M.E., Blankschtein, D., Langer, R., 1997. Evaluation of solute permeation through the stratum corneum: lateral bilayer diffusion as the primary transport mechanism. J. Pharm. Sci. 86, 1162–1172.

Kasting, G.B., Smith, R.L., Cooper, E.R., 1987. Effect of lipid solubility and molecular size on percutaneous absorption. In: Shroot, B., Schaefer, H. (Eds.), Pharmacology and The Skin, vol. 1. Karger, Basel, pp. 138–153.

Magnusson, B.M., Anissimov, Y.G., Cross, S.E., Roberts, M.S., 2004. Molecular size as the main determinant of solute maximum flux across the skin. J. Invest. Dermatol. 122, 993–999.

Majumdar, S., Thomas, J., Wasdo, S.C., Sloan, K.B., 2007. The effect of water solubility of solutes on their flux through human skin *in vitro*. Int. J. Pharm. 329, 25–36.

Mendes, E., Furtado, T., Neres, J., Iley, J., Jarvinen, J., Moreira, R., 2002. Synthesis, stability and *in vitro* dermal evaluation of aminocarbonyloxymethyl esters as prodrugs of carboxylic acid agents. Bioorg. Med. Chem. 10, 809–816.

Monene, L.M., Goosen, C., Breytenbach, J.C., Hadgraft, J., du Plessis, J., 2005. Percutaneous absorption of cyclizine and its alkyl analogues. Eur. J. Pharm. Sci. 24, 239–244.

Ni, N., Sanghvi, T., Yalkowsky, S.H., 2002. Independence of the product of solubility and distribution coefficient of pH. Pharm. Res. 19, 663–669.

Pignatello, R., Montenegro, L., Stancampiano, A.H.S., Puleo, A., Puglisi, G., 2005. Synthesis and *in vitro* skin permeation of naproxen conjugates with α-alkylamino acids. Curr. Drug Deliv. 2, 185–189.

Rautio, J., Taipale, H., Gynther, J., Vepsalainen, J., Nevalainen, T., Jarvinen, T., 1998. *In vitro* evaluation of acyloxyalkyl esters as dermal prodrugs of ketoprofen and naproxen. J. Pharm. Sci. 87, 1622–1628.

Rautio, J., Nevalainen, T., Taipale, H., Vepsalainen, J., Gynther, J., Pedersen, T., Jarvinen, T., 1999. Synthesis and *in vitro* evaluation of aminoacyloxyalkyl esters of 2-(6-methoxy-2-naphthyl) propionic acid as novel naproxen prodrugs for dermal delivery. Pharm. Res. 16, 1172–1178.

Rautio, J., Nevalainen, T., Taipale, H., Vepsalainen, J., Gynther, J., Laine, K., Jarvinen, T., 2000a. Synthesis and *in vitro* evaluation of novel morpholinyl- and methylpiperazinylacyloxyalkyl prodrugs of 2-(6-methoxy-2-naphthyl) propionic acid (naproxen) for topical drug delivery. J. Med. Chem. 43, 1489–1494.

Rautio, J., Nevalainen, T., Taipale, H., Vepsalainen, J., Gynther, J., Laine, K., Jarvinen, T., 2000b. Piperazinylalkyl prodrugs of naproxen to improve *in vitro* skin permeation. Eur. J. Pharm. Sci. 11, 157–163.

Roberts, W.J., Sloan, K.B., 1999. Correlation of aqueous and lipid solubilities with flux for prodrugs of 5-flluorouracil theophylline and 6-mercaptopurine: a Potts–Guy approach. J. Pharm. Sci. 88, 515–532.

Sloan, K.B., Wasdo, S.C., 2007. Topical delivery using prodrugs. In: Stella, V.J., Borchardt, R.T., Hageman, M.J., Oliyai, K., Tilley, J.W., Maag, H. (Eds.), Prodrugs: Challenges and Rewards. AAPS Press, pp. 83–123.

Sloan, K.B., Wasdo, S.C., Ezike-Mkparu, U., Murray, T., Nickels, D., Singh, S., Shanks, T., Tovar, J., Ulmer, K., Waranis, R., 2003. Topical delivery of 5-fluorouracil and 6-mercaptopurine by their alkylcarbonyloxymethyl prodrugs from water: vehicle effects on design of prodrugs. Pharm. Res. 20, 639–645.

Sloan, K.B., Wasdo, S.C., Rautio, J., 2006. Design for optimized topical delivery: prodrugs and a paradigm change. Pharm. Res. 23, 2729–2747.

Stinchcomb, A.L., Swaan, P.W., Ekabo, O., Harris, K.K., Browe, H., Hammell, D.C., Cooperman, T.A., Pearsall, M., 2002. Straight-chain naltrexone ester prodrugs: diffusion and concurrent esterase biotransformation in human skin. J. Pharm. Sci. 91, 2571–2578.

Swart, H., Breytenbach, J.C., Hadgraft, J., du Plessis, J., 2005. Synthesis and transdermal penetration of NSAID glycoside esters. Int. J. Pharm. 301, 71–79.

Thomas, J., Majumdar, S., Wasdo, S., Majumdar, A., Sloan, K.B., 2007. The effect of water solubility of solutes on their flux through human skin *in vitro*: an extended Flynn database fitted to the Roberts–Sloan equation. Int. J. Pharm. 339, 157–167.

Yalkowsky, S.H., Valvani, S.C., Roseman, T.J., 1983. Solubility and partitioning. VI. Octanol solubility and octanol:water partition coefficients. J. Pharm. Sci. 72, 866–870.