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Note

Determination of partition coefficients by liquid chromatography

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The availability of a rapid and accurate technique for the determination of partition coefficients (or their equivalent) became desirable when it was observed that the partition coefficient between n-octanol and water ($P_{o/w}$) was dominant in the successful Hansch correlation of phenol toxicity to aquatic species[1]. This concern has led to the development of a method using a permanently bonded long-chain alkyl packing in a high-pressure liquid chromatographic system and subsequently relating the capacity factor k' [$k' = (t_R - t_0)/t_0$, *i.e.* the net retention time relative to the non-adsorbed time] to the partition coefficient $P_{o/w}$.

EXPERIMENTAL

The separations were performed on $2\frac{1}{8}$ in. × 2 ft. Bondapak C-18/Porasil B columns that were mounted in a Waters Associates Model ALC 201 with refractive index detector. The various mole percentages of distilled water and acetone (MCB-ACS grade) were eluted at 24–26° and a flow-rate of 0.9–1.0 ml/min.

DISCUSSION

It is well recognized that a separation (*i.e.*, a difference in retention volume) in reversed-phase chromatography depends upon the partitioning characteristics of the solute between the mobile phase and the stationary phase as represented by the value of the partition coefficient. In the development of the overall approach for evaluating the elution data in the current study, it was therefore necessary to consider the relative number of moles of eluting solvent. However, the use of mole per cents resulted in a significant increase in the deviation from linearity from that previously observed in the analysis of thin-layer partition data[2] when R_M {$R_M = \log [1/(R_F - 1)]$} was plotted *vs.* volume per cent.

For example, Figs. 1 and 2 contain plots of log k' *vs.* volume per cent* (sigma $Y = 0.012$) and log k' *vs.* mole per cent (sigma $Y' = 0.035$), respectively, for a representative compound (o-chlorophenol). It was subsequently found that the linearity could be maintained and substantially extended for all the compounds studied by

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* $\log k' = R_M$, *e.g.* $k' = (t_R - t_0)/t_0 = t'_R/t_0$; $R_F = t_0/(t_0 + t_R') = 1/(1 + k')$; $R_M = \log [1/(R_F - 1)] = \log k'$ (ref. 3).
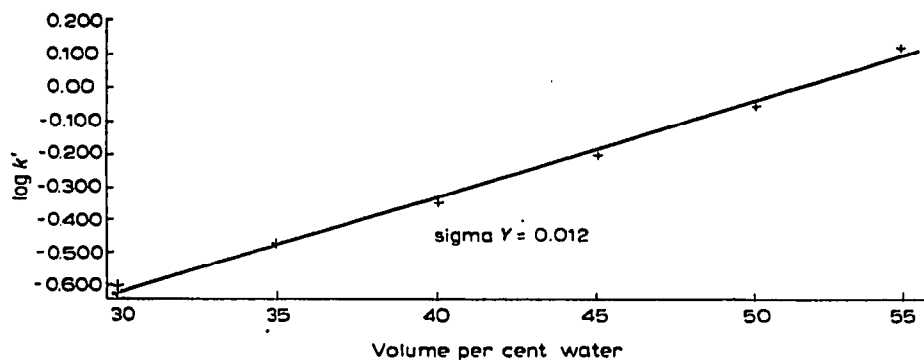
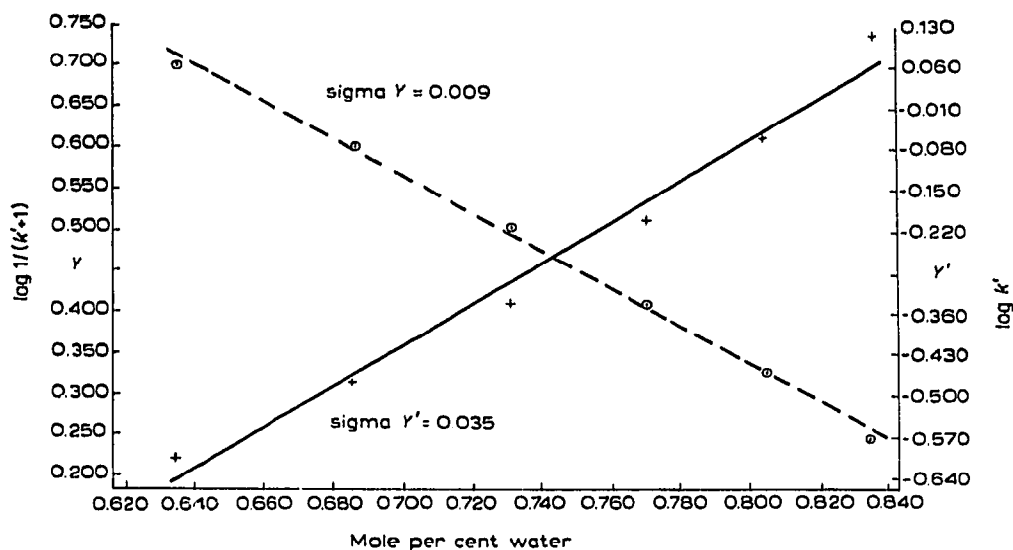Fig. 1. Plot of log k' vs. volume per cent of water.

Fig. 2. Plots of log $[1/(k' + 1)]$ and k' vs. mole per cent of water.

plotting log $[1/(k' + 1)]$ vs. mole per cent (e.g., o-chlorophenol, Fig. 2, sigma $Y = 0.009$). Such a linear correlation is observed for all compounds studied (Table I). This change is valid as both the correlations of R_M and k' are made over a range of percentages and the modification is therefore only from one empirical relationship to another.

The correlations of log k' to log P and π ($\pi = \log P_{substituted} - \log P_{parent}$) to κ ($\kappa = \log k'_{substituted} - \log k'_{parent}$) for some phenols and anilines are contained, along with their corresponding residuals, in Table II. The coefficients obtained from the individual regression analyses are found to be quite satisfactory. However, when the results from the two families of compounds are combined, the correlation of log P to log k' decreases ($r = 0.86$).

Phenols: log $P = A$ log $k' + C$; $r = 0.96$; $\pi = \kappa + C$; $r = 0.96$.
Anilines: log $P = A$ log $k' + C$; $r = 0.97$; $\pi = \kappa + C$; $r = 0.97$.

TABLE IA
PHENOL SERIES

Substituent	Range*	M^{**}	S.D.	B	S.D.
–H	73.1–88.3	2.59	0.02	2.55	0.01
3-OCH₃	73.1–88.3	2.88	0.02	2.82	0.01
2-CH₃	68.7–85.9	2.28	0.01	2.16	0.01
4-CH₃	68.7–85.9	2.36	0.01	2.26	0.01
4-NO₂	68.7–83.3	2.64	0.01	2.56	0.01
2-Cl	63.6–83.3	2.26	0.01	2.15	0.01
4-Cl	63.6–83.3	2.32	0.01	2.14	0.01
4-Br	63.6–83.3	2.32	0.01	2.10	0.01
2,6-CH₃	63.6–80.3	1.81	0.02	1.70	0.01

TABLE IB
ANILINE SERIES

Substituent	Range*	M^{**}	S.D.	B	S.D.
–H	63.6–88.3	1.81	0.02	1.93	0.02
3-OCH₃	68.7–88.3	1.98	0.02	2.03	0.01
4-OCH₃	68.7–88.3	2.00	0.02	2.16	0.02
2-CH₃	68.7–85.9	2.00	0.01	1.98	0.01
3-CH₃	68.7–85.9	1.96	0.01	1.94	0.01
4-CH₃	68.7–85.9	1.96	0.01	1.92	0.01
2-NO₂	68.7–85.9	2.26	0.01	2.14	0.01
3-NO₂	63.6–85.9	2.28	0.01	2.19	0.01
2-Cl	57.6–83.3	1.88	0.01	1.78	0.01
4-Cl	57.6–83.3	1.99	0.02	1.88	0.01
4-Br	57.6–83.3	1.99	0.01	1.85	0.01
2,4-Cl	57.6–80.3	2.06	0.01	1.82	0.01

* Mole per cent water.
** $\text{Log} [1/(k' + 1)] = MX + B$.

Although this is still an acceptable value for a regression analysis of this type, it, not unexpectedly, indicates that P and/or k' reflect more than just lipophilic character.

Related to these observations is the work of Collander[4,5], which has shown that although any two alcohol–water systems will provide a linear relationship be-

TABLE IIA
PHENOL SERIES[13]

Substituent	Log P	Log k'	Calc. log P	π	κ	Calc. π
–H	1.46	−0.164	1.61	0.00	0.000	0.15
3-OCH₃	1.56	−0.213	1.52	0.10	−0.049	0.06
4-NO₂	1.91	−0.100	1.73	0.45	0.064	0.27
4-CH₃	1.94	0.025	1.97	0.48	0.189	0.51
2-CH₃	1.95	0.090	2.09	0.49	0.254	0.63
2-Cl	2.15	0.083	2.08	0.69	0.247	0.62
2,6-CH₃	2.34	0.241	2.38	0.88	0.405	0.92
4-Cl	2.39	0.233	2.37	0.93	0.397	0.91
4-Br	2.59	0.326	2.54	1.13	0.490	1.08

TABLE IIB

ANILINE SERIES[13]

Substituent	Log P	Log k'	Calc. log P	π	κ	Calc. π
-H	0.90	−0.216	0.95	0.00	0.000	0.06
3-OCH₃	0.93	−0.145	1.11	0.03	0.071	0.21
4-OCH₃	0.95	−0.321	0.72	0.05	−0.105	−0.18
2-CH₃	1.32	−0.004	1.43	0.42	0.212	0.53
3-NO₂	1.37	0.029	1.50	0.47	0.245	0.60
4-CH₃	1.41	0.021	1.48	0.51	0.237	0.58
3-CH₃	1.43	0.004	1.45	0.53	0.220	0.55
2-NO₂	1.79	0.083	1.62	0.89	0.299	0.72
4-Cl	1.83	0.182	1.85	0.93	0.398	0.95
2-Cl	1.92	0.210	1.91	1.02	0.426	1.01
4-Br	2.26	0.262	2.02	1.36	0.478	1.12
2,4-Cl	2.69	0.585	2.75	1.79	0.801	1.85

TABLE III

COMPARISON OF OBSERVED AND CALCULATED BIOLOGICAL RESPONSES

Compound	Observed log (1/c)*	Calculated log (1/c) from κ	Calculated log (1/c) from π
Phenol	3.901	3.769	3.647
3-Methoxyphenol	3.480	3.710	3.715
4-Nitrophenol	4.219	3.845	3.952
4-Methylphenol	3.709	3.995	3.972
2-Methylphenol	3.835	4.073	3.979
2-Chlorophenol	4.238	4.065	4.114
2,6-Dimethylphenol	4.036	4.254	4.243
4-Chlorophenol	4.426	4.244	4.277
4-Bromophenol	4.463	4.356	4.412

* Toxicity to *Daphnia magna.*

tween log P values, it is not possible to extend the correlation over a wide range of solvent types (alcohols, esters, ketones, halogenated hydrocarbons). The successful correlation in the present study therefore indicates that either there is a good approximation of the solvent forces in Bondapak C-18/acetone–water to those of alcohol–water systems or that the chemical potential of either the lipophilic (μ_l) or hydrophilic (μ_h) components of the solute remains constant[6,7].

$$\log P = \frac{m\Delta\mu_l + j\Delta\mu_h}{2.3\,RT}$$

In addition, although neither the *n*-octanol–water system nor the Bondapak C-18/acetone–water chromatographic system can be construed to be structurally representative of a biological membrane, the somewhat comparable results upon substitution of κ for π in a Hansch-type biological correlation[8-11] (determined for the nine phe-

nols listed in Table III) indicate the predictive powers of k' and P in evaluating the ability of an organic molecule to pass through biological tissue[12].

$$\log (1/c) = \pi; \ r = 0.76$$
$$\log (1/c) = \kappa; \ r = 0.68$$

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