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Determination of solute lipophilicity, as $\log P(\text{octanol})$ and $\log P(\text{alkane})$ using poly(styrene–divinylbenzene) and immobilised artificial membrane stationary phases in reversed-phase high-performance liquid chromatography

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Abstract

A number of RP–HPLC systems have been characterized by the linear free energy relationship:

$$\log SP = c + r.R_2 + s.\pi_2^H + a.\sum\alpha_2^H + b.\sum\beta_2 + v.V_x \quad (i)$$

Here, SP is either $\log k'$ or $\log k_w$ for a series of solutes in a given system, where k' is the capacity factor and k_w is the capacity factor extrapolated to 100% water, and the solute descriptors are, R_2 an excess molar refraction, π_2^H the dipolarity/polarizability, $\sum\alpha_2^H$ and $\sum\beta_2$ the overall or effective hydrogen-bond acidity and basicity, and V_x the McGowan characteristic volume. Comparison of the coefficients in Eq. (1) with those for water–solvent partitions confirms that the modified electrostatically coated C_{18} phase of Pagliara et al. (J. Liq. Chromatogr., 18 (1995) 1721) can be used to obtain solute lipophilicities, as $\log P_{\text{oct}}$. For RP–HPLC systems based on poly(styrene–divinylbenzene), the coefficients in Eqs. (i) are nearer those for the correlation of water–alkane partition coefficients, as $\log P_{\text{alk}}$, than for the correlation of $\log P_{\text{oct}}$, suggesting that the RP–HPLC systems with poly(styrene–divinylbenzene) phases could be used as a rapid method for determination of solute lipophilicity, as $\log P_{\text{alk}}$ or as $\log P_{\text{cyc}}$, where the latter is the water–cyclohexane partition coefficient. Eqs. (i) has also been applied to RP–HPLC $\log k'$ values obtained with an immobilized artificial membrane (IAM) phase. A good regression equation was obtained, but the coefficients in this equation are substantially different to those for regressions with $\log P_{\text{oct}}$, $\log P_{\text{alk}}$, or $\log P_{\text{cyc}}$ as the dependent variable. On the other hand, $\log k'$ values from the RP–HPLC system of Miyake et al. [J. Chromatogr., 389 (1987) 47], consisting of silica gel coated with dipalmitoyl phosphatidyl choline as a stationary phase, with aqueous acetonitrile mobile phases, yielded coefficients in Eqs. (i) very similar to those for $\log P_{\text{oct}}$.

Keywords: Lipophilicity; Partition coefficients; Stationary phases, LC; Immobilized artificial membranes; Poly(styrene–divinylbenzene)

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1. Introduction

There is continuing interest in the development of rapid methods for the experimental determination of solute lipophilicity. Following the work of Hansch and Leo et al. [1–3], this is now mostly defined in terms of the water–octanol partition coefficient, as $\log P_{\text{oct}}$. Not surprisingly, a number of rapid chromatographic methods have been suggested for the determination of $\log P_{\text{oct}}$. These include reversed-phase high-performance liquid chromatography [4–10], thin layer chromatography [11], microemulsion electrokinetic chromatography [12], and micellar electrokinetic chromatography [12–15]. The usual method is to obtain a set of capacity factors, k' (or extrapolated capacity factors, k_w) in a given system for a training set of solutes with known water–octanol partition coefficients, and to establish a correlation equation of the type,

$$\log P = l + m \cdot \log k \quad (1)$$

where k can be k' or k_w , and, in this case, $P = P_{\text{oct}}$. Further measurements of $\log k'$ or $\log k_w$ in the same system can then be used to estimate $\log P_{\text{oct}}$ for solutes in a ‘test’ set. It is now known, however, that the properties of the training set should be well matched to properties of the test set of compounds, especially as regards hydrogen-bond acidity and basicity, otherwise incorrect values of $\log P_{\text{oct}}$ will be calculated [16]. Note that we write Eq. (1) with $\log P$ as the dependent variable rather than $\log k'$. It is the standard deviation in $\log P$ that is the crucial criterion as to whether Eq. (1) is statistically good enough to estimate $\log P$ values for the test set. The stationary phase used in such lipophilicity determinations has usually been C_{18} modified silica, but other phases have also been used, for example [7,9] an octadecyl–polyvinylalcohol copolymer (ODP), a polystyrene–divinylbenzene copolymer (PLRP-S) [7], as well as a modified C_{18} phase pretreated with an electrostatic coating [10]. Vallat et al. [9] and Pagliara et al. [10] characterised several of these phases, as well as water–solvent partitions, using the linear solvation energy relationship (LSER) of Kamlet, Taft and Abraham [17,18]. We shall refer to this work later.

Although $\log P_{\text{oct}}$ is the most common measure of solute lipophilicity, other water–organic solvent parti-

tions have proved useful. In particular, the water–cyclohexane system, either as $\log P_{\text{cyc}}$ itself, or as the $\Delta \log P$ parameter of Seiler [19] has been used to estimate blood–brain distribution [20]. Since the determination of water–cyclohexane partition, or the almost equivalent water–alkane partition, P_{alk} , by the ‘shake-flask’ method is just as time consuming as water–octanol determinations, it is not surprising that efforts have been made to develop a rapid method for these determinations. Indeed, since there are several methods for the calculation of $\log P_{\text{oct}}$ [21] but none for the calculation of $\log P_{\text{cyc}}$ or $\log P_{\text{alk}}$, there is even more point to a method for the rapid determination of $\log P_{\text{cyc}}$ or $\log P_{\text{alk}}$. Lambert et al. [22,23] have used a C_{18} derivatised polystyrene–divinylbenzene stationary phase, Act-I, in RP–HPLC in order to estimate $\log P_{\text{alk}}$. As Lambert [24] points out, such phases have an advantage in that they are stable over a wide pH range [25].

2. Methodology

Several years ago, Kamlet et al. [17,18] set out a general equation for the correlation of solute effects; since several of the descriptors were based on solvatochromic measurements on solvents, the equation has often been known as a solvatochromic equation, as well as a linear solvation energy relationship:

$$\log SP = c + d \cdot \delta + s \cdot \pi^* + a \cdot \alpha_2 + b \cdot \beta_2 + m \cdot V_1 \quad (2)$$

Here, $\log SP$ is the dependent variable, and the independent variables, or descriptors are [17,18] as follows: δ is a polarizability correction term, π^* is the dipolarity/polarizability, α_2 and β_2 are the hydrogen-bond acidity and basicity, and V_1 is the computer calculated intrinsic volume of Leahy [26]. The α_2 and β_2 descriptors were also denoted as α_m and β_m . Vallat et al. [9] and Pagliara et al. [10] characterized phases used in lipophilicity determination of $\log P_{\text{oct}}$, through Eq. (2), but we prefer to analyse data in terms of the more recent linear free energy relationship, Eq. (3), where the descriptors are derived from solute properties.

$$\log SP = c + r \cdot R_2 + s \cdot \pi_2^H + a \cdot \sum \alpha_2^H + b \cdot \sum \beta_2 + v \cdot V_x \quad (3)$$

Table 1
Coefficients in the LFER, Eq. (3), for water–organic solvent partitions

System	<i>r</i>	<i>s</i>	<i>a</i>	<i>b</i>	<i>v</i>
Water–octanol, log <i>P</i>	0.56	−1.05	0.03	−3.46	3.81
Water–isobutanol, log <i>P</i>	0.48	−0.64	−0.05	−2.28	2.76
Water–pentanol, log <i>P</i>	0.58	−0.79	0.02	−2.84	3.25
Water–alkane, log <i>P</i>	0.65	−1.66	−3.52	−4.82	4.28
Water–cyclohexane, log <i>P</i>	0.82	−1.73	−3.78	−4.91	4.65
Water–hexadecane, log <i>P</i>	0.67	−1.62	−3.59	−4.87	4.43

The independent variables in Eq. (3) are solute descriptors as follows [27]: R_2 is an excess molar refraction, π_2^H is the solute dipolarity/polarizability, $\Sigma\alpha_2^H$ and $\Sigma\beta_2$ are the solute overall or effective hydrogen-bond acidity and basicity, and V_x is the McGowan characteristic volume [28] in units of $\text{cm}^3 \text{mol}^{-1}/100$. Certain compounds such as anilines, pyridines and some heterocyclic amines have a variable basicity [29]. For partition of these particular compounds between water and solvents that contain little water at saturation, such as chloroform or cyclohexane, the parameter $\Sigma\beta_2^H$ is used, but for partition between water and solvents that contain considerable water at saturation, such as octanol, the $\Sigma\beta_2^0$ parameter is used.

Eq. (3) has been applied to partition coefficients, as log *P* values, in numerous water–organic solvent systems, and the regression coefficients obtained are presented in Table 1 [30]. It is quite clear that the solute factors affecting water–alcohol partitions are not the same as those that affect water–alkane or water–cyclohexane partitions. Hence, if an RP–HPLC system is good model for water–octanol partitions, it will not be a good model for water–

alkane partitions especially with solutes that have substantial $\Sigma\alpha_2^H$ values. This can be seen even more clearly through a comparison of coefficient ratios, (see Table 2). The *a/v* ratio in the water–alcohol partitions is effectively zero, but is quite significant in the water–alkane partitions.

In order to investigate model RP–HPLC systems for water–alkane partitions, our plan is to analyse log *k'* values, or extrapolated log *k_w* values through Eq. (3) and to compare coefficients and coefficient ratios with those for water–alkane partitions. We can then select for further examination those RP–HPLC systems that seem to be good models for water–alkane partition coefficients.

3. Results and discussion

We have previously analysed numerous sets of log *k'* values of C_{18} stationary phases with water–methanol, water–acetonitrile and water–THF eluents [31,32]. In Table 3 (first row) are given the coefficients in Eq. (3) for one particular system as an example, and in Table 4 (first row) are the coefficient

Table 2
Ratios of coefficients in the LFER, Eq. (3), for water–organic solvent partitions

System	<i>r/v</i>	<i>s/v</i>	<i>a/v</i>	<i>b/v</i>	<i>v/v</i>
Water–octanol, log <i>P</i>	0.15	−0.28	0.01	−0.91	1
Water–isobutanol, log <i>P</i>	0.17	−0.23	−0.02	−0.83	1
Water–pentanol, log <i>P</i>	0.18	−0.24	0.00	−0.87	1
Water–alkane, log <i>P</i>	0.15	−0.39	−0.82	−1.13	1
Water–cyclohexane, log <i>P</i>	0.18	−0.37	−0.81	−1.06	1
Water–hexadecane, log <i>P</i>	0.15	−0.36	−0.81	−1.10	1

Table 3
Coefficients in the LFER, Eq. (3), for RP-HPLC systems

System	<i>n</i>	<i>r</i>	<i>s</i>	<i>a</i>	<i>b</i>	<i>v</i>
C ₁₈ ; 60% MeOH ^a	126	0.25	-0.65	-0.43	-1.53	1.77
C ₁₈ -ABZ; log <i>k</i> _w ^{b,c}	75	0.30	-0.55	0.23	-3.27	3.63
C ₁₈ -ABZ; 40% MeOH ^{b,c}	69	0.26	-0.44	0.19	-2.30	2.38
PLRP-S, log <i>k</i> _w ^{b,d}	28	1.56	-1.12	-1.73	-3.04	2.60
Act-I; 60% MeOH ^b	48	0.40	-0.66	-0.92	-2.56	2.51
PRF-I; 100% MeOH ^b	45	0.46	0.06	-1.21	-0.89	0.66
PRP-I; 80% MeOH ^b	28	0.45	-0.05	-1.09	-1.70	1.46
PRP-I; 100% MeCN ^b	43	0.45	-0.26	-0.46	-0.68	0.39
PRP-I; 60% MeCN ^b	43	0.33	-0.12	-0.89	-1.71	1.81
IAM; 10% MeCN ^b	27	0.81	-0.42	0.69	-2.00	1.87
DPC; 10%, MeCN ^{b,c}	43	0.49	-0.44	0.03	-2.76	2.68
DPC; 20% MeCN ^{b,c}	46	0.57	-0.50	-0.01	-2.59	2.25
DPC; 30% MeCN ^{b,f}	36	0.31	-0.50	-0.05	-1.63	1.64

^a Equation from Ref. [31]; aromatic solutes including hydrocarbons, ethers, halides, aldehydes, ketones, esters, amides, anilines, nitriles, phenols and alcohols.

^b Equation from this work.

^c Aliphatic ethers, halides, esters, amides, nitriles, alcohols and acids and aromatic hydrocarbons, halides, ketones, esters, amides, anilines, nitriles, nitro-compounds, phenols and acids and pyridines.

^d Aromatic hydrocarbons, halides, ethers, aldehydes, ketones, esters, amides, anilines, nitriles, nitro-compounds, phenols, sulfoxides and sulfones.

^e Aromatic hydrocarbons, halides, ethers, esters, nitro-compounds, nitriles, phenols, acids and alcohols.

^f Aromatic hydrocarbons, halides, ethers, esters, phenols, acids and alcohols.

ratios. These ratios are near to the average found [32] for a large number of C₁₈ phases with the above eluents. From the coefficient ratios in Tables 2 and 4, it is clear that this RP-HPLC system is a partial, but not complete, model for the water-octanol system, and would not be a realistic model for water-alkane systems. For completeness, we have also analysed the data of Pagliara, et al. [10] who used a Supelcosil

LC-ABZ phase that is an electrostatically coated C₁₈ phase, C₁₈-ABZ. For log *k*' values with 40% methanol eluent and for log *k*_w values we find:

$$\begin{aligned} \log k'(40\% \text{ MeOH})_{\text{C}_{18} - \text{ABZ}} &= -0.599 \\ &+ 0.265R_2 - 0.440\pi_2^H + 0.187\sum\alpha_2^H - 2.299\sum\beta_2^O \\ &+ 2.379V_x \end{aligned} \quad (4)$$

Table 4
Ratios of coefficients in the LFER, Eq. (3), for RP-HPLC systems

System	<i>r/v</i>	<i>s/v</i>	<i>a/v</i>	<i>b/v</i>	<i>v/v</i>
C ₁₈ ; 60% MeOH	0.14	-0.37	-0.24	-0.86	1
C ₁₈ -ABZ; log <i>k</i> _w	0.08	-0.15	0.06	-0.90	1
C ₁₈ -ABZ; 40% MeOH	0.11	-0.18	0.08	-0.97	1
PLRP-S, log <i>k</i> _w	0.60	-0.43	-0.66	-1.17	1
Act-I; 60% MeOH	0.16	-0.26	-0.37	-1.02	1
PRP-I; 100% MeOH	0.70	0.09	-1.84	-1.35	1
PRP-I; 80% MeOH	0.31	-0.03	-0.75	-1.17	1
PRP-I; 100% MeCN	1.15	-0.66	-1.19	-1.75	1
PRP-I; 60% MeCN	0.28	-0.10	-0.76	-1.45	1
IAM; 10% MeCN	0.43	-0.23	0.37	-1.07	1
DPC; 10% MeCN	0.18	-0.16	0.01	-1.03	1
DPC; 20% MeCN	0.25	-0.22	0.00	-1.15	1
DPC; 30% MeCN	0.19	-0.31	-0.03	-1.00	1

$n = 69, r = 0.9913, SD = 0.090, F = 715$

$$\begin{aligned} \log k_w, C_{18} - ABZ = & -0.296 + 0.297R_2 \\ & - 0.549\pi_2^H + 0.228\sum\alpha_2^H \\ & - 3.268\sum\beta_2^O + 3.630V_x \quad (5) \end{aligned}$$

$n = 75, r = 0.9870, SD = 0.172, F = 522$

where n is the number of data points, r is the correlation coefficient, SD is the standard deviation in the dependent variable and F is the F -statistic. A summary of coefficients is in Tables 3 and 4. As found by Pagliara et al. [10], both the above RP–HPLC systems can be regarded as good models for water–octanol partitions.

3.1. Polystyrene–divinylbenzene phases

From the present point of view, more pertinent is

the poly(styrene–divinylbenzene) gel, PLRP-S, that was considered by Bechalany et al. [7] as a model for water–octanol partitions. For all 28 solutes we find that the regressions using $\sum\beta_2^H$ or $\sum\beta_2^O$ are almost the same; the former is:

$$\begin{aligned} \log k_w, PLRP - S = & 2.039 + 1.557 R_2 - 1.117\pi_2^H \\ & - 1.727\sum\alpha_2^H - 3.043\sum\beta_2^H \\ & + 2.596V_x \quad (6) \end{aligned}$$

$n = 28, r = 0.9740, SD = 0.299, F = 81$

Although the quality of the regression is not good, it explains why the $\log k_w$ values do not correlate too well with $\log P_{oct}$ (compare the coefficient ratios in Tables 2 and 4). But, very interestingly, the comparison of coefficient ratios suggests that $\log k_w$ should correlate with $\log P_{alk}$ or $\log P_{cyc}$ just as well as with $\log P_{oct}$. In Table 5 are details of regressions

Table 5
Regression equations based on Eq. (1)

System	P	l	m	n	r	SD	F
PLRP-S, $\log k_w$	P_{oct}	-0.81	0.786	27	0.9769	0.21	523
PLRP-S, $\log k_w$	P_{cyc}	-3.86	1.492	27	0.9740	0.40	424
PLRP-S, $\log k_w$	P_{alk}	-4.32	1.553	27	0.9743	0.45	448
PLRP-S, $\log k_w$	P_{16}	-4.20	1.517	27	0.9720	0.45	428
Act-I, 60% MeOH	P_{oct}	0.66	1.212	48	0.9572	0.29	504
Act-I, 60% MeOH	P_{cyc}	-0.94	2.156	48	0.9695	0.43	719
Act-I, 60% MeOH	P_{alk}	-1.07	2.084	48	0.9620	0.47	571
Act-I, 60% MeOH	P_{16}	-1.09	2.079	48	0.9657	0.44	637
PRP-1, 80% MeOH	P_{oct}	0.90	1.573	28	0.7677	0.60	36
PRP-1, 80% MeOH	P_{cyc}	-0.70	2.885	28	0.9397	0.48	189
PRP-1, 80% MeOH	P_{alk}	-0.74	2.644	28	0.9280	0.48	155
PRP-1, 80% MeOH	P_{16}	-0.83	2.752	28	0.9332	0.48	169
PRP-1, 60% MeCN	P_{oct}	1.04	1.895	43	0.8939	0.53	163
PRP-1, 60% MeCN	P_{cyc}	-0.59	3.415	43	0.9731	0.45	731
PRP-1, 60% MeCN	P_{alk}	-0.63	3.180	43	0.9659	0.48	571
PRP-1, 60% MeCN	P_{16}	-0.69	3.250	43	0.9722	0.44	705
IAM, 10% MeCN	P_{oct}	1.09	1.682	27	0.9170	0.69	132
IAM, 10% MeCN	P_{cyc}	-0.38	1.954	27	0.6337	2.25	17
IAM, 10% MeCN	P_{alk}	-0.59	1.904	27	0.6255	2.24	16
IAM, 10% MeCN	P_{16}	-0.58	1.897	27	0.6290	2.22	16
DPC; 10% MeCN	P_{oct}	1.58	1.287	43	0.9729	0.14	725
DPC; 20% MeCN	P_{oct}	1.85	1.446	46	0.9729	0.16	779
DPC; 30% MeCN	P_{oct}	3.08	2.051	36	0.9669	0.15	489

based on Eq. (1); in all cases, the point for benzotrifluoride is a clear outlier, and has been omitted. We give three separate regressions for $\log P_{\text{cyc}}$, $\log P_{\text{alk}}$ and $\log P_{16}$, where the latter refers to the water–hexadecane system. Most of the $\log P$ values have been reported before [33,34], but several have had to be estimated using the descriptors in Table 6 and the coefficients in Table 1, and so we thought it expedient to give all three regression equations. There is little difference in goodness of fit between the four regression equations, as expected. However, the *sd* values for the $\log P_{\text{cyc}}$, $\log P_{\text{alk}}$, and $\log P_{16}$ regressions are rather too large for the PLRP-S system to be very useful as a method of estimating such $\log P$ values.

Lambert et al. [22,23] have used a C_{18} derivatized polystyrene–divinylbenzene phase, Act-I, with 60% methanol: 40% water eluent, as a model for water–alkane partitions. The determined $\log k'$ values are in Table 6, together with the necessary descriptors, and for 48 of the 49 solutes we find:

$$\begin{aligned} \log k'(60\% \text{ MeOH})\text{Act-I} &= 0.012 + 0.399R_2 \\ &- 0.655\pi_2^H - 0.925\sum\alpha_2^H - 2.560\sum\beta_2^H + 2.508V_x \end{aligned} \quad (7)$$

$$n = 48, r = 0.9885, \text{SD} = 0.125, F = 358$$

The regression equation using $\sum\beta_2^O$ is almost the same. We did not include benzotrifluoride because we have been unable to assign descriptors. This is not helped by the divergence in the recorded values of $\log P_{\text{oct}}$, viz. 2.91 and 3.64, with 4.12 as the calculated CLOGP value [33]. Summaries of the coefficients and their ratios are in Tables 3 and 4. These suggest that the Act-I phase should be just as good a model for water–alkane partitions, as for water–octanol partitions. Regression equations based on Eq. (1) are in Table 5. The correlation coefficient and *F*-statistic are marginally better for the alkane partitions than for the octanol partition, but the *sd* value is much worse in the case of water–alkane partition (due to the larger slope in Eq. (1)). This means that $\log P_{\text{cyc}}$, for example, cannot be determined to better than 0.43 log units. The Act-I system is technically better than the PLRP-S system because (i) the $\log k'$ values fall in a more convenient range, and (ii) the $\log k'$ values do not have to be

extrapolated to give $\log k_w$. The Act-I system is therefore useful for the rapid determination of water–alkane partition coefficients with the proviso that the $\log P$ values may be in error by some 0.4 log units.

We have also studied a poly(styrene–divinylbenzene) column, PRP-1 (Hamilton, 15 cm; 5 μm particle size), with various eluents, buffered so that only the neutral species was present. $\log k'$ values are in Table 7; the descriptors for the solutes used have nearly all been given before [16,27,29–32]. When applied to the various $\log k'$ values, Eq. (3) yields:

$$\begin{aligned} \log k'(100\% \text{ MeOH})\text{PRP} - 1 &= -0.407 + 0.459R_2 \\ &+ 0.061\pi_2^H - 1.208\sum\alpha_2^H - 0.886\sum\beta_2^H \\ &+ 0.658V_x \end{aligned} \quad (8)$$

$$n = 45, r = 0.9820, \text{SD} = 0.081, F = 211$$

$$\begin{aligned} \log k'(80\% \text{ MeOH})\text{PRP} - 1 &= -0.225 + 0.451R_2 \\ &- 0.055\pi_2^H - 1.091\sum\alpha_2^H - 1.705\sum\beta_2^H + 1.461V_x \end{aligned} \quad (9)$$

$$n = 28, r = 0.9734, \text{SD} = 0.112, F = 80$$

$$\begin{aligned} \log k'(100\% \text{ MeCN})\text{PRP} - 1 &= -0.441 \\ &+ 0.449R_2 - 0.256\pi_2^H - 0.463\sum\alpha_2^H - 0.681\sum\beta_2^H \\ &+ 0.389V_x \end{aligned} \quad (10)$$

$$n = 43, r = 0.9827, \text{SD} = 0.061, F = 209$$

$$\begin{aligned} \log k'(60\% \text{ MeCN})\text{PRP} - 1 &= 0.020 + 0.326R_2 \\ &- 0.123\pi_2^H - 0.892\sum\alpha_2^H - 1.710\sum\beta_2^H + 1.181V_x \end{aligned} \quad (11)$$

$$n = 43, r = 0.9889, \text{SD} = 0.087, F = 328$$

The coefficients and their ratios are summarised in Tables 3 and 4, and from inspection of the ratios, we selected the systems with 80% methanol and 60% acetonitrile as the best models for water–alkane partitions.

Summaries of correlations using Eq. (1) are in Table 5. The system with 80% methanol eluent is much closer to water–alkane than to water–octanol

Table 6
Solute descriptors and log k' values for the Act-I column with 60% methanol mobile phase

Solute	R_2	π_2^H	$\Sigma\alpha_2^H$	$\Sigma\beta_2^H$	V_x	log k'
Nitromethane	0.313	0.95	0.06	0.31	0.4237	-0.15
Nitroethane	0.270	0.95	0.02	0.33	0.5646	0.20
1-Nitrobutane	0.227	0.95	0.00	0.29	0.8464	0.83
1-Nitrohexane	0.203	0.95	0.00	0.29	1.1282	1.51
Benzene	0.610	0.52	0.00	0.14	0.7164	1.41
Toluene	0.601	0.52	0.00	0.14	0.8573	1.77
Ethylbenzene	0.613	0.51	0.00	0.15	0.9982	2.13
Propylbenzene	0.604	0.50	0.00	0.15	1.1391	2.37
Biphenyl	1.360	0.99	0.00	0.26	1.3242	2.60
Benzotrifluoride	0.225	0.48	0.00	0.11	0.9100	1.63
Chlorobenzene	0.718	0.65	0.00	0.07	0.8288	1.76
Benzyl chloride	0.821	0.82	0.00	0.33	0.9797	1.71
Anisole	0.708	0.75	0.00	0.29	0.9160	1.38
4-Methylanisole	0.699	0.77	0.00	0.30	1.0569	1.67
4-Ethylanisole	0.728	0.80	0.00	0.30	1.1978	1.98
4-Phenylanisole	1.460	1.27	0.00	0.46	1.5238	2.64
Benzylmethylether	0.645	0.77	0.00	0.48	1.0570	1.08
Benzaldehyde	0.820	1.00	0.00	0.39	0.8730	0.81
Phenylacetaldehyde	0.755	0.70	0.00	0.64	1.0139	0.86
Acetophenone	0.818	1.01	0.00	0.48	1.0139	0.85
4-Methylacetophenone	0.842	1.00	0.00	0.52	1.1548	1.09
4-Ethylacetophenone	0.854	1.00	0.00	0.53	1.2957	1.42
4-Phenylacetophenone	1.570	1.53	0.00	0.60	1.6217	2.08
Ethylphenylketone	0.804	0.95	0.00	0.51	1.1548	1.34
Benzylmethylketone	0.748	0.90	0.00	0.66	1.1548	0.84
Benzophenone	1.447	1.50	0.00	0.50	1.4808	2.03
Methyl benzoate	0.733	0.85	0.00	0.46	1.0726	1.33
Ethyl benzoate	0.689	0.85	0.00	0.46	1.2135	1.60
Butyl benzoate	0.668	0.80	0.00	0.46	1.4953	2.29
Phenyl acetate	0.661	1.13	0.00	0.54	1.0726	0.97
Benzyl acetate	0.798	1.06	0.00	0.65	1.2135	1.29
Methyl phenylacetate	0.703	1.13	0.00	0.58	1.2135	1.25
Benzonitrile	0.742	1.11	0.00	0.33	0.8711	0.83
Phenylacetonitrile	0.751	1.15	0.00	0.45	1.0120	0.86
Aniline	0.955	0.96	0.26	0.41	0.8162	0.29
N,N-Dimethylaniline	0.957	0.81	0.00	0.41	1.0980	1.49
Benzylamine	0.829	0.88	0.10	0.72	0.9570	0.13
1-Amino-2-phenylethane	0.824	0.87	0.32	0.72	1.0980	0.32
Nitrobenzene	0.871	1.11	0.00	0.28	0.8906	1.19
Benzamide	0.990	1.50	0.49	0.67	0.9728	-0.24
N-Benzylacetamide	0.980	1.80	0.32	0.75	1.2546	0.00
Phenylacetamide	0.950	1.27	0.44	0.89	1.1137	-0.24
Acetanilide	0.870	1.40	0.50	0.67	1.1133	0.08
Benzoic acid	0.730	0.90	0.59	0.40	0.9317	0.52
Phenylacetic acid	0.730	0.97	0.60	0.61	1.0726	0.42
Phenol	0.805	0.89	0.60	0.30	0.7751	0.30
Benzyl alcohol	0.803	0.87	0.39	0.56	0.9160	0.15
Pyridine	0.631	0.84	0.00	0.52	0.6753	-0.03
Benzotrichloride	1.005		0.00		1.2240	1.29

Table 7

Values of log k' for solutes on a PRP-1 stationary phase with methanol, aqueous methanol, acetonitrile and aqueous acetonitrile mobile phases

Solute	% MeOH, v/v		% MeCN, v/v	
	100	80	100	60
2-Methylbuta-1,3-diene			-0.141	
Propanone	-0.412	-0.271	-0.602	
Butanone	-0.192			
Pentan-2-one		0.231		
Octan-2-one	0.144			
Dimethylformamide	-0.368	-0.340		-0.415
Dimethylacetamide	-0.317			-0.301
Methanol			-0.845	
Dimethylsulfoxide	-0.222			
Benzene	0.271		-0.158	0.770
Toluene	0.379		-0.054	0.932
Ethylbenzene	0.444		-0.077	1.114
<i>o</i> -Xylene	0.544		-0.025	1.120
<i>m</i> -Xylene			0.012	
<i>p</i> -Xylene	0.594			1.195
Propylbenzene	0.477		-0.030	
1,2,3-Trimethylbenzene	0.641		0.006	1.162
Butylbenzene	0.586		0.046	
1,2,3,4-Tetramethylbenzene	0.561		0.095	
Hexylbenzene	0.799		0.227	
Phenylacetylene	0.269		-0.191	
Biphenyl	0.956		0.180	
Naphthalene	0.768		0.194	1.129
Indene	0.658		0.056	
Fluorene	1.142		0.371	
Anthracene			0.617	1.941
1,2-Dichlorobenzene	0.523		0.168	1.313
2-Chlorotoluene	0.555		0.109	1.227
3-Chlorotoluene	0.506		0.084	
4-Chlorotoluene	0.457		0.084	
1,4-Dibromobenzene			0.342	1.483
2-Bromotoluene			0.172	1.316
3-Bromotoluene			0.180	
Iodobenzene			0.227	1.270
Anisole	0.428	0.979		
2-Methylanisole	0.496			
1,3-Dimethoxybenzene	0.540	1.163		0.624
1,4-Dimethoxybenzene		0.898		0.690
Acetophenone				0.466
Benzophenone				1.104
Benzonitrile				0.580
Aniline			-0.430	0.232
N-Methylaniline			-0.146	0.626
N,N-Dimethylaniline			0.000	0.944
N,N-Diethylaniline			0.089	
Nitrobenzene		0.924	-0.301	0.699
3-Nitrotoluene			-0.179	
4-Nitrotoluene	0.444	1.150	-0.195	

Table 7. Continued

Solute	% MeOH, v/v		% MeCN, v/v	
	100	80	100	60
Benzamide			−0.621	−0.222
N,N-Dimethylbenzamide		0.219		−0.017
Benzoic acid			−0.483	
Phenol	−0.350	0.089		0.055
<i>m</i> -Cresol	−0.315	0.249	−0.477	0.139
2,3-Dimethylphenol				0.363
2,4,6-Trimethylphenol				0.622
3-Ethylphenol	−0.222	0.409	−0.410	0.325
2-Isopropylphenol	−0.176	0.544	−0.339	0.516
3-Isopropylphenol	−0.180	0.519		0.340
4-Fluorophenol			−0.560	0.031
3-Chlorophenol			−0.340	0.285
4-Nitrophenol			−0.544	
Catechol		−0.342		
Benzyl alcohol			−0.324	−0.032
Methylphenylsulfide	0.647	1.388		
Pyridine	−0.140	0.038		−0.111
3-Methylpyridine	−0.062	0.240		0.049
4-Methylpyridine	−0.064	0.202		0.000
3,4-Dimethylpyridine	0.028	0.356		
3,5-Dimethylpyridine	0.125	0.356		0.190
2,4,6-Trimethylpyridine		0.054	0.490	
3-Ethylpyridine	0.054	0.301		
4-Ethylpyridine	0.029	0.394		0.193
Pyrrole	−0.398	0.019		0.076
N-Methylpyrrole		0.350		
Indole	0.000	0.623	−0.289	0.615

partition, as judged from the correlation coefficients. However, the very large slope in Eq. (1) when $\log P_{\text{alk}}$ is the dependent variable leads to a large *sd* value of 0.48 log units, so that again we find a RP-HPLC system that resembles water–alkane, and yet can only be used to obtain rough estimates of $\log P_{\text{alk}}$. The system with 60% acetonitrile eluent is slightly better, and, indeed is as good as the Act-I column with 60% methanol eluent or the PLRP-S column with $\log k_w$.

Our conclusion as to the possibility of using RP-HPLC systems as a rapid method of determining $\log P_{\text{cyc}}$, $\log P_{\text{alk}}$ or $\log P_{16}$ is that there is no advantage to be gained from the use of k_w values with the PLRP-S column, and the best that can be done is through the use of the Act-I column with 60% methanol eluent or the PRP-1 column with 60% acetonitrile eluent. Both of these will lead to estimates of the three log P values to around 0.45 log units.

3.2. Immobilised artificial membrane phases, IAM

Another new type of RP-HPLC stationary phase is based on immobilized artificial membranes, IAMs [35], and has been used to obtain lipophilicity parameters [36–40]. As pointed out by Kaliszan et al. [37], lipophilicity scales obtained from $\log k'$ on IAMs did not correlate well with lipophilicity taken as $\log P_{\text{oct}}$. Ong et al. [40] also found that $\log k'$ on an IAM phase correlated only poorly with $\log P_{\text{oct}}$ for 22 varied solutes. Since no analysis of the solute factors that influence $\log k'$ values on IAM columns has yet been reported, the reasons for this lack of correlation are not known. We therefore determined $\log k'$ values with the IAM column of Nasal et al. [39] for a set of solutes, in order to rectify this situation. The column is a commercially available IAM.PC.MG column (150-mm length, 4.6 mm I.D., particle diameter 12 μm , and pore diameter 300 \AA) from Regis (Morton Grove, IL, USA) formed by

1-myristoyl-2-[(13-carboxyl)-tridecoyl]-sn-3-glycero-phosphocholine(licithin-CO₂H) bonded to silica-propylamine with unreacted propylamine end-capped with methylglycolate; it was used with a mobile phase of 10% acetonitrile: 90% aqueous buffer at pH 7. The determined log *k'* values are in Table 8, and application of Eq. (3) leads to:

$$\log k'(10\% \text{ MeCN})\text{IAM} = -1.037 + 0.808R_2 - 0.424\pi_2^H + 0.690\sum\alpha_2^H - 1.998\sum\beta_2^H + 1.872V_x \quad (12)$$

$$n = 27, r = 0.9928, \text{SD} = 0.124, F = 287$$

For the solutes used, $\sum\beta_2^H$ and $\sum\beta_2^O$ are the same. The regression equation is quite good, and the coefficients and their ratios are in Tables 3 and 4. Eq. (12) shows that solute dipolarity and hydrogen-bond basicity lead to lower values of log *k'*, but that solute

Table 8

Values of log *k'* for solutes on an IAM.PC.MG column with 10% acetonitrile mobile phase

Compound	log <i>k'</i>
Hexachlorobutadiene	1.998
Cyclohexanone	-0.607
Benzene	0.093
Toluene	0.436
Hexylbenzene	2.056
1,3,5-Trisopropylbenzene	2.428
Naphthalene	1.330
Chlorobenzene	0.655
Methylphenylether	0.310
Acetophenone	0.290
Benzonitrile	0.154
1,4-Dinitrobenzene	0.157
Acetanilide	0.177
Benzamide	-0.099
Phenol	0.366
4-Chlorophenol	1.124
3,5-Dichlorophenol	1.895
4-Iodophenol	1.593
3-Trifluoromethylphenol	1.234
4-Cyanophenol	0.771
Benzyl alcohol	-0.004
N-Methyl-2-pyrrolidinone	-1.497
Indazole	0.710
Caffeine	-0.396
Dibenzothiophene	2.132
Cortisone	0.761
Hydrocortisone	0.939

polarizability, hydrogen-bond acidity, and volume all lead to an increase in log *k'*. Thus the IAM phase is less dipolar and less hydrogen-bond acidic than 10% acetonitrile, but is more polarizable, more hydrogen-bond basic and more hydrophobic than the mobile phase. It is quite unusual for RP-HPLC stationary phases to be appreciably stronger hydrogen-bond bases than aqueous organic mobile phases, see the *a*-coefficients in Table 3, and so this particular IAM phase has rather different properties to C₁₈ or poly(styrene-divinylbenzene) phases.

From the coefficient ratios in Table 4, log *k'* values in the IAM system are not likely to be well correlated with either log *P*_{oct} or with log *P*_{alk}; in the former comparison, there are differences in the *a/v* and *b/v* ratios, and in the latter comparison there is a very large difference in the *a/v* ratio, as well as differences in the *s/v* and *b/v* ratios. Details of regressions of log *k'* against the various log *P* values are in Table 5. These confirm that if the particular IAM system we have studied is used for the determination of lipophilicity of solutes, this lipophilicity scale will not be the same as either log *P*_{oct} or log *P*_{alk}. We can show how the coefficient ratios can be used to interpret the various regressions of log *P* against log *k'* by using the log *P*_{alk} vs log *k'* (IAM) regression as an example. The various ratios for these two dependent variables in Eq. (3) are:

Variable	<i>r/v</i>	<i>s/v</i>	<i>a/v</i>	<i>b/v</i>	<i>v/v</i>
log <i>P</i> _{alk}	0.15	-0.39	-0.82	-1.13	1
log <i>k'</i> (IAM)	0.43	-0.23	0.37	-1.07	1

Hence in correlations of log *P*_{alk} vs log *k'*(IAM), compounds with large $\sum\alpha_2^H$ values will not fit at all, and compounds with large $\sum\beta_2^H$ values (nearly all of which also have reasonably large π_2^H values) will deviate in the same direction, but to a lesser extent. If we select the 10 compounds in Table 8 with the smallest $\sum\alpha_2^H$ and $\sum\beta_2^H$ values, we can obtain a 'baseline correlation'

$$\log P_{\text{alk}} = 1.46 + 1.95 \log k'(10\% \text{ MeCN})\text{IAM} \quad (13)$$

$$n = 10, r = 0.9208, \text{SD} = 0.66, F = 45$$

and then show how acidic and basic compounds

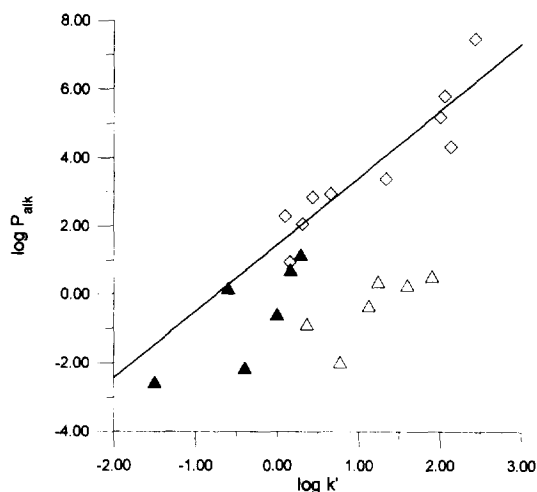


Fig. 1. Plot of $\log P_{\text{alk}}$ vs $\log k'$ (10% methanol) IAM. The 10 solutes used to obtain Eq. (13) are denoted as (\diamond); solutes with large values of $\Sigma\alpha_2^H$ are shown as (Δ); solutes with large values of $\Sigma\beta_2^H$ are shown as (\blacktriangle).

deviate in accord with our deductions using correlation coefficient ratios. This is clearly seen in Fig. 1, where the strongly acidic compounds are way off the 'baseline' given by Eq. (13), and strongly basic (and dipolar) compounds deviate to a lesser extent.

3.3. Dipalmitoyl phosphatidylcholine coated silica phase, DPC

Some years before IAM phases were investigated using RP-HPLC, Miyake et al. [41] prepared a new stationary phase by physically coating silica gel with dipalmitoyl phosphatidylcholine, DPC. Although such a phase would not be expected to emulate properties of biological membranes [37], it might still be of interest in the determination of lipophilicity. Indeed, Miyake et al. [41] showed that $\log k'$ values obtained with 10–30% aqueous acetonitrile correlated well with $\log P_{\text{oct}}$ for a variety of aromatic solutes. Analysis of the reported [41] $\log k'$ values through Eq. (3) yielded the following equations:

$$\begin{aligned} \log k'(10\% \text{ MeCN})\text{DPC} = & -1.220 + 0.492R_2 \\ & -0.441\pi_2^H + 0.029\Sigma\alpha_2^H - 2.757\Sigma\beta_2^H + 2.680V_x \end{aligned} \quad (14)$$

$$n = 43, r = 0.9754, \text{SD} = 0.106, F = 145$$

$$\begin{aligned} \log k'(20\% \text{ MeCN})\text{DPC} = & -1.084 + 0.572R_2 \\ & -0.502\pi_2^H - 0.006\Sigma\alpha_2^H - 2.589\Sigma\beta_2^H + 2.245V_x \end{aligned} \quad (15)$$

$$n = 46, r = 0.9809, \text{SD} = 0.096, F = 203$$

$$\begin{aligned} \log k'(30\% \text{ MeCN})\text{DPC} = & -1.248 + 0.311R_2 \\ & -0.504\pi_2^H - 0.047\Sigma\alpha_2^H - 1.635\Sigma\beta_2^H + 1.643V_x \end{aligned} \quad (16)$$

$$n = 36, r = 0.9716, \text{SD} = 0.068, F = 101$$

The coefficients are summarised in Table 3, and the coefficient ratios are in Table 4. For all three regressions, Eqs. (14–16), the coefficient ratios are remarkably similar to those for the $\log P_{\text{oct}}$ regression, see Table 1, first row. There is no point in carrying out regressions of $\log P_{\text{alk}}$ against the DPC $\log k'$ values, because the very different a/v ratios preclude any substantial correlation. However, we give in Table 5 a summary of the regression equations using $\log P_{\text{oct}}$ as the dependent variable, for the exact set of solutes used in Eqs. (14–16). There are minor differences to the regressions of $\log P_{\text{oct}}$ against $\log k'$ given by Miyake et al. [41], but the equations listed in Table 5, and those given originally [41], show that the RP-HPLC system of Miyake et al. [41] is an excellent match to water–octanol partition coefficients, exactly as indicated by the coefficient ratios of Eqs. (14–16).

It is interesting to compare the coefficients and their ratios for the DPC phase and the IAM phase, both at 10% acetonitrile. The most outstanding difference is in the a -coefficient and the a/v ratio, which indicate that the IAM phase is appreciably more basic (in the hydrogen-bond sense) than is the DPC phase. This may be due to structural features of the two phases. In the DPC phase, the phospholipid physically adsorbed on silica is probably a rather disordered arrangement of multilayers of lipid molecules, whereas the chemically bonded phospholipid molecules in the IAM phase may form a more ordered arrangement with the polar phosphatidylcholine head groups pointing away from the hydro-

carbon chains and solvated by the mobile phase. Even when solvated, the head groups will act as strong hydrogen-bond bases, they will interact with solute hydrogen-bond acids, and hence give rise to a positive a -coefficient.

4. Conclusions

In conclusion, we confirm the suggestion of Pagliara et al. [10] that the C_{18} -ABZ column with k' values at 40% methanol eluent or with k_w values is a good model for $\log P_{\text{oct}}$, and the earlier suggestion of Miyake et al. [41] that the DPC column with 10–30% acetonitrile eluent is a good model for $\log P_{\text{oct}}$. These two sets of systems are possibly the best RP-HPLC models for lipophilicity based on $\log P_{\text{oct}}$ yet found.

The recently studied phases based on poly-(styrene-divinylbenzene), i.e., PLRP-S, Act-I, and PRP-1, all yield $\log k'$ or $\log k_w$ values that correlate quite well with the three sets of water-alkane $\log P$ values, as shown in Table 5. However, the slopes of the correlations using Eq. (1) are very large, from 1.5 up to 3.4, so that any error in $\log k'$ or $\log k_w$ is magnified in the determination of $\log P_{\text{alk}}$. This means that lipophilicity determinations, as $\log P_{\text{alk}}$, will be subject to possible errors of 0.4–0.5 log unit, thus limiting the usefulness of the RP-HPLC systems as rapid methods for the determination of this particular scale of lipophilicity.

We can also confirm the analysis of Kaliszan et al. [37] who noted that lipophilicity obtained using an IAM column would not correspond to the $\log P_{\text{oct}}$ lipophilicity scale, and we have extended this analysis by showing that the IAM lipophilicity does not correspond to the $\log P_{\text{alk}}$ or $\log P_{\text{cyc}}$ scales, either.

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References

- [1] C. Hansch, P.P. Maloney, T. Fujita and R.M. Muir, *Nature*, 194 (1962) 178.
- [2] C. Hansch and T. Fujita, *J. Am. Chem. Soc.*, 86 (1964) 1616.
- [3] A. Leo, C. Hansch and D. Elkins, *Chem. Rev.*, 71 (1971) 525.
- [4] A. Nahum and Cs. Horváth, *J. Chromatogr.*, 192 (1980) 315.
- [5] A. Tsantili-Kakoulidou, N. El Tayar, H. van de Waterbeemd and B. Testa, *J. Chromatogr.*, 389 (1987) 33.
- [6] D.J. Minick, D.A. Brent and J. Frenz, *J. Chromatogr.*, 461 (1989) 177.
- [7] A. Bechalany, T. Rothlisberger, N. El Tayar and B. Testa, *J. Chromatogr.*, 473 (1989) 115.
- [8] C. Yamagami and N. Takao, *Chem. Pharm. Bull.*, 40 (1992) 123.
- [9] P. Vallat, W. Fan, N. El Tayar, P.-A. Carrupt and B. Testa, *J. Liq. Chromatogr.*, 15 (1992) 2133.
- [10] A. Pagliara, E. Khamis, A. Trinh, P.-A. Carrupt, R.-S. Tsai and B. Testa, *J. Liq. Chromatogr.*, 18 (1995) 1721.
- [11] K. Dross, C. Sonntag and R. Mannhold, *J. Chromatogr. B*, 673 (1994) 113.
- [12] Y. Ishihama, Y. Oda, K. Uchikawa and N. Asakawa, *Anal. Chem.*, 67 (1995) 1588.
- [13] B.J. Herbert and J.G. Dorsey, *Anal. Chem.*, 67 (1995) 744.
- [14] M. Adlard, G. Okafo, E. Meenan and P. Camilleri, *J. Chem. Soc. Chem. Commun.*, (1995) 2241.
- [15] J.T. Smith and D.V. Vinjamoori, *J. Chromatogr. B*, 669 (1995) 59.
- [16] M.H. Abraham, H.S. Chadha and A. Leo, *J. Chromatogr. B*, 685 (1994) 203.
- [17] M.J. Kamlet, R.M. Doherty, J.-L.M. Abboud, M.H. Abraham and R.W. Taft, *CHEMTECH*, 16 (1986) 566.
- [18] M.H. Abraham, R.M. Doherty, M.J. Kamlet and R.W. Taft, *Chem. Br.*, 22 (1986) 551.
- [19] P. Seiler, *Eur. J. Med. Chem.*, 9 (1974) 473.
- [20] R.C. Young, R.C. Mitchell, T.H. Brown, C.R. Ganellin, R. Griffiths, M. Jones, K.K. Rana, D. Saunders, I.R. Smith, N.E. Sore and T.J. Wilks, *J. Med. Chem.*, 31 (1988) 656.
- [21] A. Leo, *Chem. Rev.*, 93 (1993) 1281.
- [22] W.J. Lambert and L.A. Wright, *J. Chromatogr.*, 464 (1989) 400.
- [23] W.J. Lambert, L.A. Wright and J.K. Stevens, *Pharm. Res.*, 7 (1990) 577.
- [24] W.I. Lambert, *J. Chromatogr. A*, 656 (1993) 469.
- [25] D.P. Lee, *J. Chromatogr. Sci.*, 20 (1982) 203.
- [26] D.E. Leahy, *J. Pharm. Sci.*, 75 (1986) 629.
- [27] M.H. Abraham, *Chem. Soc. Rev.*, 22 (1993) 73.
- [28] M.H. Abraham and J.C. McGowan, *Chromatographia*, 23 (1987) 243.
- [29] M.H. Abraham, *J. Phys. Org. Chem.*, 6 (1993) 660.
- [30] M.H. Abraham and H.S. Chadha, in *Lipophilicity in Drug Action and Toxicology*, V. Pliska, H. van de Waterbeemd and B. Testa (Editors), VCH, Weinheim, Germany, 1996.

- [31] M.H. Abraham and M. Roses, *J. Phys. Org. Chem.*, 7 (1994) 672.
- [32] M.H. Abraham, M. Roses, C.F. Poole and S.K. Poole, *J. Phys. Org. Chem.*, in press.
- [33] A. Leo and C. Hansch, *The Pomona Medicinal Chemistry Project*, Pomona, Claremont, CA 91711.
- [34] M.H. Abraham, H.S. Chadha, G.S. Whiting and R.C. Mitchell, *J. Pharm. Sci.*, 83 (1994) 1085.
- [35] C. Pidgeon and U.V. Venkatarum, *Anal. Biochem.*, 176 (1989) 36.
- [36] K.M.R. Kallury, W.E. Lee and M. Thompson, *Anal. Chem.*, 64 (1992) 1062.
- [37] R. Kaliszan, A. Kaliszan and I.W. Wainer, *J. Pharm. Biomed. Sci.*, 11 (1993) 505.
- [38] C. Pidgeon, S. Ong, H. Choi and H. Lui, *Anal. Chem.*, 66 (1994) 2701.
- [39] A. Nasal, M. Sznitowska, A. Bucinski and R. Kaliszan, *J. Chromatogr. A*, 692 (1995) 83.
- [40] S. Ong, H. Lui, X. Qui, G. Bhat and C. Pidgeon, *Anal. Chem.*, 34 (1995) 755.
- [41] K. Miyake, F. Kitaura, N. Mizuno and H. Terada, *J. Chromatogr.*, 389 (1987) 47.