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REVERSED-PHASE THIN-LAYER CHROMATOGRAPHY OF POLYNUCLEAR AROMATIC HYDROCARBONS AND CHLORINATED BIPHENYLS

RELATIONSHIP WITH HYDROPHOBICITY AS MEASURED BY AQUEOUS SOLUBILITY AND OCTANOL–WATER PARTITION COEFFICIENT

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SUMMARY

Reversed-phase thin-layer chromatography with methanol–water as eluent was used to study relationships between retention and hydrophobicity of non-polar aromatic compounds. For polynuclear aromatic hydrocarbons, n-alkyl- and chloro-substituted benzenes, good correlation was observed between R_m values and log $P_{octanol}$. Significantly different correlations were obtained for polychlorinated biphenyls (PCBs) containing chlorine atoms in the positions *ortho* to the phenyl–phenyl bond. This effect could not be explained by the measured lower $\pi_{octanol}$ values for *ortho* chlorine in PCBs. Estimation of hydrophobicity from aqueous solubility, S, combined with melting point data is restricted to S values higher than 1 μg/l. A group-contribution approach, including a retention index scale, will be very useful for correlation and prediction of reversed-phase retention, hydrophobicity and environmental and toxicological properties of chemicals.

INTRODUCTION

Hydrophobicity is now recognized as the driving force for the typical distribution processes of non-polar organic chemicals in aqueous environments, such as solubility in water, octanol–water partitioning, bioconcentration in aquatic animals and soil sorption phenomena[1]. It is often directly related to toxicological and pharmacokinetic properties of drugs and other chemicals, as expressed by correlations between the parameter studied and the octanol–water partition coefficient (as log P_{ow}) of structurally related compounds[2]. The abundance of octanol–water partitioning data in the literature and the existence of extensive data compilations have led to methods of calculating partition coefficients, based on the additivity of structural group contributions to the hydrophobicity of a molecule[3,4]. Hydrophobicity may be expressed as an increase in the free energy (ΔG), which is related to log γ_w, the activity coefficient in aqueous solution.

Correlations between octanol–water partition coefficient and aqueous solubility have been described by several authors[5–9]. Recently, Yalkowsky and Valvani[10] and Mackay and co-workers[11,12] discussed an important improvement in such correlations, made by introduction of a crystal energy term based on the melting point of the substance.

The retention mechanism in reversed-phase liquid chromatography (RP-LC) is also attributed to solvophobic effects[13,14]. In "classical" liquid–liquid chromatography, the partition coefficients between the apolar stationary phase and the polar eluent are related to the capacity factors

$$P = \frac{V_0}{V_s} \times k' = \frac{V_r - V_0}{V_s} \qquad (1)$$

where P = partition coefficient, V_r = retention volume, V_0 = volume of mobile phase in column, V_s = volume of stationary phase and k' = capacity factor. Tomlinson[15] has discussed the use of these chromatographic hydrophobic parameters for correlation analysis of structure–activity relationships.

In modern RP-LC, however, the stationary phase is not a viscous liquid coating, but is chemically bonded to an inorganic support. This has made the method accessible to high-performance liquid chromatographic (HPLC) techniques, but, at the same time, it reduces eqn. 1 to a rather theoretical expression without much practical use for the determination of partition coefficients from retention data (and vice versa), since partitioning of solutes is now restricted to a very thin "brush" layer of apolar molecular tails (e.g., octadecyl groups)[16]. Direct comparison of RP-LC capacity factors with other, "pure", hydrophobic parameters is also precluded by the use of eluents which often contain water only as a minor constituent.

The first choice for any attempt to correlate RP-LC retention with hydrophobicity now seems to be a methanol–water eluent system, since methanol has nearly the same type of proton donor and acceptor properties as water, which makes it useful as a kind of "organic diluent". A linear increase in log k', often observed when the percentage of methanol in solvent mixtures used for isocratic elution is decreased, is in accordance with this approach. Several authors have found a linear relationship between log k' and alkyl chain length for a homologous series of solutes, and substituents such as carboxylic acid, hydroxyl and phenyl groups also give constant contributions to log k', analogous to the Hansch constants (π) in the octanol–water partitioning system[17–23]. Direct relations between log k' (R_M) and log P_{ow} have been described for chlorinated benzene derivatives in C_{18}-HPLC[24] and for a number of aromatic and organochlorine compounds in C_{18} thin-layer chromatography (TLC)[25,26], whereas HPLC gradient elution (22–75% methanol) resulted in a nonlinear relationship[27].

The main purpose of these correlations was to find an alternative to the direct measurement of octanol–water partition coefficients. This is especially useful for highly lipophilic and impure compounds, for which detection and phase separation may become problematic when the classical method is applied, since the concentrations to be measured in the two phases can differ by a factor of 10^5 or more. Several compounds of environmental interest, such as certain polyaromatic hydrocarbons, polychlorinated benzenes, biphenyls and terphenyls, and non-polar polymeric struc-

tures, will fall into this category. A reliable estimation of their hydrophobicity will greatly facilitate prediction and determination of their environmental mobility and bioaccumulation potential[28]. The advantage of hydrophobicity determinations from RP-LC retention parameters alone is clear: compounds with different hydrophobicities would be separated and quantification would not be required. At the same time, a systematic and quantitative approach using linear free energy relationships might provide tools for prediction of the RP-LC retention behaviour of chemicals with known structures[23].

In this study, C_{18} reversed-phase thin-layer chromatography (RP-TLC), using methanol–water mixtures as eluent, was chosen as a relatively simple and inexpensive, neutral reversed-phase system to compare the retention of polynuclear aromatic hydrocarbons (PAHs) and polychlorinated biphenyls (PCBs) with the octanol–water partition coefficients, and with aqueous solubility as an important physico-chemical property related to hydrophobicity.

EXPERIMENTAL

Measurement of octanol–water partition coefficients

Approximately 5 mg of the test chemical were dissolved in 2.5 ml of water-saturated 1-octanol (Baker, analytical grade). Of this solution, 1 ml was equilibrated with 500 ml octanol-saturated distilled water in a volumetric flask by magnetic stirring during 30 min. Layers were separated by centrifugation at *ca.* 1000 *g* for 10 min, and the octanol layer was then pipetted from the water layer. A 100-ml sample of the water was extracted with *ca.* 100 ml distilled toluene in three portions. Care was taken to prevent contamination of the water sample by octanol droplets. The combined toluene extracts were evaporated to 1 ml to enable quantitation of the chlorinated compounds by gas chromatography with electron-capture detection, whereas a 0.25-ml sample of the octanol phase was sufficiently diluted (*e.g.,* × 400) with distilled toluene to obtain nearly the same concentration.

Partition coefficients were then determined from concentration ratios, measured by successive injections of the concentrated water extract and the diluted sample of the octanol layer on a Tracor 550 GC system equipped with a linearized ^{63}Ni electron-capture detector (ECD) and Spectra-Physics 4100 computing integrator. GC conditions: glass column (1 m × 2 mm I.D.) packed with 2% Dexsil 300 GC on Chromosorb 750 (100–120 mesh); carrier gas, argon–methane (95:5), flow-rate 25 ml/min; detector make-up, 75 ml/min; isothermal operation at temperatures between 90 and 150°C (injector and outlet, 250°C; detector, 325°C).

For a direct comparison, partition coefficients of isomers were determined simultaneously in one partition experiment, if the GC conditions allowed separate detection and quantification of the compounds.

Reversed-phase thin-layer chromatography

Chemicals were spotted with the aid of glass capillaries on Whatman KC-18 RP-TLC plates (20 × 20 cm, thickness 0.2 mm), with fluorescent indicator. Chromatograms were obtained by ascending elution with mixtures of methanol (Baker, analytical grade) and de-ionized water in a closed 2-l glass tank system, after equilibration with the aid of wet filter-paper, or by horizontal "sandwich" elution (Camag;

Varo-KS-Kammer). Aromatic, UV-quenching substances were detected as dark spots on fluorescent plates in 254 nm UV-light. The most important retention parameter, R_M, was calculated for each spot

$$R_M = \log k' = \log(1/R_F - 1) \tag{2}$$

where R_F = distance travelled by spot divided by distance travelled by the eluent front.

Chemicals

Polyaromatic hydrocarbons (PAHs), 1,2,3,4-tetra- and pentachlorobenzene were obtained from Aldrich, and n-alkylbenzenes from Poly Science Corporation. Polychlorinated biphenyls (PCBs) were purchased from Analabs, except 2,2',5-tri- and 2,2',5,5'-tetrachlorobiphenyl, which were synthesized according to Sundström[29], and decachlorobiphenyl which was prepared by perchlorinated[30]. Each chemical was at least 97 % pure.

RESULTS

The octanol–water partition coefficients (as log P) of chlorinated biphenyls and of 1,2,3,4-tetrachlorobenzene, measured in this study (Table I), show that substitution of chlorine in the position *ortho* to the phenyl–phenyl bond has a less pronounced effect on log P than substitution in the *meta* or *para* position. π_{Cl} values derived from these measurements are given at the bottom of Table I. They are somewhat lower than the π value (0.66) derived from log P of tetrachlorobenzene, and the values for substitution of chlorine in an aromatic ring given by Rekker[3] and Hansch and Leo[4] (0.71–0.74). Log P values for higher chlorinated biphenyls were calculated using these new differential substitution constants, which resulted in considerably lower values than those estimated previously.

TABLE I

MEASURED log P_{ow} VALUES FOR TETRACHLOROBENZENE, MONO- AND DICHLOROBIPHENYLS

The reproducibility and the accuracy of GC determinations were better than 0.10 log P units.

Compound	*log P_{ow}*
1,2,3,4-Tetrachlorobenzene	4.75 ± 0.1
2-Chlorobiphenyl	4.59 ± 0.1
3-Chlorobiphenyl	4.71 ± 0.1
4-Chlorobiphenyl	4.61 ± 0.1
2,2'-Dichlorobiphenyl	5.00 ± 0.1
3,5-Dichlorobiphenyl	5.37 ± 0.1
3,3'-Dichlorobiphenyl	5.30 ± 0.1
4,4'-Dichlorobiphenyl	5.36 ± 0.1
Calculated π_{Cl} values for PCBs (log P biphenyl = 4.10)	
π_{Cl}-*ortho*	0.46
π_{Cl}-*meta*	0.62
π_{Cl}-*para*	0.59

Correlation with aqueous solubility

As discussed by Mackay *et al.*[11], octanol–water partition coefficients can be correlated with aqueous solubilities of solids if a melting point correction is included. For aqueous solubilities measured at 25°C, the simplest form of the correlation equation is

$$\log P_{ow} = - \log S - 0.01 \times \text{m.p.} + a \tag{3}$$

where S = aqueous solubility (mol/l) and m.p. = melting point (°C). The term a, related to the logarithm of the activity coefficient in octanol, γ_o, is only slightly dependent on the type of compounds in the correlation. Mackay *et al.*[11] found a mean γ_o value of 4.8 for 45 organic, mainly aromatic, compounds, which is in agreement with the value of 0.55 for the term a in eqn. 3 calculated from the PAH data of Yalkowsky and Valvani[10]. The relationship between the calculated log P values of PCBs and log S + 0.01 × m.p. (Table IIa) was fitted to eqn. 3, as shown in Fig. 1 where it is compared with the same correlation for PAHs, mentioned above. The solubility data of PCBs in Table IIa were taken from Weil *et al.*[31], who gave the most complete set obtained by one, reliable method which is also suitable for other almost insoluble compounds. The melting point data in Table IIa were collected by Hutzinger *et al.*[32]. The resulting correlation equation for PCBs containing up to six chlorine atoms is:

$$\log P_{ow} = \log S - 0.01 \times \text{m.p.} + 0.05 \tag{4}$$

The root mean square of the deviation in log P for the 20 points is 0.28, corresponding to a factor of 1.9 in P_{ow}.

The difference between the a terms (eqn. 3) of the PCB and the PAH correlations is 0.5. Following the interpretation of Mackay *et al.*[11], it must be concluded that the mean γ_o of PCBs (up to hexachlorobiphenyl) is about 14, three times as high as γ_o for PAHs.

As shown by Fig. 1, the correlation between P and S failed for PCBs with calculated log P higher than 7.5 or solubilities below 0.5 μg/l (*ca.* 10^{-9} mol/l). Apparently, the hydrophobicity that can be measured by aqueous solubility has reached its maximum here. Similarly, coronene (log P = 7.7, S = 0.14 μg/l or $10^{-9.3}$ mol/l, m.p. = 438°C[33]) was not included by Yalkowsky and Valvani[10] in their PAH correlation.

RP-TLC

Whatman KC-18 TLC plates, compared with other makes, have the advantage of larger surface areas and faster elution[34], which is especially important in ascending tank elution systems. The reproducibility of the ascending tank elution was moderate, presumably due to insufficient acclimatization of the elution chamber, coupled with formation of a solvent gradient on the plate. A better equilibration and reproducibility was obtained by horizontal elution in the sandwich system. Comparison of the behaviour of PAHs in both systems (Table IIb) shows that the retention of relatively fast eluting compounds, at the top of the table, was stronger in the tank system. This might be the result of a decreasing percentage of methanol at the solvent front during

TABLE II

RP-TLC RETENTION, R_M, COMPARED WITH HYDROPHOBIC PARAMETERS OF PCBs (a), PAHs (b) AND SUBSTITUTED BENZENES (c)

m.p. = Melting point (°C); S = aqueous solubility (mol/l); P_{ow} = octanol–water partition coefficient; R_M = log $(1/R_F - 1)$; N_{ab} = n-alkylbenzene retention index. Whatman KC-18 RP-TLC, methanol–water (95:5).

| Compound	m.p.	$-log S$	$-log S + -0.01 \times m.p.$	$log P_{ow}$	R_M		N_{ab}
(a) PCBs							
Biphenyl	71	4.28	3.57	4.10	−0.21		2.56
2-Chloro–	34	4.66	4.32	4.56	−0.24		2.28
3–	17	5.16	4.91	4.72	−0.10		3.57
4–	78	5.32	4.54	4.69	−0.13		3.29
2,2′-Dichloro–	61	5.45	4.84	5.02	−0.32		1.55
2,4–	24	5.55	5.31	5.15	−0.12		3.39
2,5–	23	5.58	5.35	5.18	−0.05		4.43
3,3′–				5.34	0.02		4.67
4,4′–	149	6.60	5.11	5.28	−0.10		3.57
2,2′,5-Trichloro–	44	5.60	5.16	5.64	−0.11		3.48
2,4,4′–	57	5.99	5.42	5.74			
2,4,5–	78	6.44	5.66	5.77			
2,4′,5–				5.77	−0.01		4.40
3,4,4′–	88	7.23	6.35	5.90			
2,2′,5,5′-Tetrachloro–				6.26	0.00		4.49
2,2′,6,6′–				5.94	−0.22		2.47
2,3,4,5–	92	7.18	6.26	6.39			
2,3′,4′,5–				6.39	0.15		5.87
3,3′,4,4′–	180	8.59	6.79	6.52			
3,3′,5,5′–				6.58	0.34		7.62
2,2′,3,4,5′-Pentachloro–	112	7.86	6.74	6.85			
2,2′,4,5,5′–	77	7.89	7.12	6.85			
2,3,4,5,6–	124	7.68	6.44	6.85			
2,2′,3,3′,4,4′-Hexachloro–	150	8.91	7.41	7.44	0.24		6.70
2,2′,4,4′,5,5′–	103	8.47	7.44	7.44			
2,2′,4,4′,6,6′–	114	8.60	7.46	7.12			
2,2′,3,4,5,5′,6-Heptachloro–	149	8.92	7.43	7.93	0.38		7.99
2,2′,3,3′,4,4′,5,5′-Octachloro–	159	9.19	7.60	8.68	0.58		9.83
2,2′,3,3′,5,5′,6,6′–	162	9.37	7.75	8.42			
2,2′,3,3′,4,4′,5,5′,6-Nonachloro–	206	9.61	7.55	9.14	0.67		10.65
Decachlorobiphenyl	305	10.49	7.44	9.60	0.90		12.77
(b) PAHs					*Tank*	*Sandwich*	
Naphthalene	80	3.61	2.81	3.35	−0.08	−0.25	2.19
Fluorene	116	4.93	3.77	4.18	0.00	−0.10	3.57
Anthracene	216	6.38	4.22	4.63	0.07	−0.03	4.21
Phenanthrene	101	5.15	4.14	4.63	0.03	−0.06	3.94
Fluoranthene	111	5.90	4.79	5.22	0.10	0.05	4.95
Pyrene	156	6.18	4.62	5.22	0.16	0.12	5.59
Chrysene	255	8.06	5.51	5.91	0.22	0.16	5.96
Benzo[a]pyrene	175	7.82	6.07	6.50	0.40	0.38	7.99
Benzo[g,h,i]perylene	277	9.02	6.25	7.10	0.54	0.54	9.46
(c) Substituted benzenes							
n-Butyl–				4.18	−0.05		4.03
n-Hexyl–				5.24	0.16		5.96
n-Octyl–				6.30	0.38		7.99
n-Decyl–				7.35	0.60		10.01
1,2,3,4-Tetrachloro–				4.94	0.05		4.95
Pentachloro–				5.69	0.23		6.61
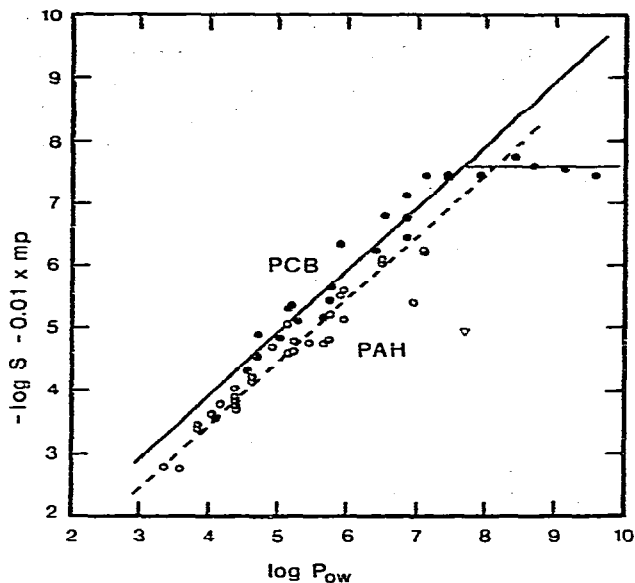
Fig. 1. Relationship between octanol–water partition coefficient. P_{ow}. and aqueous solubility. S (mol/l), when corrected for melting point (m.p.). ●—●. PCBs; O----O. PAHs; ▽, coronene.

tank elution. Consequently, the correlation of R_M with log P_{ow} obtained by linear regression analysis was somewhat better for the retention data from the sandwich systems (eqns. 5 and 6):

sandwich

$$\log P_{ow} = 4.52\ R_M + 4.81$$

$$n = 9, r = 0.98, s = 0.20$$

(5)

tank

$$\log P_{ow} = 5.31\ R_M + 4.39$$

$$n = 9, r = 0.96, s = 0.26$$

(6)

a non-linear relationship would give a better fit to the tank data. Therefore, the sandwich system was preferred for further experiments.

The relationship between calculated log P_{ow} and R_M for 20 PCBs (Table IIa) is given by:

$$\log P_{ow} = 4.52\ R_M + 5.79$$

$$n = 20, r = 0.95, s = 0.51$$

(7)

This correlation is far inferior to that of the PAHs (eqn. 5), as demonstrated by the root mean square deviation in log P. Two compounds with equal R_M value have completely different log P values, viz., 4.1 and 5.9 for biphenyl and 2,2′,6,6′-tetrachlorobiphenyl respectively. In addition, eqns. 5 and 7 show a difference of one log P unit.

Important improvements are obtained when the PCBs are distinguished ac-

cording to their number of chlorine atoms in *ortho* positions (numbers 2 and 6 of both phenyl rings). As indicated by the four compounds at the top of Table IIa and the four tetrachlorobiphenyls, substitution of hydrogen by chlorine in one of the *ortho* positions gives no contribution, or even a small negative one, to R_M, in contrast to *meta*- or *para*-chlorine substitution (positions 3, 4 and 5: $\Delta R_{MCl} = 0.13 \pm 0.06$). The new lines in Fig. 2 correspond to the following equations:

$$\text{no } o\text{-Cl} \qquad\qquad : \log P_{ow} = 4.5\ R_M + 5.2\ (n = 6) \qquad\qquad (8)$$

$$\text{one } o\text{-Cl} \qquad\qquad : \log P_{ow} = 4.5\ R_M + 5.6\ (n = 5) \qquad\qquad (9)$$

$$\text{two, three or four } o\text{-Cl} \quad : \log P_{ow} = 4.2\ R_M + 6.3\ (n = 9) \qquad\qquad (10)$$

Deviations between calculated and observed values were now generally below 0.25 log P units (factor 1.75 in P); exceptions were 4,4'-di-, 2,2',6,6'-tetra- and decachlorobiphenyl, with log P deviations of *ca.* 0.5 (factor of 3 in P). This accuracy is comparable to that obtained by the PAH correlation (eqn. 5).

The four *n*-alkylbenzenes (Table II) fitted the equation:

$$\log P_{ow} = 4.85\ R_M + 4.44 \qquad\qquad (11)$$

Tetra- and pentachlorobenzene were shown to be in intermediate positions between alkylbenzenes and PAHs (Fig. 2).

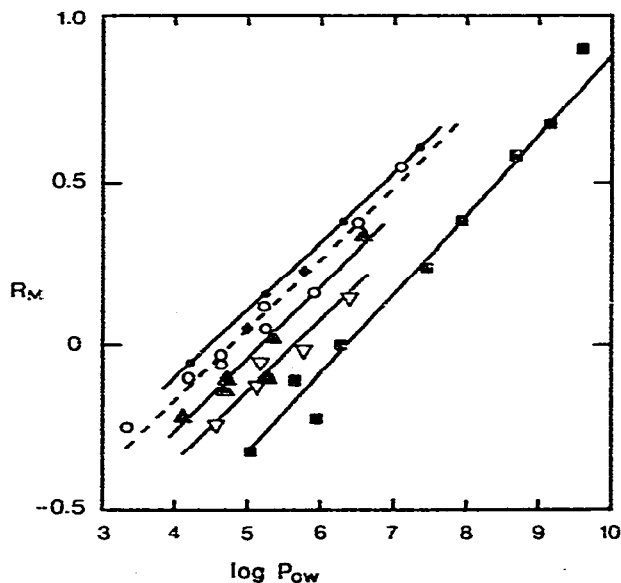Direct comparison of aqueous solubility and RP-TLC retention was only

Fig. 2. Relationship between octanol–water partition coefficient, P_{ow}, and reversed-phase retention, R_M. ●, *n*-Alkylbenzenes; ◆, polychlorinated benzenes; ○, polycyclic aromatic hydrocarbons; ▲, polychlorinated biphenyl (PCB) without *ortho*-Cl; ▽, PCB with one *o*-Cl; ■, PCB with two or more *o*-Cl. Whatman KC-18 RP-TLC; methanol–water (95:5).

possible for a restricted number of compounds from Table II (Fig. 3). The correlation between R_M and $\log S + 0.01$ m.p. is evident for the PAHs:

$$- \log S - 0.01 \times \text{m.p.} = 4.3 + 4.7 \, R_M \quad (n = 9) \tag{12}$$

mean deviation: 0.3 log S units

max. deviation: 0.5 log S units

For the higher chlorinated biphenyls, (log $S + 0.01$ m.p.) had reached a maximum value, whereas too few data were available for the lower chlorinated biphenyls to allow reasonable correlations according to the different degrees of *ortho*-chlorine substitution.

Changes in the eluent composition resulted in a linear increase of the R_M values of PCBs with the water content of the eluent, in the range of 0–20% (v/v) (Fig. 4)

2,2',3,4,5,5',6-heptachlorobiphenyl:	$R_M = \quad 0.06 + 7.6 \times \Phi$	(13)
2,2',5,5'-tetrachlorobiphenyl:	$R_M = -0.24 + 6.4 \times \Phi$	(14)
2-chlorobiphenyl:	$R_M = -0.34 + 4.5 \times \Phi$	(15)
biphenyl:	$R_M = -0.41 + 4.5 \times \Phi$	(16)

where Φ = the volume fraction of water in the eluent.
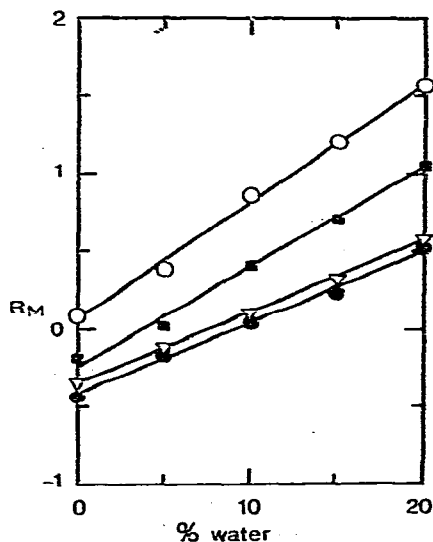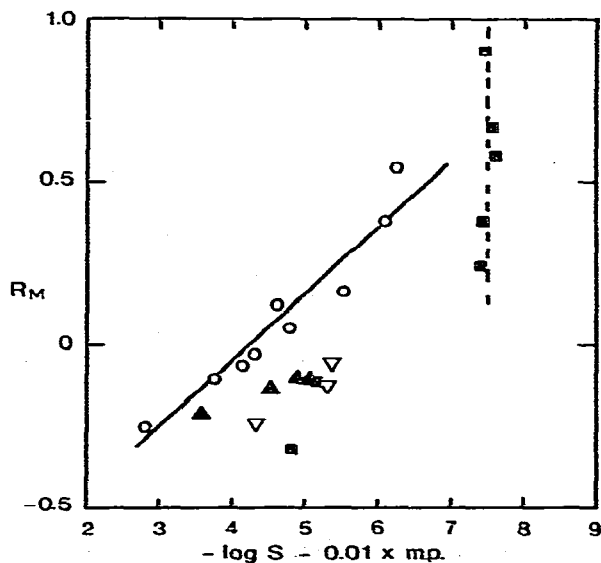

Fig. 3. Relationship between aqueous solubility, S (mol/l), corrected for melting point (m.p.), and reversed-phase retention, R_M. O, Polycyclic aromatic hydrocarbons; polychlorinated biphenyls: ▲, no *ortho*-Cl; ▽, one o-Cl; ■, two or more o-Cl. Whatman KC-18 RP-TLC; methanol–water (95:5).

Fig. 4. Retention of polychlorinated biphenyls at different percentages of water in the eluent (Whatman KC-18 RP-TLC, methanol–water). ●, Biphenyl; ▽, 2-chloro-; ■, 2,2',5,5'-tetrachloro-; O, 2,2',3,4,5,5',6-heptachlorobiphenyl.

CONCLUSIONS AND DISCUSSION

Aqueous solubility and octanol–water partition coefficient can be used to give satisfying and consistent estimations of the hydrophobicity of polyaromatic hydrocarbons and polychlorinated biphenyls, provided that the appropriate correction is made for the crystal energy of the compounds. However, the use of aqueous solubilities is limited to values higher than 0.5 $\mu g/l$, corresponding to log $P_{ow} < 7.5$. Estimation of higher log P values by calculation from fragmental or substitution constants is still possible for compounds with known structures, if sufficient log P data are available from smaller, structurally related chemicals. The difference between the π values obtained for chlorine attached to different sites of an aromatic nucleus, previously suggested by Tulp and Hutzinger[9], demonstrates the pitfalls of a too simple application of "literature values".

Especially for mixtures of highly lipophilic unknown chemicals, RP-LC might be a reasonable alternative. However, the results of the present C_{18}-RP-TLC study, by use of methanol–water mixtures, show that pure hydrophobicity data are not obtained. One single correlation of log P_{ow} and R_M for all types of aromatic compounds together will produce systematic errors in log P determinations. The differences may be due to different solvophobic effects, as a result of the high methanol concentrations in the eluent, or to specific interactions with the stationary phase. It is possible that the fit in a semi-crystalline matrix of C_{18}-alkyl chains is affected by any distortion of the planarity of the biphenyl nucleus, resulting from steric hindrance by ortho-chlorine atoms. On the other hand, the influence of the other substituting groups (chlorine in other positions, n-alkyl, aromatic rings in PAHs) on the RP-TLC retention of aromatic compounds correlates well with π values in the octanol–water system. Therefore, it is expected that R_M values from RP-LC can still be useful for structure–activity correlations of closely related compounds. However, the use of "log P_{ow} derived from reverse phase chromatography" should be avoided, since these values are highly dependent on the type of reference compounds chosen for the basic log P vs. R_M correlation.

As a reproducible and reliable intermediate, it is suggested that retention indices be based on the linear relationship between the R_M or log P value and the number of carbon atoms of the components of a homologous series of n-alkyl substituted compounds, such as the alkylbenzenes in this study[35,36]. Eventually, "apparent log P_{ow}" values can be calculated for comparison with other hydrophobic parameters, as exemplified by eqn. 17.

$$\log P_{ow} = 2.07 + 0.527 \times N \qquad (17)$$

where N represents the n-alkylbenzene retention index. It can be considered as the number of carbon atoms in the alkyl chain of an imaginary n-alkylbenzene having the same retention; N is determined from R_M by interpolation in a series of n-alkylbenzenes.

It should be emphasized, however, that the retention index thus defined is an independent parameter, which can be used directly for structure–activity correlations without conversion into log P. In addition, it will be a practical basis for the determination of relationships between chemical structure and retention in reversed-phase

liquid chromatography. Besides the homologous series on which the retention index is based, only the type of reversed-phase material and the eluent composition should be specified. It is expected that the retention index will show only a minor change with variation in the percentage of methanol in the eluent. The exact determination of this dependence will be simplified by linearity of the relationship between R_M and the methanol percentage over a certain range (see Fig. 3).

The reversed-phase retention index may replace the unreliable log P_{ow} determinations of highly lipophilic chemicals (log $P > 5$), for prediction of biological activity and environmental distribution coefficients, such as bioaccumulation factors and sorption coefficients. For this purpose, more knowledge of different group contributions to RP-LC retention and their influence on environmental and biological parameters is desirable.

Thin-layer chromatography is a rapid, simple and inexpensive method. However, high-performance liquid column chromatography may be preferred for reasons of accuracy, sensitivity and reproducibility. In addition, HPLC offers the possibility of gradient elution, which may be very useful for mixtures of unknown chemicals, as found in chemical wastes, to estimate environmental mobility and accumulation potential[28].

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