

# Relationship between immobilised artificial membrane chromatographic retention and the brain penetration of structurally diverse drugs

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## Abstract

Retention factors were determined for a set of 26 drugs, for which brain/blood concentration data are available, using immobilised artificial membrane (IAM) chromatography. The compound set represented acidic, basic and neutral drugs from various structural classes. The relationship between IAM retention and lipophilicity (*n*-octanol–water partition coefficient  $K_{\text{oct}}$ ), molecular size and acid/base character of the drugs and the relationship between brain distribution and IAM retention and the other parameters were analysed. IAM retention was increased with increases in lipophilicity and solute size, and decreased by the ionisation of acidic groups. Ionisation of basic groups had no significant effect. A three-parameter regression model with  $\log K_{\text{oct}}$ , molecular weight and an indicator parameter for the presence of carboxyl group explained 93% of the variation in  $\log k_{\text{IAM}}$ . The concentration ratio between brain and blood ( $\log \text{BB}$ ) was only weakly correlated with the IAM chromatographic retention or *n*-octanol–water partitioning. Three-parameter models taking ionisation and size into account, in addition to either  $\log K_{\text{oct}}$  or  $\log k_{\text{IAM}}$ , explained about 85% of the variation of  $\log \text{BB}$  in the test set. Although IAM chromatography offers no advantage in these models, it seems to provide a better model than *n*-octanol–water partitioning for the membrane distribution of ionised compounds.

**Keywords:** Brain/blood concentration ratio; Immobilised artificial membrane chromatography; Quantitative structure–activity relationships

## 1. Introduction

During the last few decades the *n*-octanol–water partition coefficient ( $K_{\text{oct}}$ ) has been the standard hydrophobicity parameter used in the

field of quantitative structure–activity relationships (QSAR) and drug design.  $\log K_{\text{oct}}$  has been extensively employed to explain the interactions of drugs with receptors and biological membranes. For instance, CNS activity [1,2] and the brain/blood concentration ratio of drugs [3] have been reported to show parabolic dependence on *n*-octanol–water partitioning. These and other

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similar findings have led to wide acceptance of the *n*-octanol–water partition coefficient as the main design parameter for CNS entry, and an ideal  $\log K_{\text{oct}}$  value of 2–2.5 for penetration of the blood–brain barrier (BBB) [4].

In recent years, however, several cases have been reported which show that very little connection may exist between *n*-octanol–water partitioning and the brain/blood concentration ratio or permeability of the blood–brain barrier [5,6]. One rationale for originally choosing  $K_{\text{oct}}$  as a standard lipophilicity scale was the superficial similarity between *n*-octanol and the membrane lipids. Liquid bulk *n*-octanol, however, is not a very realistic model for the liquid crystalline phospholipid bilayers of biological membranes. Phospholipid liposomes have more structural similarities to membranes, and similar partition coefficients have been observed for the partitioning of solutes to liposomes and to endogenous membranes [7]. Liposome systems have therefore attracted much interest as an alternative model for studying drug–membrane interactions [8–10].

Recently, immobilised artificial membranes (IAMs) have been introduced as HPLC column packing materials [11,12]. The IAMs are prepared by covalently binding a hydrocarbon chain of membrane phospholipids or their mimics on the silica surface. Good correlations have been demonstrated between IAM chromatographic retention and the partitioning of organic solutes between the aqueous phase and phosphatidylcholine liposomes [13]. Because of the convenience of chromatographic methods compared with shake-flask partition methods, great interest has arisen in studying the applicability of IAM chromatography for predicting the transport of drugs across biological membranes. Pidgeon et al. [14] have suggested that IAM chromatography always gives better correlations than ODS chromatography or *n*-octanol–water partitioning systems with respect to the prediction of solute transport through any biological barrier. These workers also proposed that IAM chromatography, unlike *n*-octanol–water partitioning, can predict membrane transport of structurally unrelated drugs.

In this work IAM chromatographic retention factors were measured for a set of structurally

diverse drugs. Concentration ratios between brain and blood have been published for most of these drugs. The IAM retention factor ( $K_{\text{IAM}}$ ) was compared with the *n*-octanol–water partition coefficient as a descriptor in multiple regression models for predicting the brain/blood concentration ratios.

## 2. Materials and methods

### 2.1. Materials

The following drugs were purchased from University Pharmacy (Helsinki, Finland): acetaminophen, acetylsalicylic acid, antipyrine, diphenhydramine hydrochloride, caffeine, cimetidine (Tagamet<sup>®</sup>, Orion-Farmos, Turku, Finland), codeine, ibuprofen, pentobarbital sodium, ranitidine hydrochloride (Ranimex<sup>®</sup>, Orion-Farmos, Vantaa, Finland), salicylic acid and theophylline. The following drugs were gifts: alprazolam, chlorpromazine hydrochloride, hydroxyzine hydrochloride, imipramine hydrochloride, indomethacin, promazine hydrochloride, thioridazine hydrochloride, sodium valproate and verapamil (from Orion-Farmos, Espoo, Finland); clonidine hydrochloride and oxazepam (from Leiras, Turku, Finland); ketoprofen (from Medifon, Helsinki, Finland); tolfenamic acid (from The Hospital Pharmacy, Turku University Central Hospital, Turku, Finland); desipramine hydrochloride (from Ciba-Geigy, Basle, Switzerland); midazolam (from Hoffmann-La Roche, Basle, Switzerland); pyrilamine maleate and trifluoperazine dihydrochloride (from May & Baker Ltd., Dagenham, UK).

### 2.2. Brain/blood distribution data

Brain/blood concentration ratios for the drugs were taken from the published literature. The references are given in Table 1. In cases where several references were found for the same drug, data measured using rats as the experimental animal, i.v. administration and selective analytical methods were preferred. In cases where more than one reference is given in Table 1, the average was used.

Table 1  
Physicochemical data,  $\log k_{IAM}$  and  $\log BB$  values of the compounds

No.	Compound	$M_r$	$V_{mol}^a$	$\log K_{oct}^b$	$\log D_{oct,7.4}^c$	$I_3^d$	$\log k_{IAM}^e$	$\log BB^f$	Ref.
1.	Acetylsalicylic acid	180.16	136.75	1.10	-2.80	-1	-1.022	-0.50	[29]
2.	Valproic acid	144.22	155.44	2.72	0.32	-1	-0.745	-0.22	[30]
3.	Theophylline	180.17	138.97	-0.25	-1.5	0	-0.432	-0.29	[31–33]
4.	Caffeine	194.19	153.27	0.07	0.07	0	-0.229	-0.055	[32–38]
5.	Antipyrine	188.23	166.69	0.19	0.19	0	-0.155	-0.097	[39]
6.	Salicylic acid	138.12	103.28	2.19	-2.24	-1	-0.101	-1.10	[40]
7.	Ranitidine	314.41	280.64	0.27	-0.59	1	0.129	-1.23	[6]
8.	Acetaminophen	151.17	128.05	0.49	-1.82	0	0.185	-0.31	[41,42]
9.	Ibuprofen	206.29	205.97	3.68	0.68	-1	0.409	-0.18	[43]
10.	Clonidine	230.10	177.12	1.37 <sup>g</sup>	0.63	0	0.434	0.11	[6]
11.	Codine	299.37	266.33	0.82 <sup>g</sup>	-0.05	1	0.442	0.55	[44]
12.	Ketoprofen	254.29	214.39	2.79	-0.01	-1	0.496		
13.	Pentobarbital	226.28	211.24	2.11	2.01	0	0.540	0.12	[45–47]
14.	Cimetidine	252.34	223.41	0.21	0.11	0	0.564	-1.42	[6]
15.	Diphenhydramine	255.36	248.09	3.36	1.75	1	1.064		
16.	Alprazolam	308.77	241.50	3.2	3.20	0	1.157	0.044	[48–50]
17.	Pyrilamine	285.39	274.50	2.77	1.24	1	1.213	0.50	[6,51]
18.	Indomethacin	357.80	279.84	4.23	1.33	-1	1.452	-1.26	[52]
19.	Oxazepam	286.72	215.47	2.1	2.10	0	1.594	0.61	[48,50,53]
20.	Hydroxyzine	374.91	324.74	4.16	3.98	1	1.678	0.39	[54]
21.	Tolfenamic acid	261.71	209.43	5.70		-1	1.790		
22.	Imipramine	280.42	277.42	4.41	2.31	1	1.818	1.30	[55,56]
23.	Desipramine	266.39	261.15	4.09	1.05	1	1.866	1.20	[57,58]
24.	Midazolam	325.78	250.71	3.7	3.67	0	1.965	0.36	[48,59]
25.	Verapamil	454.61	442.04	3.53	2.00	1	1.982	-0.70	[60]
26.	Promazine	284.43	259.10	4.28	2.28	1	2.146	1.23	[61,62]
27.	Chlorpromazine	318.87	274.88	5.20	3.29	1	2.551	1.06	[23,62]
28.	Trifluoperazine	407.50	340.29	6.48	5.70	1	3.022	1.44	[63]
29.	Thioridazine	370.58	339.13	6.42	4.32	1	3.035	0.24	[62,64]

<sup>a</sup> Molecular volume was calculated using the Cerius<sup>2</sup> program.

<sup>b</sup> Logarithm of *n*-octanol-water partition coefficient was taken from Ref. [15].

<sup>c</sup> Logarithm of the apparent distribution coefficient at pH 7.4 was calculated using Eq. (1) for acids and Eq. (2) for bases and published  $pK_a$  values from Ref. [15].

<sup>d</sup> Indicator parameter: 1 if amino-nitrogen is present, -1 for carboxylic acid, and 0 for all the other compounds.

<sup>e</sup> Logarithm of IAM chromatographic retention factor.

<sup>f</sup> Logarithm of brain/blood concentration ratio. In cases where more than one reference is given in the Ref. column, the average was used.

<sup>g</sup> Value was calculated using ClogP for Macintosh v. 1.03 program (BioByte, Claremont, CA).

### 2.3. Physicochemical parameters

Experimental  $\log K_{\text{oct}}$  values were not available for all the compounds and therefore published [15] calculated  $\log K_{\text{oct}}$  values were used. The apparent distribution constant,  $\log D_{\text{oct},7.4}$ , was calculated using Eq. (1) for acids and Eq. (2) for bases and published  $\text{p}K_{\text{a}}$  values [15]. Molecular volumes were calculated using the Cerius<sup>2</sup> program (Molecular Simulations, San Diego, CA).

$$\log D = \log K - \log[1 + 10^{(\text{pH} - \text{p}K_{\text{a}})}] \quad (1)$$

$$\log D = \log K - \log[1 + 10^{(\text{p}K_{\text{a}} - \text{pH})}] \quad (2)$$

### 2.4. IAM chromatography

The chromatographic system consisted of an LKB 2150 HPLC pump, and LKB 2151 variable wavelength UV-Vis detector (LKB-Produkter, Bromma, Sweden), a Rheodyne 7125 injector module equipped with a 5  $\mu\text{l}$  loop (Rheodyne, Cotati, CA) and a Merck-Hitachi D-2000 chromatographic integrator (E. Merck, Darmstadt, Germany). A commercially distributed IAM.PC.DD 30 mm  $\times$  4.6 mm i.d. column was purchased from Regis Technologies, Inc. (Morton Grove, IL). The column had a particle diameter of 5  $\mu\text{m}$  and a pore diameter of 300 Å. The stationary phase of the column is formed by bonding 11-carboxylundecylphosphocholine on spherical aminopropyl silica with the unreacted propylamine moieties end-capped with C10 and C3 anhydrides. The mobile phase was phosphate-buffered saline (pH 7.4; 0.01 M) with a flow rate of 1 ml  $\text{min}^{-1}$ . For all studies, the injection volume was 5  $\mu\text{l}$  of an aqueous solution of the compound (0.1 mg  $\text{ml}^{-1}$ ) in the mobile phase, the detection wavelength varying according to the individual compounds. The experiments were carried out at ambient temperature. All retention factors given represent the mean of 2–4 determinations of each sample solution. The retention factor  $k$  was calculated as  $k = (t_{\text{m}} - t_0)/t_0$ , where  $t_{\text{m}}$  is the retention time of the compound in minutes and  $t_0$  (the column dead time or void volume) is the retention time of the unretained compound. The solvent disturbance given by water was used as the column dead time. The repeatability of retention times (intra-assay)

was better than 0.5% (RSD), and the intermediate precision (inter-assay) varied from 2%–5% (RSD) within a period of 2 weeks, except for cimetidine for which it was 10% (RSD). The retention times of compounds determined several weeks after first using the column were corrected by chromatographing three earlier determined compounds in the same batch. This was considered necessary because the retention of the compounds tended to decrease over time. For the compounds not eluting with an aqueous mobile phase, five concentrations of acetonitrile were used as isocratic mobile phases and linear plots of  $\log k_{\text{IAM},x}$  vs. percent acetonitrile ( $x$ ) were plotted and extrapolated to obtain the  $\log k_{\text{IAM},x}$  value that theoretically corresponded to 0% acetonitrile. All the data given represent  $\log k_{\text{IAM}}$  values corresponding to 100% aqueous mobile phase.

### 2.5. Quantitative structure–property relationships

The Cerius<sup>2</sup> program and the SPSS for Windows Release 6.1 program (SPSS Inc., Chicago, IL) were used to compute the correlation and regression analyses. The leave-one-out method was used to calculate cross-validated  $r^2$  values [16].

## 3. Results and discussion

### 3.1. IAM chromatographic retention

The compounds studied represented structurally diverse drugs with various pharmacodynamic and pharmacokinetic properties and varying degrees of ionisation at pH 7.4, resulting in either a positive or negative charge. The IAM chromatographic retention factors measured for 29 compounds and the physicochemical and structural parameters used in the correlation analyses are shown in Table 1. Two acidic compounds (ketoprofen and tolfenamic acid) for which brain distribution data were not available were included in the study to increase the lipophilicity span of the compounds of this class. The retention factors were measured using phosphate-buffered saline (pH 7.4; 0.01 M) as the mobile phase, except for the four most hydrophobic compounds which re-

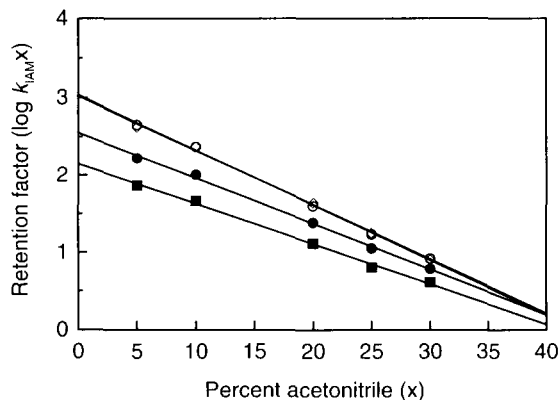


Fig. 1. The logarithm of retention factors ( $\log k_{IAM,x}$ ) at different acetonitrile concentrations in the mobile phase (% v/v) for (■) promazine, (●) chlorpromazine, (◇) trifluoperazine and (○) thioridazine.

quired an organic modifier for elution as a measurable peak. Retention factors for these compounds were determined by extrapolating to zero acetonitrile percentage as shown in Fig. 1. Excellent linearity of the relationships were found over the whole eluent composition range studied, the correlation coefficient,  $r$  being  $> 0.999$  for all the compounds, with the exception of promazine ( $r = 0.998$ ).

As the first approximation, the retention of an IAM column depended on lipophilicity. The  $\log k_{IAM}$  values for all the compounds are plotted

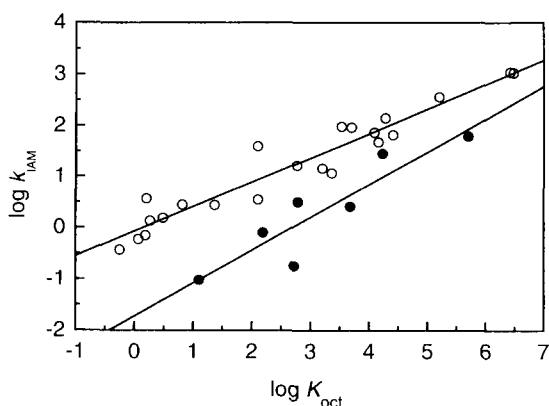


Fig. 2. Correlation between IAM chromatographic retention factor ( $\log k_{IAM}$ ) and calculated *n*-octanol–water partition coefficient ( $\log K_{oct}$ ): (●) carboxylic acids; (○) all other compounds.

against  $\log K_{oct}$  in Fig. 2. The coefficient of correlation for the whole set was  $r = 0.837$ . Correlation with the apparent distribution coefficient  $\log D_{oct,7.4}$  was slightly higher:  $r = 0.876$ . However, if the seven compounds containing a carboxyl group and the other compounds are treated separately, the following equations are obtained:

$$\log k_{IAM} = 0.48 \log K_{oct} - 0.07$$

$$n = 22 \quad r = 0.961 \quad r^2 = 0.924 \\ s = 0.29 \quad F = 241.7 \quad (3)$$

$$\log k_{IAM} = 0.64 \log K_{oct} - 1.72$$

$$n = 7 \quad r = 0.912 \quad r^2 = 0.832 \\ s = 0.47 \quad F = 24.8 \quad (4)$$

All the compounds, with the exception of the carboxylic acids, showed a similar dependence of retention on lipophilicity regardless of the degree of ionisation. The parameter coefficients and  $r$  values for the subset containing the 12 basic compounds with a degree of ionisation of 80–100% and for the other subset containing the seven compounds with a degree of ionisation of 0–20% were close to those in Eq. (3). Correlation with  $\log D_{oct,7.4}$  for the 22 non-carboxyl compounds, however, was not so good ( $r = 0.876$ ).

Carboxylic acids, which are almost completely in the anionic form under the experimental conditions used, clearly form a subgroup that behaves differently in IAM chromatography. The intercept of the regression line for these compounds was 1.6 log units lower than the line for the other compounds and the dependence of retention on hydrophobicity was steeper, thus implying that, with increasing lipophilicity, their behaviour will approach that of the other compounds.

It is generally believed that the apparent distribution constant of ionisable compounds measured at the relevant pH rather than  $\log K_{oct}$ , should correlate with membrane distribution and transport of drugs [17], because only the un-ionised form is supposed to be able to partition significantly into the lipid phase. However, it was shown recently by Austin et al. [18] that the charged form of certain amines, but not of carboxylic acids, is able to partition into phospholipid vesicles as well as the uncharged form. These results

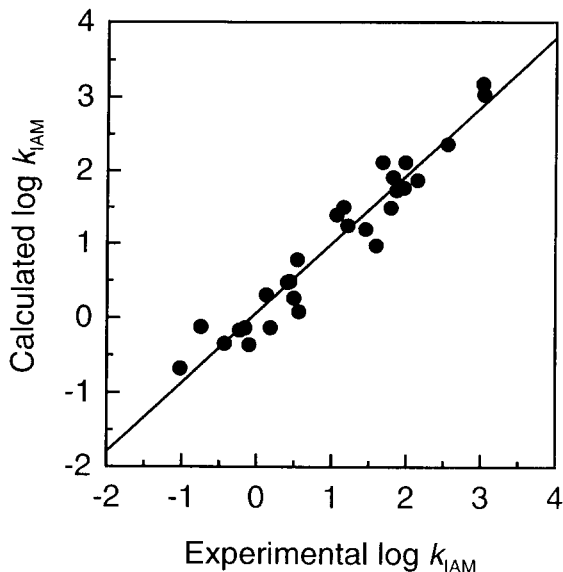


Fig. 3. Correlation between calculated (from Eq. (5)) and experimentally determined  $\log k_{IAM}$  values.

are in accordance with the IAM chromatographic behaviour of acidic and basic drugs observed in this work.

The size of the compound expressed either as the molecular weight ( $M_r$ ) or molecular volume ( $V_m$ ) showed almost as high a correlation ( $r = 0.799$  and  $0.770$  respectively) with  $\log k_{IAM}$  as with lipophilicity. The collinearity of these two properties with  $\log K_{oct}$  was not very high in this set ( $r = 0.592$  and  $0.594$ ).

A three-parameter model explained 93% of the variation in  $\log k_{IAM}$ :

$$\log k_{IAM} = 0.41 \log K_{oct} - 0.89I_1 + 0.0003M_r - 0.83$$

$$n = 29 \quad r = 0.966 \quad r^2 = 0.932$$

$$s = 0.300 \quad F = 114.8 \quad (5)$$

$I_1$  is an indicator parameter having a value of one for carboxylic acids and zero for other compounds.

The cross-validated  $r^2$  value for model (5) was 0.909. Experimental  $\log k_{IAM}$  values are plotted against the values calculated from Eq. (5) in Fig. 3.

### 3.2. Relationship between brain/blood distribution and chromatographic and other parameters

Brain/blood distribution data were available for all the compounds apart from the two acidic drugs mentioned above. Diphenhydramine was excluded from this analysis, because its very high brain concentration is reported to be due to active transport [19]. The brain/blood concentration ratios ( $\log BB$ ) correlated weakly with the IAM retention factors ( $\log k_{IAM}$ ) and with the *n*-octanol–water partition coefficient ( $\log K_{oct}$ ) ( $r = 0.576$  and  $0.537$  respectively).

Taking into account the effect of ionisation and solute size improved the regression models:

$$\log BB = 0.62 \log k_{IAM} + 1.00I_2 - 0.01V_m + 1.18$$

$$n = 26 \quad r = 0.762 \quad r^2 = 0.581$$

$$s = 0.56 \quad F = 10.2 \quad (6)$$

$$\log BB = 0.32 \log K_{oct} + 0.96I_3 - 0.01V_m + 1.06$$

$$n = 26 \quad r = 0.839 \quad r^2 = 0.705$$

$$s = 0.47 \quad F = 17.5 \quad (7)$$

When outliers are omitted, the following models of comparable quality are obtained:

$$\log BB = 0.58 \log k_{IAM} + 0.89I_2 - 0.01V_m + 1.28$$

$$n = 21 \quad r = 0.921 \quad r^2 = 0.848$$

$$s = 0.27 \quad F = 31.5 \quad (8)$$

$$\log BB = 0.35 \log K_{oct} + 0.99I_3 - 0.01V_m + 1.25$$

$$n = 23 \quad r = 0.921 \quad r^2 = 0.848$$

$$s = 0.32 \quad F = 35.2 \quad (9)$$

The cross validated  $r^2$  values for models (8) and (9) were 0.627 and 0.776 respectively. The outliers are cimetidine, indomethacin, ranitidine, salicylic acid, and thioridazine in the case of Eq. (6), and the latter three in the case of Eq. (7). In Eqs. (6) and (8) the parameter  $I_2$  has the value one if amino-nitrogen is present and the value zero for all the other compounds. The model implies that the brain favours cationic compounds over phospholipid membranes. The indicator parameter  $I_3$  in Eqs. (7) and (9) has, in addition, the value of  $-1$  for compounds with a carboxyl group. This is in accordance with the relationship between  $\log k_{IAM}$  and  $\log K_{oct}$  modelled by Eq. (5).

The rationale for predicting the transport across membranes by means of partition coefficients between an aqueous phase and a lipid phase is based on the solubility–diffusion model of membrane permeability [7,20]:

$$P = K_m D_m / L \quad (10)$$

where  $P$  is the permeability coefficient ( $\text{cm s}^{-1}$ , the speed with which the solute moves across a slice of the medium),  $K_m$  is the equilibrium distribution constant in the membrane,  $D_m$  is the diffusion constant in the membrane and  $L$  is the membrane thickness. Solute size has the opposite effect on  $K_m$  and  $D_m$ , a larger size favouring distribution to lipid phase and decreasing diffusion rate. The effect of solute size on permeability is a balance between these two effects. Both negative [17,21] and positive [22] coefficients for the size term in QSAR equations modelling brain distribution have been reported depending on the other parameters used. Membrane distribution, however, has been considered as the main factor controlling permeability.

The IAM retention factor can be rationalised as a better model for membrane distribution than alkane–water or  $n$ -octanol–water partition coefficients, which are conventionally used in this context. The parallel behaviour of ionised compounds in IAM chromatography and in liposome partitioning, as discussed above, and the high correlation reported earlier [13] between  $\log k_{\text{IAM}}$  and the logarithm of the distribution constant in liposome systems [23] support this assumption. Consequently, IAM retention can be expected to outplay  $n$ -octanol–water partitioning as a better predictor for membrane permeation if the solubility–diffusion model is valid. In the case of brain distribution data, however, the only advantage of  $\log k_{\text{IAM}}$  over  $\log K_{\text{oct}}$  is that it accounts for the effect of carboxylate anions.

Eq. (10) is based on a simple membrane model, in which both the membrane and the outside and inside aqueous phases are homogeneous and symmetrical [7,20]. The model is obviously an oversimplification for penetration of the blood–brain barrier. For instance, the effect of ionisation on brain distribution seems to be even more complicated than its effect on membrane distribution, as

is shown by Eqs (6)–(9).

Some data concerning the correlation of IAM chromatography with in-vitro or in-vivo membrane permeability were published earlier. Two reports deal with IAM chromatography and skin permeation of steroids. Nasal et al. [24] observed good correlation ( $r = 0.942$ ) between logarithms of IAM retention factors and skin permeability coefficients for a set of 10 steroids. The retention factors on IAM and on C18 columns showed comparable correlation ( $r \approx 0.84$ ) with skin permeability in the study of Alvarez et al. [25] using a structurally more diverse set of 15 steroids. When 8 structurally similar analogues were used, the IAM column gave superior correlation ( $r = 0.913$ ). Pidgeon et al. [14] demonstrated weak correlation of  $k_{\text{IAM}}$  with permeability through Caco-2 cells ( $r = 0.762$ ) and for rat intestinal absorption ( $r = 0.791$ ) for sets of structurally diverse drugs. After correction for molecular weight, improved correlations ( $r = 0.854$  and  $0.858$  respectively) were obtained. Good correlation ( $r = 0.941$ ) of  $k_{\text{IAM}}$  with oral absorption in mice was observed for a set of structurally related cephalosporin analogues.

A successful method for predicting the brain/blood concentration ratio of structurally diverse drugs has been published by Abraham et al. [22]. They applied the general solvation equation of Abraham et al. [22] to the brain/blood distribution data published by Young and co-workers [6,26]. The model with five solvatochromic parameters had a correlation coefficient of  $r = 0.776$  for the whole set ( $n = 30$ ) and a value of  $r = 0.941$  after removing eight outliers. The three-parameter models constructed in this work showed a comparable fit to the data. The effect of the solvatochromic parameters, especially molecular volume and hydrogen bond basicity, is actually embedded in  $\log K_{\text{oct}}$ , as has been shown by Abraham et al. [27]. They have also shown that in the case of skin permeability [28] a  $\log K_{\text{oct}}$  model exhibits comparable performance with the solvatochromic model if a corrective term for the excess size effect of  $\log K_{\text{oct}}$  is included. The use of a  $V_m$  term with a negative coefficient was also found to be necessary for the brain distribution models in this work, and in addition, another corrective term for

ionisation was required. In conclusion, IAM chromatography seems to be a useful method for predicting solute distribution in membranes. Log  $k_{IAM}$ , however, offered no advantage over log  $K_{oct}$  as a parameter in models predicting brain/blood concentration ratios of drugs, in particular because log  $K_{oct}$  can be calculated with sufficient accuracy.

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