Hydrogen-bonding properties of reversed-phase liquid chromatographic stationary phases used for measuring solute hydrophobic/lipophilic parameters

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Reversed-phase liquid chromatographic (RPLC) methods may provide a suitable alternative to shake-flask methods for the measurement of solute hydrophobic/lipophilic parameters. Stationary phases generally used for this purpose are of two types, i.e. siliceous supports coated with a layer of oil (for example, octan-1-ol, (Lewis et al., 1983)), and silicas chemically modified with alkyl groups (Hafkenscheid and Tomlinson, 1983). For practical reasons the use of physically adsorbed liquid stationary phases is minimal, and alkyl-bonded stationary phases are almost exclusively used.

A main advantage in using these latter systems is that non-aqueous solvents may be incorporated into the aqueous mobile phase to reduce its polarity and (consequently) solute retention — resulting in a considerable expansion of the measurable range of hydrophobic/lipophilic property values. In general, the relationships found between distribution coefficients (K_d) and RPLC capacity factors (k') may be represented by:

$$\log K_d = a \cdot \log k' + b \tag{1}$$

where a and b are constants. Eqn. 1 is a derivative of the relationship found by Collander (1951) existing between distribution coefficients obtained in two different phase systems, with water as the polar phase, and the non-aqueous phase containing the same functionalities, i.e.

$$\log K_{d,1} = a \cdot \log K_{d,11} + b \tag{2}$$

with subscripts I and II referring to different non-aqueous solvents. Collander showed that Eqn. 2 is of significant value when comparing different alcohol/water phase systems. Leo and Hansch (1971), Leo et al. (1971) and Rekker (1977) have used the Collander equation to study the relationships between distribution coefficients obtained in the octan-1-ol/water system, and those measured for various other organic solvent/water systems. Although they found Eqn. 2 to hold well for systems with organic solvents similar to octan-1-ol, the relationship failed with increasing difference in hydrogen-bonding properties between octan-1-ol and the second solvent studied (e.g. chloroform, alkanes). In such cases a more reliable use of Eqn. 2 required the generation of separate equations for two or more solute groups, to be distinguished according to their proton donor/acceptor properties.

To quantitatively account for differences observed between octan-1-ol/water and alkane (cyclohexane)/water distribution coefficients ($K_{d,oct}$ and $K_{d,alk}$, respectively), Seiler (1974) proposed the introduction of a functional group hydrogen-bonding parameter I_h. By postulating the a coefficient in Eqn. 2 to be unity, (assuming I_h to fully account for differences in solvent hydrogen-bonding capacities), he obtained a modified Collander equation of the form:

$$\log K_{d,oct} = \log K_{d,oct} + I_h + b \tag{3}$$

By least-squares regression analysis of measured literature $\log K_d$ values, Seiler calculated I_h values for 23 different functionalities (including electronic and neighbourhood effects), and found these to adequately describe the differences between experimental $\log K_{d,oct}$ and $\log K_{d,alk}$ values.

In alkyl-bonded RPLC, where mobile phase effects are generally perceived to be largely responsible for (differences in) solute retention, the stationary phase is often considered to be an approximately "indifferent" environment. If this were correct, a priori one would expect to find a general correlation between k' and solute alkane/water distribution coefficients. Fig. 1 shows this not to be the case. Here logarithmic capacity factors, $\kappa_{0.50}$, determined in the RPLC system Hypersil octadecylsilane (ODS)/aqueous methanol with a methanol volume fraction of 0.50, have been plotted against solute $\log K_{d,alk}$ values, (averaged values for various alkane/water phase systems, Hansch and Leo, 1979). Logarithmic capacity factors and alkane/water distribution coefficients are given in Table 1. (For full experimental details, see Hafkenscheid and Tomlinson, 1983.) For most neutral and basic solutes studied, a distinct relationship exists between $\log K_{d,alk}$ and $\kappa_{0.50}$, although very weak bases, (compounds 28, 32, 33, 44, Table 1), are found to behave differently. Acids and alcohols are displaced in a somewhat irregular manner from the general relationship. Such observations are analogous to the findings of Hansch and Leo (1971), Leo et al. (1971) and Rekker (1977) for relationships between $\log K_{d,alk}$ and $\log K_{d,oct}$. Moreover, it is interesting to note that the deviations observed in Fig. 1 may be accounted for using Seiler's hydrogen-bonding term values. This is shown in Fig. 2, where $\kappa_{0.50}$ plotted against ($\log K_{d,alk} + \Sigma I_h$) — gives a linear relationship, although some significant outliers exist (i.e. chloramphenicol, p-hydroxybenzoic acid, progesterone and phenobarbitone). However, these solutes ex-

TABLE 1

SOLUTE LOGARITHMIC CAPACITY FACTORS AND ALKANE/WATER DISTRIBUTION COEFFICIENTS

	$\kappa_{0.50}$	$\log K_{d,alk}$
1. Chloroform	0.480	1.74
2. n-Butanol	−0.054	−0.80
3. n-Pentanol	0.314	−0.33
4. n-Hexanol	0.694	0.45
5. n-Caproic acid	0.588	−0.65
6. n-Caprylic acid	1.288	0.65
7. Ethylether	0.055	0.63
8. Ethylacetate	−0.075	0.31
9. Cyclohexane	1.633	4.15
10. Benzene	0.663	2.23
11. Toluene	1.001	2.84
12. Ethylbenzene	1.302	3.30
13. n-Propylbenzene	1.648	3.47
14. Chlorobenzene	1.011	2.94
15. Nitrobenzene	0.471	1.46
16. Phenol	0.020	−0.82
17. Aniline	−0.146	−0.02
18. Benzoic acid	0.276	−0.71
19. Methylbenzoate	0.663	2.08
20. Benzylalcohol	0.023	−0.65
21. p-Xylene	1.346	3.44
22. p-Cresol	0.347	- 0.17
23. p-Toluidine	0.165	0.49
24. p-Dichlorobenzene	1.337	3.48
25. p-Chlorophenol	0.538	−0.10
26. p-Chloroaniline	0.310	0.62
27. p-Nitrophenol	0.218	−1.96
28. p-Nitroaniline	−0.069	−1.03
29. Hydroquinone	−0.84	−4.25
30. p-Hydroxybenzoic acid	−0.33	−1.84
31. Methylparaben	0.230	−0.95
32. Methyl-p-aminobenzoate	−0.021	−0.22
33. Ethyl-p-aminobenzoate	0.288	−0.07
34. 1,3,5-Trichlorobenzene	2.098	4.40
35. Benzophenone	1.140	3.29
36. Naphthalene	1.288	3.38
37. Barbitone	−0.222	−2.05
38. Amobarbitone	0.679	−1.52
39. Pentobarbitone	0.679	−1.46
40. Phenobarbitone	0.159	−2.70
41. Phenytoin	0.544	−2.33
42. Primidone	−0.050	−3.22
43. Chloramphenicol	0.107	−1.48
44. Methaqualone	0.728	0.55
45. Progesterone	1.743	1.23
46. Testosterone	1.304	0.37
47. Cortisone	0.575	− 0.44
48. Hydrocortisone	0.686	−2.04

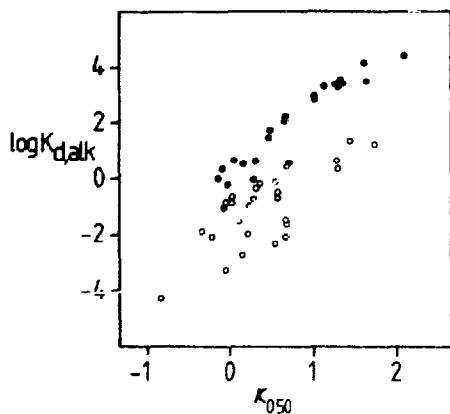Fig. 1.   Fig. 2.

Fig. 1. Relationship between the isocratic logarithmic RPLC capacity factor $\kappa_{0.50}$ (measured aqueous methanol mobile phases with a methanol volume fraction of 0.50, see Discussion) and logarithmic alkane/water distribution coefficients, log $K_{d,alk}$. Closed circles: neutral, basic and amphiprotic solutes; open circles: acids and alcohols.
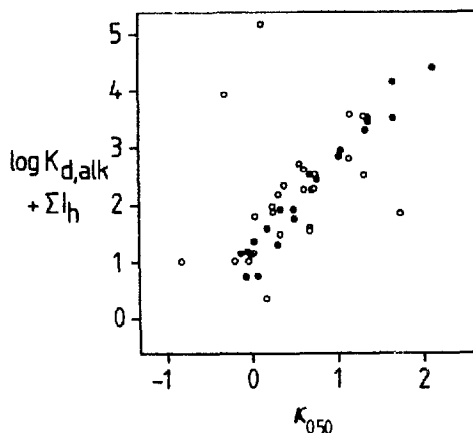
Fig. 2. Relationship between the isocratic logarithmic RPLC capacity factor $\kappa_{0.50}$, and logarithmic alkane/water distribution coefficients with added contributions for solute hydrogen-bonding abilities according to Seiler (1974). Key as for Fig. 1.

hibit similar behaviour when (log $K_{d,alk} + \Sigma I_h$) is related to log $K_{d,oct}$.

This indicates that in the RPLC system used, the stationary phase has (octan-1-ol-like) hydrogen-bonding properties. We believe this to be due to adsorption of mobile phase components by the ODS material. This assumption is supported by the fact that, where necessary, the presence of an appropriate concentration of NH_4^+ in the mobile phase (~ 41 mmol $\cdot$ dm^{-3}) has been found to eliminate peak tailing and dependence of solute retention times on amounts injected, which are caused by adsorption effects due to residual silanol groups (Sugden et al., 1978). Whilst the adsorption of methanol from aqueous methanol mobile phases by alkyl-bonded silicas is generally accepted, water has also been shown to be adsorbed to a considerable extent (Yonker et al., 1982a and b).

Confirmatory evidence for the hydrogen-bonding abilities of alkyl-bonded stationary phases can be obtained upon examination of selected retention data. For example, Tanaka and Thornton (1977) have determined the capacity factors of benzoic acid and 13 aliphatic alcohols and carboxylic acids using μ-Bondapak C-18 as stationary phase, and aqueous phosphate buffer mobile phases (with all acids chromatographed in their unionized form). Fig. 3 shows the plot of the resulting $\kappa_{0.00}$ values (with subscript 0.00 referring to zero mobile phase modifier content) versus solute log $K_{d,oct}$ and log $K_{d,alk}$ values (Hansch and Leo, 1979), clearly indicating the hydrogen-bonding ability of the μ-Bondapak C-18 when using "non-organic" mobile phases. Examination of the retention data given by Berendsen and de Galan (1980) for 3 n-alcohols and acetone using custom-made RP-10, RP-14, RP-18 and RP-22 silicas with water as mobile phase, shows this property to be common to other related alkyl-bonded phases. Although no direct, unambiguous cause for the hydro-
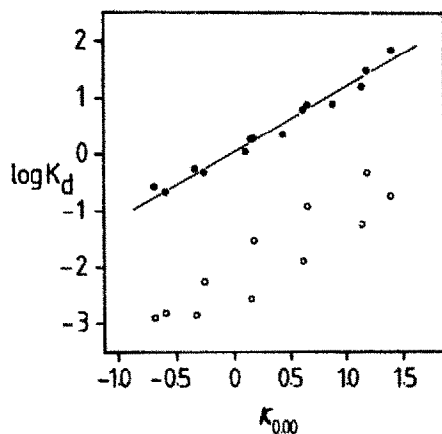
Fig. 3. Relationships between isocratic logarithmic capacity factors for aqueous mobile phases from Tanaka and Thornton (1977), $\kappa_{0.00}$, and solute logarithmic octan-1-ol/water distribution coefficients (closed circles), and logarithmic alkane/water distribution coefficients (open circles). The solid line is the calculated linear regression line between $\kappa_{0.00}$ and logarithmic octan-1-ol/water distribution coefficients.

gen-bonding properties of these materials can be indicated, Berendsen's finding that solute peaks were fronting rather than tailing makes it improbable that hydrogen-bonding effects are due to adsorptive properties of residual silanol groups. A remaining probability is that these effects are caused by adsorbed water, implying the octan-1-ol/water-like behaviour of alkyl-bonded/aqueous methanol RPLC systems to be the result of adsorption of water as well as methanol. This finding suggests that, instead of using RPLC retention data to estimate octan-1-ol/water distribution behaviour, such data can be used independently as descriptors of solute hydrophobic/lipophilic properties.

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