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Measurement of lipophilicity indices by reversed-phase high-performance liquid chromatography: comparison of two stationary phases and various eluents

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ABSTRACT

Twenty-eight benzene derivatives spanning a broad range of lipophilicities were used as model compounds to examine the optimum stationary phase and eluent conditions for the determination of lipophilic indices by reversed-phase high-performance liquid chromatography. This was assessed by linear regressions comparing published octanol–water partition coefficients with isocratic capacity factors and capacity factors extrapolated to 100% water in the eluent. Methanol–water eluents are always to be preferred to acetonitrile–water and tetrahydrofuran–water eluents. The octadecylsilane (ODS) phase yielded good correlations especially when a masking agent was added to the eluent, but this introduced an additional experimental variable. The octadecyl–polyvinyl copolymer (ODP) phase was just as satisfactory as the ODS phase without the need for a masking agent, and thus appears to be a valuable alternative.

INTRODUCTION

Lipophilicity, a medicinally relevant physico-chemical property, plays an influential role in many biological processes and therefore finds numerous applications in quantitative structure–activity relationship (QSAR) studies [1–4].

Partition coefficients have been measured in nearly 100 solvent–water systems, mainly by means of the traditional shake-flask method [2]. *n*-Octanol–water is widely accepted as the reference system because of its analogy with biomembranes [3]. However, practical disadvantages and the limitation to log P values between -2 and $+4$ have led researchers to investigate other methods for measuring lipophilicity [4,5]. In recent years, reversed-phase high-performance liquid chromatography (RP-HPLC) has become a popular alternative, capacity factors frequently being used as substitutes for octanol–water partition coefficients in QSAR studies.

The measurement of lipophilicity by RP-HPLC is based on the principle of the partition of a solute between a polar eluent and a stationary phase of low polarity.

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Under most conditions pure water cannot be used as the eluent and an organic modifier must be added to shorten the retention of solutes.

In order to suppress the effect of the organic modifier and to establish lipophilicity indices independent of eluent conditions the isocratic capacity factors determined at different organic modifier to water ratios are extrapolated to 100% water, yielding log k_w values [6,7]. Generally, the extrapolation is based on a quadratic relationship between isocratic capacity factors and the volume fraction x of the organic modifier [8,9]. When methanol is used as the organic modifier, a linear relationship is obtained for many solutes over a wide range of volume fractions; exceptions include very polar compounds such as caffeine or protonated bases [10]. Deviations from linearity have been attributed to silanophilic interactions, conformational changes of the solute, organic modifier absorbed on the stationary phase or changes in ionization in the case of ionizable solutes [11]. Further, methanol is unique among organic modifiers as it provides a strong hydrogen bond donor and acceptor capability and thus does not markedly alter the hydrogen-bonded network of water or affect polar interactions of solutes [12]. However, as methanol leads to inconveniently long retention times for the more lipophilic solutes, acetonitrile and tetrahydrofuran have also been used as organic modifiers in order to reduce retention times and to broaden the lipophilicity range measurable by RP-HPLC [13].

Octadecylsilane (ODS) is the most frequently used lipophilic stationary phase. This type of column, however, possesses a high proportion of free silanol groups which induce silanophilic interactions with basic and other polar compounds. This adsorption mechanism severely affects the partition behaviour of solutes between the eluent and the stationary phase; the addition of a masking agent such as *n*-decylamine or N,N-dimethyloctylamine to the mobile phase may decrease [14,15] but not necessarily suppress [16] such interactions. Unfortunately, a masking agent introduces an additional variable into the conditions owing to its own selective effect on retention [17]. In addition, its applicability is limited, as it cannot be used with acidic compounds owing to ion pair formation.

Recently, an octadecyl–polyvinyl copolymer [ODP, a poly(vinyl alcohol) gel esterified with octadecanoyl groups] has become available; being devoid of silanophilic interactions, it was shown to provide a valuable alternative as a stationary phase in lipophilicity measurements [18–20].

To assess better the relative merits of the ODS and ODP phases, we compared their performance under a variety of eluent conditions. Specifically, three organic modifiers were used, namely methanol as a hydrogen bond donor and acceptor, acetonitrile as a hydrogen bond acceptor of high polarity and tetrahydrofuran (THF) as a less polar hydrogen bond acceptor. The effects of *n*-decylamine as a masking agent of silanol groups were also investigated with each organic modifier in conjunction with the ODS stationary phase. Extrapolated lipophilicity indices (log k_w) using methanol as the organic modifier were taken from a previously published study [19]. In the latter, lipophilicity indices were used to compare different stationary phases only.

EXPERIMENTAL

Materials

Monosubstituted benzenes purchased from Fluka (Buchs, Switzerland) and

Merck (Darmstadt, Germany) were of analytical-ragent grade and used without further purification. Methanol, acetonitrile and THF were purchased from Merck and were of adequate purity for HPLC.

Chromatography

A Siemens S 101 chromatograph equipped with an Orlita type DMP-AE 10.4 pump was used. The detector was a Uvikon 760 LC from Kontron operating at 254 nm. A Spectra-Physics SP 4100 computing integrator was used for peak registration and calculation of retention times.

Columns

The ODS column (25 cm × 4 mm I.D.) was prepacked with LiChrosorb RP-18, particle size 10 μm (Knauer, Berlin, Germany). The ODP column (15 cm × 6 mm I.D.) was prepacked with the copolymer gel, particle size 5 μm (Asahi Chemicals, Kawasaki, Japan).

Mobile phase preparation

Mobile phases were made up volumetrically from various combinations of methanol, acetonitrile and THF with a 0.02 *M* 3-morpholinopropanesulphonate buffer (pH 7.4), once with *n*-decylamine (0.2%, v/v) and once without. All solutions were purified by filtration using a Millipore Q system. Retention times, t_r, were obtained at ambient temperature (21 ± 1°C). The flow-rate was adjusted to 1.5 ml/min and the column dead time, t_0, was determined using the organic modifier as the non-retained compound. Capacity factors, log k_i, defined as $\log[(t_r - t_0)/t_0]$, were determined at 4 to 8 different fractions of organic modifier (range 90–10%) and extrapolated to 100% water as the mobile phase to yield log k_w values.

RESULTS AND DISCUSSION

The log k_w values were derived by extrapolation of the isocratic capacity factors, log k_i, and are presented in Tables I–III. Extrapolation was performed linearily when methanol was used as the organic modifier. Quadratic extrapolation was necessary when acetonitrile and THF were the organic modifiers. It should be noted that quadratic extrapolation has drawbacks, particularly when insufficient data are available in water-rich volume fractions. In such instances a possible error in isocratic capacity factors may be amplified, leading to unreliable log k_w values. Such extrapolation errors are apparent for log k_w values of some lipophilic compounds in our series, *e.g.*, the "unreasonably" high log k_w value of benzene obtained using acetonitrile on ODS. Indeed benzene, under these conditions, appears to be more lipophilic than toluene and benzophenone and almost as lipophilic as naphthalene.

To assess the validity of the different sets of log k_w values as lipophilicity indices, it was useful to establish their relationships with the corresponding octanol–water log *P* values obtained from the Hansch and Leo database [21] (Tabel IV). The isocratic capacity factors with organic solvent–water (50:50, v/v) eluents (data not given) were also included in such regression analyses in order to illustrate some limitations of extrapolating to 100% water.

TABLE I

EXTRAPOLATED CAPACITY FACTORS OF 28 MONOSUBSTITUTED BENZENES DETERMINED WITH VARIOUS ORGANIC SOLVENTS USING AN ODS STATIONARY PHASE AND A MASKING AGENT

No.	Compound	Log k_w		
		CH_3OH–D[a]	ACN–D[b]	THF–D[c]
1	Benzenesulphonamide	0.77 ± 0.21	1.43 ± 0.09	0.97 ± 0.06
2	Methyl phenyl sulphone	0.93 ± 0.01	1.13 ± 0.02	0.76 ± 0.03
3	Methyl phenyl sulphoxide	0.77 ± 0.02	1.02 ± 0.05	0.44 ± 0.03
4	Benzamide	0.81 ± 0.04	0.91 ± 0.06	0.73 ± 0.02
5	Aniline	0.95 ± 0.02	1.11 ± 0.03	1.56 ± 0.07
6	Benzyl alcohol	1.05 ± 0.02	1.17 ± 0.03	1.03 ± 0.02
7	Acetanilide	1.17 ± 0.01	1.31 ± 0.04	1.20 ± 0.01
8	2-Phenylethanol	1.42 ± 0.03	1.50 ± 0.07	1.38 ± 0.02
9	Phenol	1.28 ± 0.01	1.45 ± 0.04	1.55 ± 0.05
10	Benzaldehyde	1.54 ± 0.06	1.42 ± 0.08	1.31 ± 0.05
11	Benzonitrile	1.51 ± 0.02	1.76 ± 0.05	1.56 ± 0.05
12	Nitrobenzene	1.70 ± 0.04	2.01 ± 0.06	2.30 ± 0.02
13	N-Methylaniline	1.51 ± 0.02	1.74 ± 0.03	1.93 ± 0.03
14	N,N-Dimethylaniline	2.28 ± 0.01	2.32 ± 0.12	2.50 ± 0.02
15	Phenyl acetate	1.57 ± 0.04	1.87 ± 0.04	1.52 ± 0.04
16	Methyl benzoate	2.15 ± 0.02	2.09 ± 0.09	2.17 ± 0.02
17	Thioanisole	2.72 ± 0.04	2.26 ± 0.09	2.89 ± 0.04
18	Anisole	2.01 ± 0.03	2.22 ± 0.06	2.33 ± 0.27
19	Benzene	1.91 ± 0.04	2.06 ± 0.09	2.31 ± 0.15
20	Fluorobenzene	2.07 ± 0.04	2.28 ± 0.08	2.57 ± 0.02
21	Chlorobenzene	2.72 ± 0.03	2.30 ± 0.08	3.11 ± 0.08
22	Bromobenzene	2.88 ± 0.03	2.78 ± 0.14	3.10 ± 0.07
23	Iodobenzene	3.14 ± 0.04	2.81 ± 0.17	2.69 ± 0.14
24	Toluene	2.62 ± 0.02	2.11 ± 0.13	2.70 ± 0.05
25	Trifluoromethylbenzene	3.11 ± 0.03	2.69 ± 0.10	3.37 ± 0.08
26	Biphenyl	3.92 ± 0.08	3.46 ± 0.57	3.40 ± 0.11
27	Benzophenone	3.45 ± 0.16	2.75 ± 0.19	2.97 ± 0.12
28	Naphthalene	3.29 ± 0.05	2.90 ± 0.21	2.70 ± 0.09

[a] Log k_w (CH_3OH–D) is the lipophilic index extrapolated linearly to 100% water using the ODS column, methanol as the organic solvent and *n*-decylamine as a masking agent. Data from ref. 19.

[b] Log k_w (ACN–D) is the lipophilic index extrapolated quadratically to 100% water using the ODS column, acetonitrile as the organic solvent and *n*-decylamine as a masking agent.

[c] Log k_w (THF–D) is the lipophilic index extrapolated quadratically to 100% water using the ODS column, THF as the organic solvent and *n*-decylamine as a masking agent.

Methanol as organic modifier

Using methanol as the organic modifier and ODS as the stationary phase, eqns. 1 and 2 (Table IV) were established between octanol–water partition coefficients (log *P*) and both extrapolated and isocratic capacity factors (50% methanol).

In these equations the slope is larger than 1 and the intercept is significantly different from 0. The isocratic capacity factors result in a slightly better relationship with log *P* values. Adding *n*-decylamine to the mobile phase as a masking agent led to eqns. 3 and 4. The slope and intercept in eqn. 3 are close to 1 and 0, respectively,

TABLE II

EXTRAPOLATED CAPACITY FACTORS OF 28 MONOSUBSTITUTED BENZENES DETERMINED WITH VARIOUS ORGANIC SOLVENTS USING AN ODS STATIONARY PHASE WITHOUT A MASKING AGENT

Compound No.	Log k_w			Log P^d
	CH_3OH^a	ACN[b]	THF[c]	
1	0.90 ± 0.06	0.89 ± 0.05	0.78 ± 0.02	0.31
2	1.29 ± 0.07	1.34 ± 0.08	0.79 ± 0.02	0.49
3	1.33 ± 0.06	1.28 ± 0.07	0.49 ± 0.07	0.55
4	1.04 ± 0.07	1.00 ± 0.06	0.74 ± 0.02	0.64
5	1.13 ± 0.04	1.12 ± 0.06	1.03 ± 0.02	0.90
6	1.32 ± 0.04	1.27 ± 0.06	1.10 ± 0.02	1.10
7	1.45 ± 0.03	1.44 ± 0.06	1.25 ± 0.02	1.16
8	1.73 ± 0.04	1.66 ± 0.06	1.47 ± 0.01	1.36
9	1.30 ± 0.03	1.27 ± 0.04	1.58 ± 0.05	1.46
10	1.67 ± 0.04	1.71 ± 0.04	1.46 ± 0.02	1.45
11	1.85 ± 0.09	1.74 ± 0.15	1.67 ± 0.04	1.56
12	2.00 ± 0.06	2.07 ± 0.06	2.40 ± 0.04	1.85
13	1.74 ± 0.06	1.79 ± 0.04	2.02 ± 0.08	1.66
14	2.36 ± 0.04	2.33 ± 0.09	2.60 ± 0.07	2.31
15	2.04 ± 0.09	1.89 ± 0.03	1.64 ± 0.04	1.49
16	2.26 ± 0.04	2.34 ± 0.06	2.25 ± 0.10	2.12
17	2.71 ± 0.11	2.87 ± 0.09	3.25 ± 0.10	2.74
18	2.20 ± 0.05	2.11 ± 0.06	2.43 ± 0.02	2.11
19	2.08 ± 0.04	3.05 ± 0.13	2.25 ± 0.14	2.13
20	2.18 ± 0.05	2.16 ± 0.08	2.75 ± 0.06	2.27
21	2.75 ± 0.08	2.86 ± 0.10	3.26 ± 0.09	2.84
22	2.89 ± 0.09	2.59 ± 0.11	2.87 ± 0.23	2.99
23	3.16 ± 0.09	3.09 ± 0.11	3.33 ± 0.12	3.25
24	2.62 ± 0.06	2.79 ± 0.08	2.95 ± 0.08	2.73
25	3.11 ± 0.09	2.58 ± 0.07	3.40 ± 0.10	2.79
26	3.88 ± 0.10	3.52 ± 0.30	3.75 ± 0.13	4.09
27	3.11 ± 0.16	2.64 ± 0.14	2.91 ± 0.07	3.18
28	3.22 ± 0.10	3.11 ± 0.07	2.85 ± 0.39	3.30

[a] Log k_w (CH_3OH) is the lipophilicity index extrapolated linearly to 100% water using an ODS column and methanol as the organic solvent without any masking agent. Data taken from ref. 19.

[b] Log k_w (ACN) is the lipophilicity index extrapolated quadratically to 100% water using an ODS column and acetonitrile as the organic solvent without any masking agent.

[c] Log k_w (THF) is the lipophilicity index extrapolated quadratically to 100% water using an ODS column and tetrahydrofuran as the organic solvent without any masking agent.

[d] Log P is the logarithm of *n*-octanol–water partition coefficient (data from ref. 21).

indicating a slightly hyperdiscriminative capacity of this partition system compared with the octanol–water system. The addition of *n*-decylamine increases the discriminative capability of the HPLC system. This is reflected in the higher extrapolated capacity factors found for some lipophilic compounds. Thus log k_w values determined in this system offer the advantage of being very similar to log P values over a wide range of lipophilicity and may be used directly as substitutes for log P. In this case also isocratic capacity factors lead to a slightly better relationship with log P values (eqn. 4).

TABLE III

EXTRAPOLATED CAPACITY FACTORS OF 28 MONOSUBSTITUTED BENZENES DETERMINED WITH VARIOUS ORGANIC MODIFIERS USING AN ODP STATIONARY PHASE

Compound No.	Log k_w		
	ODP–CH_3OH[a]	ODP–ACN[b]	OD–THF[c]
1	1.12 ± 0.03	1.24 ± 0.12	1.11 ± 0.04
2	1.18 ± 0.05	1.59 ± 0.15	1.20 ± 0.04
3	0.68 ± 0.05	1.09 ± 0.15	0.67 ± 0.14
4	1.24 ± 0.17	1.24 ± 0.09	0.87 ± 0.04
5	1.46 ± 0.07	1.29 ± 0.06	1.47 ± 0.02
6	1.33 ± 0.03	1.29 ± 0.07	1.21 ± 0.01
7	1.52 ± 0.06	1.77 ± 0.14	1.32 ± 0.03
8	1.93 ± 0.14	1.45 ± 0.09	1.44 ± 0.05
9	1.81 ± 0.08	1.33 ± 0.04	1.91 ± 0.06
10	1.74 ± 0.03	2.00 ± 0.17	1.82 ± 0.02
11	2.35 ± 0.15	1.78 ± 0.04	2.05 ± 0.03
12	2.62 ± 0.04	2.01 ± 0.05	2.56 ± 0.09
13	2.26 ± 0.07	1.59 ± 0.07	2.21 ± 0.05
14	2.87 ± 0.07	1.75 ± 0.09	2.62 ± 0.05
15	2.21 ± 0.19	1.80 ± 0.14	1.82 ± 0.04
16	2.48 ± 0.04	1.72 ± 0.14	2.21 ± 0.05
17	3.23 ± 0.07	2.16 ± 0.21	2.87 ± 0.05
18	2.46 ± 0.03	1.83 ± 0.10	2.26 ± 0.07
19	2.40 ± 0.05	1.75 ± 0.16	2.44 ± 0.11
20	2.93 ± 0.13	1.56 ± 0.09	2.50 ± 0.11
21	3.25 ± 0.14	1.85 ± 0.14	2.77 ± 0.10
22	3.64 ± 0.15	1.90 ± 0.15	3.00 ± 0.13
23	3.89 ± 0.22	2.06 ± 0.10	3.22 ± 0.14
24	3.25 ± 0.14	2.13 ± 0.19	2.85 ± 0.09
25	3.68 ± 0.25	1.83 ± 0.16	2.88 ± 0.09
26	4.63 ± 0.14	2.28 ± 0.13	3.43 ± 0.12
27	3.71 ± 0.17	1.94 ± 0.09	2.78 ± 0.11
28	3.94 ± 0.21	2.03 ± 0.15	3.09 ± 0.09

[a] Log k_w (ODP–CH_3OH) is the lipophilicity index extrapolated linearly to 100% water using an ODP stationary phase and methanol as the organic solvent. Data from ref. 19.

[b] Log k_w (ODP–ACN) is the lipophilicity index extrapolated quadratically to 100% water using an ODP column and acetonitrile as the organic modifier.

[c] Log k_w (ODP–THF) is the lipophilicity index extrapolated quadratically to 100% water using an ODP column and tetrahydrofuran as the organic solvent.

Using ODP as the stationary phase, similar equations were obtained; the slope and intercept in eqns. 5 and 6 indicate that the log k values obtained in this system are larger than the corresponding log P values. Unlike eqns. 2 and 4, the isocratic capacity factors do not lead to an improved correlation with log P values, presumably because measurements at low methanol fractions are not possible owing to the high hydrophobicity of the ODP phase.

TABLE IV

RELATIONSHIP BETWEEN LIPOPHILICITY INDICES DETERMINED BY RP-HPLC AND THE SHAKE-FLASK METHOD

n is the number of compounds in the analysis, r is the correlation coefficient, s is the standard deviation of the equation and F is the Fischer test.

(A) Methanol as the organic modifier				
(A1) ODS as the stationary phase, no masking agent				
$\log P = 1.23(\pm 0.05)\log k_w - 0.65(\pm 0.12)$	(1)	$\log P = 1.71(\pm 0.05)\log k_{50} + 0.95(\pm 0.04)$	(2)	
$n = 28, r = 0.976, s = 0.216, F = 532$		$n = 28, r = 0.988, s = 0.156, F = 1060$		
(A2) ODS stationary phase using n-decylamine as a masking agent				
$\log P = 0.91(\pm 0.03)\log k_w + 0.18(\pm 0.12)$	(3)	$\log P = 1.67(\pm 0.04)\log k_{50} + 1.05(\pm 0.03)$	(4)	
$n = 28, r = 0.983, s = 0.181, F = 763$		$n = 28, r = 0.993, s = 0.118, F = 1830$		
(A3) ODP stationary phase				
$\log P = 0.94(\pm 0.04)\log k_w - 0.35(\pm 0.22)$	(5)	$\log P = 1.57(\pm 0.07)\log k_{50} + 0.39(\pm 0.09)$	(6)	
$n = 28, r = 0.978, s = 0.206, F = 573$		$n = 28, r = 0.980, s = 0.209, F = 736$		
(B) Acetonitrile as the organic modifier				
(B1) ODS stationary phase, no masking agent				
$\log P = 1.33(\pm 0.07)\log k_w + 0.78(\pm 0.14)$	(7)	$\log P = 2.43(\pm 0.13)\log k_{50} + 0.73(\pm 0.08)$	(8)	
$n = 27^a, r = 0.971, s = 0.245, F = 408$		$n = 28, r = 0.963, s = 0.268, F = 337$		
(B2) ODS stationary phase using n-decylamine as a masking agent				
$\log P = 1.41(\pm 0.08)\log k_w - 0.80(\pm 0.17)$	(9)	$\log P = 2.38(\pm 0.12)\log k_{50} + 0.88(\pm 0.07)$	(10)	
$n = 28, r = 0.957, s = 0.288, F = 286$		$n = 28, r = 0.969, s = 0.247, F = 401$		
(B3) ODP stationary phase				
$\log P = 2.53(\pm 0.35)\log k_w - 2.40(\pm 0.06)$	(11)	$\log P = 2.24(\pm 0.17)\log k_{50} + 1.05(\pm 0.10)$	(12)	
$n = 28, r = 0.815, s = 0.580, F = 51.3$		$n = 28, r = 0.932, s = 0.363, F = 171$		
(C) Tetrahydrofuran as the mobile phase				
(C1) ODS as the stationary phase, no masking agent				
$\log P = 1.00(\pm 0.05)\log k_w - 0.15(\pm 0.13)$	(13)	$\log P = 2.95(\pm 0.25)\log k_{50} + 1.11(\pm 0.10)$	(14)	
$n = 28, r = 0.964, s = 0.267, F = 338$		$n = 28, r = 0.918, s = 0.397, F = 408$		
(C2) ODS as the stationary phase using n-decylamine as a masking agent				
$\log P = 1.07(\pm 0.07)\log k_w - 0.22(\pm 0.16)$	(15)	$\log P = 2.75(\pm 0.24)\log k_{50} + 1.34(\pm 0.09)$	(16)	
$n = 28, r = 0.943, s = 0.331, F = 211$		$n = 28, r = 0.916, s = 0.402, F = 135$		
(C3) ODP stationary phase				
$\log P = 1.23(\pm 0.07)\log k_w - 0.69(\pm 0.16)$	(17)	$\log P = 2.26(\pm 0.23)\log k_{50} + 1.59(\pm 0.10)$	(18)	
$n = 28, r = 0.960, s = 0.281, F = 303$		$n = 28, r = 0.886, s = 0.464, F = 94.7$		

[a] Benzene excluded.

Acetonitrile as organic modifier

As with methanol, several equations were established (Table IV). Eqn. 7 (benzene excluded) does not actually differ from eqn. 1 when the ODS stationary phase is used with methanol. We thus observe that the selective effect of the solvent is not reflected in log k_w values and no difference should be expected when either methanol or acetonitrile is used. However, it is interesting that when the data for the

same compound are compared using either methanol or acetonitrile, the log k_w values are different. The addition of *n*-decylamine (eqns. 9 and 10) does not improve the correlation compared with eqns. 7 and 8. As acetonitrile has a weak hydrogen bonding ability, it does not attract sufficient water to the stationary phase [22], thus presumably preventing *n*-decylamine (which exists in the protonated form) from reaching the stationary phase.

Eqns. 11 and 12 demonstrate that acetonitrile is not suitable as an organic modifier when the ODP stationary phase is used. Indeed, a very long equilibration time between the mobile and stationary phase is needed, particularly at volume fractions rich in water. Large errors are expected for the measurements performed in this region and consequently will be reflected in the extrapolation values. Indeed, isocratic capacity factors (eqn. 12) lead to better correlations with log *P* than the extrapolated values (eqn. 11). In addition, the experimental conditions could not be kept sufficiently stable.

Tetrahydrofuran as organic modifier

Under comparable conditions, eqns. 13–18 (Table IV) were obtained using THF as the mobile phase. Eqn. 13, which correlates the extrapolated values obtained by using THF and the ODS column with log *P*, is still acceptable. Interestingly, the coefficients of the equation denote a remarkable similarity with the octanol–water system, with a slope close to 1 and an intercept not significantly different from 0. This similarity may be due to an attenuation of silanophilic interactions caused by the large amount of water brought into contact with the stationary phase by THF [22].

The addition of *n*-decylamine (eqns. 15 and 16) does not improve the correlation compared with eqns. 13 and 14, as the effects of silanol groups have already been attenuated by the associated THF–water.

Using THF with an ODP stationary phase (eqn. 17), a reasonable correlation between log k_w and log *P* values is obtained compared with eqn. 5. However, the selective effect of THF is clear in eqn. 18. Generally, all relationships between log *P* and isocratic capacity factors have deteriorated (eqns. 14, 16 and 18) compared with eqns. 13, 14 and 17.

CONCLUSIONS

As far as the organic modifier is concerned, methanol clearly appears to be the solvent of choice for the determination of lipophilicity by RP-HPLC. *n*-Decylamine, although effective as a masking agent, should not be used unless essential, as it introduces an additional variable and exerts its own effects on retention. The ODP stationary phase is a promising alternative to ODS for the assessment of lipophilicity as it leads to good lipophilicity indices without the necessity for a masking agent. However, the ODP stationary phase cannot be used with acetonitrile as the organic modifier.

From the present and previous studies, we conclude that the best system currently available for the determination of lipophilicity indices by RP-HPLC consists of ODP as the stationary phase and water–methanol as the eluent.

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