

CHROM. 12,135

## Note

### Determination of log $P_{\text{oct}}$ values of chloro-substituted benzenes, toluenes and anilines by high-performance liquid chromatography on ODS-silica

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(First received May 1st, 1979; revised manuscript received June 18th, 1979)

The quantitative structure-activity relationship (QSAR) developed by Hansch and co-workers<sup>1</sup> has been of proven utility in aquatic toxicology for several years<sup>2-4</sup>. This relationship is based partly on the correlation between partition coefficients  $P$  in the partitioning systems m and n given by Collander<sup>5</sup>:

$$\log P_m = a \log P_n + b \quad (1)$$

In order to simulate the properties of a bio-system, octanol-water was chosen as the reference system. The partition coefficients in this system ( $P_{\text{oct}} = C_{\text{oct}}/C_{\text{water}}$ ) can be determined by the shake-flask method<sup>1</sup>. After equilibration, the octanol and/or aqueous phase is analyzed by means of an appropriate technique. Further, it is possible to estimate  $P_{\text{oct}}$  values from liquid chromatographic retention data. To this end, the capacity ratio ( $k'$ ) can be used, since it is proportional to the partition coefficient  $P$  and hence directly related to  $P_{\text{oct}}$  according to eqn. 1. The most convenient and precise method of obtaining  $k'$  values is high-performance liquid chromatography (HPLC), which has been applied in this field by several authors<sup>6-10</sup>.

The most reliable procedure to get log  $P_{\text{oct}}$  values from log  $k'$  values by HPLC has been outlined by Mirrlees *et al.*<sup>9</sup>. They covered trimethyl-chlorosilane (TMCS)-treated silica *in situ* with a thin layer of octanol and used water (saturated with octanol) as the eluent. The correlation of  $k'$  with shake-flask  $P_{\text{oct}}$  values was excellent, as expected. A disadvantage of this method is that its applicability is limited ( $-0.3 < \log P < 3.7$ ) by solute detectability, which decreases strongly with increasing  $P$  values due to poor solute solubility and band-spreading. Hence, the method cannot be applied to the highly hydrophobic chlorinated benzene derivatives presented in Table I.

In this respect alkylsilylated silicas offer an interesting alternative, as the

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amount of organic modifier in the aqueous mobile phase (and hence the  $k'$  values) can be varied at will without affecting the column stability.

In order to avoid adsorption of the solute to residual silanol sites, which decreases the precision of the  $\log k' - \log P_{\text{oct}}$  correlation, a large surface concentration of bonded alkyl groups is required and even a TMCS treatment of the sorbent may be necessary. Even for apolar solutes, relative  $\log k'$  values appear to depend on the surface coverage of the alkyl layer. Thus, Hennion *et al.*<sup>11</sup> obtained relative  $\log k'$  values for some phenolic and polycyclic aromatic compounds on octadecylsilyl (ODS)-silicas which were nearly constant, only when the surface coverage was larger than  $2 \mu\text{mole}/\text{m}^2$ . Similar results have been reported by Hemetsberger *et al.*<sup>12</sup> for some halogenated benzenes at surface coverages larger than  $3.5 \mu\text{mole}$  of pentadecylsilyl groups per  $\text{m}^2$ .

In our work with ODS-silica (*ca.*  $4.4 \mu\text{mole ODS}/\text{m}^2$ ), methanol-water (70:30) was used as the eluent. This rather large methanol content was chosen in order to obtain reasonably short retention times for highly hydrophobic solutes. Methanol is preferable to higher alcohols because of its relative low viscosity, which is an advantage for chromatography. The water content of the eluent (and hence its polarity) is much less than that of the aqueous phase of the octanol-water system. Therefore, it can be expected that  $\log k' - \log P_{\text{oct}}$  correlations obtained in this manner will be relatively poor if they are based on  $\log k'$  values of different solute types (especially with respect to polarity). Generally, eluent compositions of less than 10% methanol are less suitable because of the poor peak symmetry obtained. Karger *et al.*<sup>13</sup> have suggested that wetting of the ODS layer is not complete at these large water contents.

The examined solutes are series of chlorinated benzenes, toluenes and anilines of similar size, shape and polarity. As buffering of the eluent can be omitted (the  $\text{p}K_{\text{b}}$  values of the anilines are  $\geq 9.4$ ), salt effects need not be taken into account.

The  $\log k'$  values are compared with calculated rather than with experimental  $\log P$  values because shake-flask results appeared to be inaccurate if  $\log P \geq 4$ . Usually,  $\log P_{\text{calc}}$  data are obtained with the  $\pi$ -method, developed by Hansch *et al.*<sup>14</sup>, which is based on the additivity of solute group contributions to  $\log P$ . A more refined approach has been outlined by Rekker<sup>15</sup>. In his  $f$ -method the contributions of small molecular fragments are summed.  $\log P_{\text{calc}}$  values after Rekker are used in this work.

## EXPERIMENTAL

### *Chemicals and characterization of the column packing material*

All solutes had the highest available purity. Methanol (analyzed reagent grade) was obtained from J. T. Baker (Deventer, The Netherlands). Elemental analysis showed that LiChrosorb RP-18 (E. Merck, Darmstadt, G.F.R.) contains 0.286 g carbon per g bare LiChrosorb Si 100 ( $S_{\text{BET}} = 282 \text{ m}^2/\text{g}$  according to Hemetsberger *et al.*<sup>12</sup>). The ODS surface concentration is  $4.4 \mu\text{mole}/\text{m}^2$  if it is assumed that dichloromethyloctadecylsilane has reacted with the silica.

### *Determination of $\log P_{\text{oct}}$*

A 100-mg amount of each of the di- and trichlorobenzenes, 25 mg of the

tetrachlorobenzenes and 2.5 mg of penta- and hexachlorobenzene was dissolved in 10 ml octanol in glass-stoppered centrifuge tubes and to each tube 10 ml distilled water were added. The tubes were shaken mechanically for 15 min at 22°. After 3 h the octanol phase was discarded and the tubes were centrifuged for 10 min at 1000 g to promote the removal of octanol droplets from the aqueous phase. The octanol was removed with a tap pump. From each tube a 3-ml aliquot of aqueous phase was transferred into a glass-stoppered centrifuge tube and extracted by shaking with 3 ml *n*-hexane for 5 min. The tubes were centrifuged at 1000 g for 10 min. Finally, the hexane-solution was analyzed with a Tracor 550 gas chromatograph equipped with a <sup>63</sup>Ni electron capture detector. The glass column (2 m × 2.5 mm I.D.) was filled with Chromosorb W HP (80–100 mesh) coated with 3 wt. % OV-1. All measurements were repeated five times. The standard error of the log  $P_{\text{oct}}$  values was better than 0.04.

#### *Determination of log k'*

The apparatus was a Packard-Becker (Delft, The Netherlands) Model 8200 chromatograph equipped with a UV and a refractive index detector. The eluent flow-rate was monitored continuously with a calibrated siphon counter (Waters Assoc., Milford, Mass., U.S.A.). The column (precision-bore stainless steel, 25 cm × 2.1 mm I.D.) was packed by forcing a de-gassed and homogenized slurry (10 wt. % LiChrosorb RP-18 in amyl alcohol) into the column with *n*-hexane at 350 atm. The packing was settled and rinsed by flushing 200 ml each of hexane and methanol through the column at 350 atm. The weight of sorbent in the column was 0.61 g.

The sample size was ≤ 20 μg, except for octanol and the chloroalkanes, to be monitored by the refractive index detector (*ca.* 50 μg). The influence of eluent flow-rate and of sample size on the  $k'$  values was negligible. Retention volumes ( $V_R$ ) were corrected for eluent hold-up in the column and dead volumes by means of the retention volume of methanol ( $V_0$ ). The  $k'$  values were obtained from:

$$k' = (V_R - V_0)/V_0$$

The eluent was methanol-water (70:30) and the pressure drop was 100 atm. All measurements were made at least in duplicate and at 35°. The reproducibility of the retention volumes was *ca.* 10 μl or 2% for strongly retained solutes. Hence, log  $k'$  values are precise to 0.02–0.03.

## RESULTS AND DISCUSSION

The experimental log  $P_{\text{oct}}$  and log  $k'$  values along with log  $P_{\text{calc}}$  values after Rekker are collected in Table I for the series of chlorinated benzenes, toluenes, anilines and for some miscellaneous compounds.

Comparison of the log  $P_{\text{oct}}$  and log  $P_{\text{calc}}$  values of the chlorobenzenes shows good agreement for log  $P$  values up to *ca.* 4, becoming increasingly worse for higher values. Inspection of the differences shows that the steric interference of the chloro substituents cannot explain this discrepancy. The simplest explanation for the deviating log  $P_{\text{oct}}$  values is the occurrence of non-centrifugeable octanol droplets in

TABLE I

EXPERIMENTAL  $\log P_{\text{oct}}$  AND  $\log k'$  VALUES AND  $\log \hat{P}$  VALUES ESTIMATED FROM EQN. 3, CALCULATED  $\log P$  VALUES AFTER REKKER AND DEVIATIONS ( $\Delta$ ) BETWEEN  $\log P_{\text{calc}}$  AND  $\log \hat{P}$  FOR A NUMBER OF CHLOROBENZENES, -TOLUENES, -ANILINES AND SOME MISCELLANEOUS SOLUTES

<i>Solute series</i>	<i>Code</i>	$\log P_{\text{oct}}$	$\log k'_i$	$\log \hat{P}$	$\log P_{\text{calc}}$	$\Delta$
<i>Benzenes</i>						
H	1	2.14*	0.09	2.34	2.13	-0.21
Cl	2	2.84*	0.29	2.84	2.81	-0.03
1,2-diCl	3	3.55	0.51	3.39	3.53	0.14
1,3-diCl	4	3.60	0.60	3.62	3.53	-0.09
1,4-diCl	5	3.62	0.51	3.39	3.53	0.14
1,2,3-triCl	6	4.11	0.75	3.99	4.20	0.21
1,2,4-triCl	7	3.93	0.80	4.12	4.20	0.08
1,3,5-triCl	8	4.15	0.95	4.49	4.20	-0.29
1,2,3,4-tetraCl	9	4.46	1.01	4.64	4.94	0.30
1,2,3,5-tetraCl	10	4.50	1.12	4.92	4.94	0.02
1,2,4,5-tetraCl	11	4.52	1.08	4.82	4.94	0.12
pentaCl	12	4.88	1.36	5.52	5.69	0.17
hexaCl	13	5.00	1.66	6.27	6.44	0.17
<i>Toluenes</i>						
H	14	2.69*	0.33	2.94	2.59	-0.35
2-Cl	15	3.42*	0.56	3.52	3.31	-0.21
4-Cl	16	3.33*	0.52	3.42	3.31	-0.11
2,4-diCl	17	—	0.85	4.24	3.98	-0.26
2,6-diCl	18	—	0.87	4.29	3.98	-0.31
1-CH <sub>2</sub> Cl-2,4-diCl	19	—	0.68	3.82	3.87	0.05
1,3-diCH <sub>2</sub> Cl	20	—	0.24	2.72	2.87	0.15
<i>Anilines</i>						
H	21	0.93*	-0.48	0.91	1.03	0.12
2-Cl	22	1.91*	-0.15	1.74	1.76	0.02
3-Cl	23	1.90*	-0.22	1.57	1.76	0.19
4-Cl	24	1.83*	-0.22	1.57	1.76	0.19
2,4-diCl	25	—	0.21	2.64	2.42	-0.22
2,5-diCl	26	—	0.15	2.49	2.42	-0.07
3,4-diCl	27	—	0.00	2.12	2.42	0.30
3,5-diCl	28	—	0.19	2.59	2.42	-0.17
2,3,4-triCl	29	—	0.40	3.12	3.17	0.05
2,4,5-triCl	30	—	0.46	3.27	3.17	-0.10
2,3,4,5-tetraCl	31	—	0.73	3.94	3.92	-0.02
<i>Miscellaneous</i>						
Hexachloroethane			0.68	3.82	3.58	-0.24
Hexachloro-1,3-butadiene			1.05	4.74	4.63**	-0.11
Octanol			0.34	2.97	2.95	-0.02

\* Experimental values given by Rekker<sup>15</sup>.

\*\* Corrected for proximity effects<sup>15</sup>.

the aqueous phase with a total volume fraction  $F$ . Suppose that the  $\log P_{\text{calc}}$  values represent true partition data, then it holds that

$$P_{\text{oct}}/P_{\text{calc}} = 1/(1 + F \cdot P_{\text{calc}})$$

From this equation,  $F = (2.0 \pm 1.5) \cdot 10^{-5}$ . Centrifugation for 2 h at 450 g, as proposed by Rekker<sup>15</sup>, had no influence on the magnitude of  $F$ . Therefore, it is con-

cluded that values of  $\log P_{\text{oct}} > 4$  obtained with the shake-flask method are unreliable if usual centrifugation techniques are applied. The same conclusion emerges from data given by Tulp and Hutzinger<sup>16</sup>.

As regards the chromatographic data, it must be determined whether the  $\log k'$  values of the chlorobenzenes, -toluenes and -anilines obey the additivity rule because this is a prerequisite for a good correlation with  $\log P_{\text{oct}}$  values. For this purpose, most precise results can be expected if the  $\log k'$  values of the chloro-toluenes (CT) and chloroanilines (CA) are compared with those of the corresponding chlorobenzene homomorphs (CB), since trivial steric shielding effects are eliminated in this manner. The following results are obtained:

$$\begin{aligned} o-, p\text{-Cl} &: \log k' (\text{CT}) - \log k' (\text{CB}) = +0.05 \pm 0.02 \quad (n = 5) \\ o\text{-Cl} &: \log k' (\text{CA}) - \log k' (\text{CB}) = -0.63 \pm 0.03 \quad (n = 6) \\ m-, p\text{-Cl} &: \log k' (\text{CA}) - \log k' (\text{CB}) = -0.78 \pm 0.04 \quad (n = 5) \end{aligned} \quad (2)$$

These differences are constant within their experimental error (0.03–0.04). It can be concluded that anilines, which have a chloro group in *ortho* position, are slightly more retained than anilines with a *meta*- or *para*-chloro substituent. This is presumably due to reduced aniline–eluent interaction (hydrogen-bonding) by steric interference of the *ortho*-chloro group. Both the  $\pi$ -, and  $f$ -methods fail to account for this effect. Consequently, the precision of the  $\log k' - \log P_{\text{calc}}$  correlation will be affected to some extent. Adsorption of the anilines to any present silanol sites can be ruled out, as its contribution to  $\log k'$  values would decrease with increasing number of (electron-withdrawing) chloro substituents (see results given in ref. 17). This must be due to the large polar eluent strength (towards silanol groups) and to the high ODS surface concentration on LiChrosorb RP-18 (4.4  $\mu\text{mole}/\text{m}^2$  of ODS groups which have reacted largely bifunctionally<sup>18</sup> with the 8–9  $\mu\text{mole}/\text{m}^2$  of silanol sites<sup>19</sup>).

Finally, the correlation of  $\log k'$  and  $\log P_{\text{calc}}$  values will be examined. The regression of  $\log P_{\text{calc}}$  on  $\log k'$  is:

$$\log P_{\text{calc}} = (2.50 \pm 0.07) \log k' + (2.12 \pm 0.05) \quad (3)$$

The standard error of fit,  $s = 0.183$ . This equation can be used to estimate values of  $\log P_{\text{oct}}$  ( $\log \hat{P}$ ) from  $\log k'$  data. The correlation between  $\log \hat{P}$  and  $\log P_{\text{calc}}$  values is good [the linear correlation coefficient,  $r = 0.989$  ( $n = 20$ )]. Nevertheless, it follows from Table I that the discrepancies between  $\log P_{\text{calc}}$  and  $\log \hat{P}$  data ( $\Delta$ ) exceed the accuracy of  $\log P_{\text{calc}}$  (*ca.* 0.1) in many cases. These discrepancies will be discussed in terms of  $f$  values.

From the  $f$  values of molecular fragments<sup>15</sup> ( $\text{C}_6\text{H}_5 = 1.886$ ;  $\text{C}_6\text{H}_4 = 1.688$ ;  $\text{C}_6\text{H}_3 = 1.431$ ;  $\text{C}_6\text{H}_2 = 1.256$ ;  $\text{C}_6\text{H} = 1.081$ ; and  $\text{C}_6 = 0.906$ ) the (mean)  $\hat{f}(\text{Cl})$  value can be obtained from the  $\log \hat{P}$  values of the chlorobenzenes. This  $\hat{f}(\text{Cl})$  value was used in the calculation of  $\hat{f}(\text{CH}_3)$  and  $\hat{f}(\text{NH}_2)$  from the  $\log \hat{P}$  values of the toluenes and anilines. The results obtained are given in Table II along with the  $f$  values after Rekker. It can be concluded that: (1) the selectivities of both systems (ODS/methanol–water and octanol–water) towards aromatic chloro groups are roughly the same, but the former system shows a significantly larger selectivity towards methyl groups; (2) the  $\hat{f}(\text{NH}_2)$  value is strongly affected by *ortho*-chloro groups. This is due to specific aniline–eluent interactions.

TABLE II

$f(\text{Cl})$ ,  $f(\text{CH}_3)$  AND  $f(\text{NH}_2)$  VALUES FROM  $\log \hat{P}$  (BASED ON ODS-SILICA/METHANOL-WATER) AND FROM  $\log P_{\text{Oct}}$  VALUES ACCORDING TO REKKER  
Numbering refers to compounds involved in the calculation (Table I).

Solute series	Group	Position	$f \pm s$	
			this work	after Rekker
Chlorobenzenes (2-13)	Cl	<i>o, m, p</i>	$0.90 \pm 0.02$	0.922
Chlorotoluenes (14-18)	CH <sub>3</sub>	<i>o, p</i>	$0.98 \pm 0.05$	0.702
Chloroanilines (21-31)	NH <sub>2</sub>	<i>o</i>	$-0.75 \pm 0.04$	-0.854
		<i>m, p</i>	$-0.96 \pm 0.09$	-0.854

The  $\Delta$  values for some miscellaneous compounds ( $\log k'$  values were left out of the preceding calculations) illustrate the accuracy of  $\log \hat{P}$  values estimated from  $\log k'$  values by means of eqn. 3.

*Mutatis mutandis*, Rekker's  $f$ -method can be used to predict  $\log k'$  values (the standard error of fit of the regression of  $\log k'$  on  $\log P_{\text{calc}}$  is 0.08).

Summarizing, the following conclusions can be drawn. For lipophilic compounds  $\log P_{\text{Oct}}$  values from the shake-flask method are unreliable if  $\log P_{\text{Oct}} \geq 4$ . Distinct advantages of the bonded phase HPLC technique over the shake-flask method are its simplicity, precision and large application range ( $\log P \leq 8$ ). However, the obtained  $\log \hat{P}$  values show significant systematic deviations from those of the octanol-water standard system (represented by  $\log P_{\text{calc}}$  values). These deviations are due to slightly different interaction forces at the hydrophilic (amino group of the anilines) and lipophilic moieties of isomeric solute molecules in both systems. As a result, this HPLC method is not accurate enough for the determination of  $\pi$ - or  $f$ -constants.

If high accuracy is not a priority (as is often the case in QSARs), HPLC results on ODS-silica may be usable provided that  $\log \hat{P}$  values of similar compounds are compared, especially in those cases where  $\log P_{\text{calc}}$  values ( $> 4$ ) may be erroneous (see examples given by Rekker<sup>15</sup>).

#### ACKNOWLEDGEMENT

One of the authors (H.K.) gratefully acknowledges support by the Netherlands organization for the advancement of pure research (Z.W.O.).

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