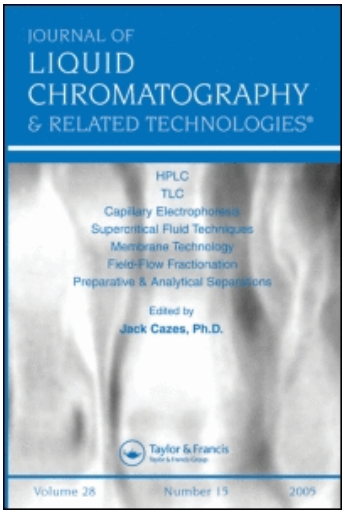

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SOLVATOCHROMIC ANALYSIS OF THE RETENTION MECHANISM OF TWO NOVEL STATIONARY PHASES USED FOR MEASURING LIPOPHILICITY BY RP-HPLC

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ABSTRACT

The lipophilicity of a large set of solute was measured by reversed-phase high-performance liquid chromatography using two novel stationary phases, namely an octadecyl polyvinyl-alcohol copolymer (ODP) and an octyl-silane (OS) phase. Solvatochromic analysis of the results showed the ODP/buffer system to bear a close resemblance to 1-octanol/buffer system. In contrast, the OS phase is a stronger H-bond acceptor than 1-octanol and will lead to overestimating the lipophilicity of strong H-bond donor solutes.

INTRODUCTION

Lipophilicity, as expressed by the partition coefficient P, is a physicochemical parameter of importance in QSAR studies. Since the pioneering

work of Meyer [1] and Overton [2], several techniques have been developed to determine lipophilicity experimentally, namely the "shake-flask" (SF) method [3,4], the AKUFVE method [5], reversed-phase thin-layer chromatography [6,7], reversed-phase high-performance liquid chromatography (RP-HPLC) [8-11], and more recently in our laboratory centrifugal partition chromatography (CPC) [12,13]. While the CPC technique has advantages, RP-HPLC still remains a method of choice for assessing lipophilicity, in particular for highly lipophilic compounds (log P > 3).

Silica-gel bonded phases such as octadecylsilane (ODS) are the most frequently used lipophilic stationary phases. These types of stationary phases, however, possess a high proportion of free acidic silanol groups (pKa = 6.8 ± 0.2 [14]), which elicit silanophilic interactions with basic and other very polar compounds. The proportion of unreacted silanol groups (up to one-half [15,16]) can be reduced by "end-capping" treatment consisting of secondary silanization reaction with short alkyl groups like trimethylsilane. However, and as warned by many authors [17-19], an end-capped silica still bears unreacted silanols, which strongly affect the retention behaviour of solutes. The addition of a masking agent such as n-decylamine or N,N-dimethyloctylamine to the mobile phase decreases [20,21] but not necessarily suppresses [22] such interactions. Furthermore, a masking agent introduces an additional variable in the mechanism of retention by virtue of its own selective effect on retention.

In recent years, Kamlet, Taft and co-workers have developed a new set of parameters expressing the dipolarity/polarizability (π^*), the hydrogen-bond donor acidity (α) and the hydrogen-bond acceptor basicity (β) of monofunctional solutes. These parameters have proven useful in identifying and evaluating the relative contribution of structural factors encoded in various physical properties such as water solubility and lipophilicity [23-28], as described by equation 1:

$$XYZ = m(V_I/100) + s\pi^* + d\delta + b\beta + a\alpha + XYZ_0 \quad (1)$$

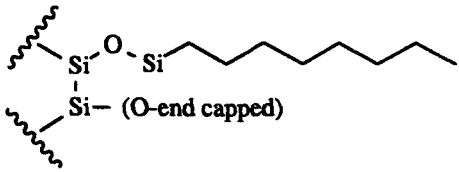
where XYZ is a solubility or solubility-related property, V_I the intrinsic molar volume of the solutes, δ the polarizability correction parameter (Hildebrand's solubility). The regression coefficients *m*, *s*, *b*, and *a* reflect the relative contribution of each parameter to the solute property in a given solvent system, and XYZ_0 is the intercept. In eq.1, $V_I/100$ is used so that the parameter measuring the cavity term will roughly cover the same numerical range

(~0.0-1.0) as the other independent variables π^*, β and α. Thus, the relative contribution of the various terms to the property XYZ is easy to evaluate.

Using these parameters, Sadek et al. have investigated the partitioning behaviour of well selected "*non-silanophilic*" solutes in several silica-gel bonded phases [29]. The results show a typical 1-octanol/water partitioning behaviour, i.e., an important contribution from both molar volume ($V_I/100$) and hydrogen-bond acceptor basicity (β), a small but significant contribution of dipolarity/polarizability (π^*), and a non-significant contribution of the hydrogen-bond donor acidity term (α) due to an equal and balanced hydrogen-bond acceptor capacity of both the eluent and the stationary phase. Unfortunately, the limited range of solutes in this study, i.e. the lack of strong H-bond donor solutes, the strongest being alkanols ($\alpha \sim 0.3$), restricts its usefulness. More recently, Kamlet et al. have applied the same approach to another set of compounds and compared the retention behaviour in ODS stationary phase with 1-octanol/water partition [30]. They have recommanded the use of ODS-C18 stationary phase with a 30/70 methanol-water mobile phase for predicting log P_{oct}.

Recently, two novel lipophilic stationary phases, namely an octadecyl polyvinyl-alcohol copolymer (ODP) and an octyl-silane (OS), have become commercially available (figure 1). Being devoid of reactive silanol groups and presenting many other advantages like sharp resolution with a good number of theoretical plates, efficient separation of basic compounds without the help of a masking agent, stability over a wide pH range, reduced swelling and shrinkage, and the possibility to have a fair flow rate without undesired pressure increases at the column inlet, the ODP stationary phase was shown to provide a valuable alternative for measuring lipophilicity [31-34]. The purpose of the present study is to assess the intermolecular forces elicited by these lipophilic stationary phases (ODP and OS) using the solvatochromic parameters and to verify whether they mimic the lipophilic character of 1-octanol, the lipophilic solvent of reference. A large number of solutes, belonging to different chemical classes and covering a wide range in lipophilicity and polarity, were selected. Isocratic capacity factors (log k_i) at various concentrations of co-solvent methanol were measured and linearly extrapolated to 100% aqueous mobile phase providing the lipophilic index log k_w, a better descriptor of solute lipophilicity [11,35].

Octylsilane (OS) stationary phase



Octadecylpolyvinyl (ODP) stationary phase

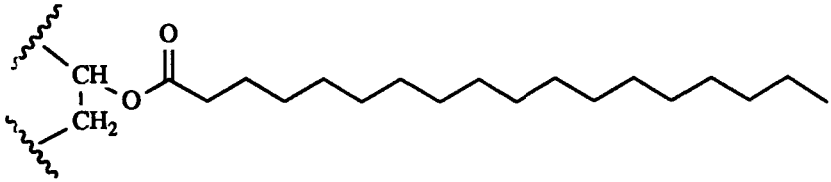


FIGURE 1. Simplified structure of the two stationary phases used in this study.

MATERIALS

All compounds were obtained from commercial sources (Merck, Darmstadt, Germany; Fluka, Buchs, Switzerland; Janssen Chimica, Beerse, Belgium; Aldrich-Chemie, Steinheim, Germany) and in the highest available purity. Analytical grade 1-octanol and morpholinopropane sulfonic acid (MPS) were purchased from Merck, HPLC grade methanol from Machler (Basel, Switzerland) and PIC-B8 buffer from Waters (Millipore, Volketswil, Switzerland).

METHODS

Measurement of Capacity Factors by RP-HPLC

The chromatograph was equipped with a MSI 660 Auto-Sampler with a 20 µl injection loop, a HPLC Pump 420, a Column Oven 480 and an Oven Controller 480, a Detector 432 (all from Kontron, Switzerland), and a SP4100 Computing Integrator (Spectra-Physics). We used an octadecyl polyvinyl-alcohol copolymer (ODP) stationary phase (15 cm x 6 mm I.D., 5µm, Asahi Chemicals, Kawasaki Japan), and a deactivated octyl-silane (OS-C8) stationary phase (Lichrosorb RP-Select B, 5µm) packed in a Hibar LichroCART column (manu-fix, 25 cm, Cat 15543, Merck). For all analyses, the flow-rate was set at 1 ml/min, the column oven temperature at 37°C and the detection wavelength at 254 nm or 190 nm. The column dead time (t_0) was defined as the retention time of an unretained compound ($K_2Cr_2O_7$). The experimental procedure has already been described [32].

The log k_w values were either determined directly using an 100% aqueous buffer mobile phase (phosphate, PIC-B8 and morpholinopropane sulfonic acid 10^{-2}M buffers, pH range 3-10), or extrapolated to 100% water from the isocratic capacity factors determined using methanol/buffer mixtures containing between 30 and 80% (v/v) methanol. For acids and bases, equations 2 and 3 were used to correct for ionization:

$$\log k_w = \log k_w^{app} + \log (1 + 10^{pH-pka}) \qquad \text{for acids} \qquad (2)$$

$$\log k_w = \log k_w^{app} + \log (1 + 10^{pka-pH}) \qquad \text{for bases} \qquad (3)$$

where log k_w^{app} is the logarithm of the apparent capacity factor determined at 100% water.

Measurement of Partition Coefficients by CPC

1-Octanol/water partition coefficients (log P_{oct}) were assessed for some compounds by the CPC method using a Flow-Through Multilayer Coil Planet Centrifuge instrument (P.C. Inc., Kim Place, Potomac, MD, USA) or a

Horizontal Flow-Through Multilayer CPC instrument (Pharma-Tech Research, Baltimore, MD, USA) (see table 1). The equipment and experimental procedures have been described previously [12,13].

RESULTS AND DISCUSSION

Table 1 reports various lipophilicity indices expressed as the logarithm of partition coefficients between 1-octanol and water (log P_{oct}), and the logarithm of capacity factors at 100% aqueous phase using OS and ODP stationary phases (log k_w^{OS} and log k_w^{ODP}, respectively). Also listed are the solvatochromic parameters taken from the literature [26,28] or calculated according to Hickey et al. [36]. In order to avoid introducing erroneous values in the regression analysis, and as Hickey's rules are neither accurate for compounds containing S-methyl groups nor sensitive to the difference between ortho and para substitution, we calculated the solvatochromic parameters only for compounds lacking ortho-substitutents or sulfur atom. The polarizability correction parameter δ, not listed in Table 1, is 1 for aromatic compounds, 2 for biphenyl and 0 for non-polyhalogenated aliphatics. Log P_{oct} values range from -0.77 (methanol) to 3.90 (biphenyl), log k_w^{OS} from 0.26 (2,6-difluorobenzamide) to 3.63 (1,3,5-tribromobenzene) and log k_w^{ODP} from -0.94 (methanol) to 4.63 (biphenyl); methanol and biphenyl were not measured on the OS-stationary phase.

Table 2 shows the solvatochromic analyses of lipophilicity data, log P_{oct}, log k_w^{OS} and log k_w^{ODP}; the original solvatochromic equation for log P_{oct} [26] is included for comparison. The OS data set is constituted only of aromatic compounds, hence the δ parameter has a value of 1 for all compounds and is not taken into account in the regression analysis. For unknown reasons, ethylamine was found to be an outlier in the regression analysis (equations not shown) and was removed from the ODP data set.

Comparing the solvatochromic analyses of the log P values of the three data sets (equations 4, 5, and 7) reveals that the regression coefficients are very similar. This is consistent with Kamlet's statement that having a data set composed of only aliphatic or aromatic solutes does not affect significantly the regression coefficients of the equation [26]. Our postulate is therefore that it is permissible to compare solvatochromic analyses based on different sets of

TABLE 1. Lipophilicity and Solvatochromic Parameters of Investigated Solutes.

Compound	log P_{oct} [a]	log k_w^{OS} [b]	log k_w^{ODP} [c]	$V_I/100$ [d]	π^* [e]	β [f]	α [g]
1. Methanol	-0.77	- [h]	-0.94	0.21	0.40	0.42	0.35
2. Ethanol	-0.31	-	-0.43	0.31	0.40	0.45	0.33
3. 1-Propanol	0.25	-	0.14	0.41	0.40	0.45	0.33
4. Isopropanol	0.05	-	0.01	0.40	0.40	0.51	0.31
5. 1-Butanol	0.75	-	0.73	0.50	0.40	0.45	0.30
6. 2-Butanol	0.76	-	0.49	0.52	0.40	0.51	0.31
7. Isobutanol	0.76	-	0.46	0.50	0.40	0.51	0.31
8. Cyclohexanol	1.23	-	1.11	0.64	0.45	0.51	0.31
9. Acetone	-0.24	-	0.13	0.38	0.71	0.48	0.04
10. Diethyl ether	0.89	-	0.85	0.52	0.27	0.47	0.00
11. Formic acid	-0.54	-	-0.61	0.23	0.65	0.38	0.65
12. Acetic acid	-0.17	-	-0.20	0.32	0.60	0.45	0.56
13. Propionic acid	0.30	-	0.47	0.42	0.58	0.45	0.56
14. Ethyl acetate	0.73	-	0.98	0.52	0.55	0.45	0.00
15. Ethylamine	-0.13	-	-0.29	0.34	0.32	0.70	0.05
16. Acetonitrile	-0.34	-	-0.09	0.27	0.75	0.31	0.09
17. N,N-Dimethylformamide	-1.01	-	-0.32	0.47	0.88	0.69	0.00
18. Tetrahydrofurane	0.46	-	0.53	0.42	0.27	0.47	0.00
19. Benzene	2.13	1.40	2.40 [i]	0.49	0.59	0.10	0.00
20. Biphenyl	3.90	-	4.63 [i]	0.92	1.18	0.20	0.00
21. Naphthalene	3.30	-	3.94 [i]	0.75 [j]	0.70 [j]	0.20 [j]	0.00 [j]
22. Toluene	2.68	-	3.25 [i]	0.59	0.55	0.11	0.00

(continued)

TABLE 1. *(Continued).*

Compound	log P_{oct} [a]	log k_w^{OS} [b]	log k_w^{ODP} [c]	$V_I/100$ [d]	π^* [e]	β [f]	α [g]
23. Trifluoromethylbenzene	2.79	-	3.68 [i]	0.68 [j]	0.84 [j]	-0.11 [j]	0.15 [j]
24. Fluorobenzene	2.27	1.50	2.93 [i]	0.52	0.62	0.07	0.00
25. Chlorobenzene	2.89	1.98	3.25 [i]	0.58	0.71	0.07	0.00
26. Bromobenzene	2.99	2.12	3.64 [i]	0.62	0.79	0.06	0.00
27. Iodobenzene	3.25	2.21	3.89 [i]	0.67	0.81	0.05	0.00
28. Nitrobenzene	1.85	-	2.62 [i]	0.63	1.01	0.30	0.00
29. 1,3-Difluorobenzene	2.33 [k]	1.72	-	0.55 [j]	0.67 [j]	0.03 [j]	0.00 [j]
30. 1,3-Dichlorobenzene	3.60	2.62	-	0.67 [j]	0.75 [j]	0.03 [j]	0.00 [j]
31. 1,3-Dibromobenzene	3.75	2.91	-	0.75 [j]	0.89 [j]	0.01 [j]	0.00 [j]
32. 1,3,5-Trichlorobenzene	4.15	3.22	-	0.76 [j]	0.70 [j]	0.00 [j]	0.00 [j]
33. 1,3,5-Tribromobenzene	4.51	3.63	-	0.88 [j]	0.94 [j]	-0.03 [j]	0.00 [j]
34. Benzyl alcohol	1.10	0.77	1.33 [i]	0.63	0.99	0.52	0.39
35. 2-Fluorobenzyl alcohol	1.30 [k]	0.84	-	-	-	-	-
36. 4-Fluorobenzyl alcohol	1.32 [k]	0.89	-	0.66 [j]	1.02 [j]	0.47 [j]	0.47 [j]
37. 2-Chlorobenzyl alcohol	1.77	1.33	-	-	-	-	-
38. 4-Chlorobenzyl alcohol	1.96	1.42	-	0.72	1.11	0.42	0.40
39. 2-Bromobenzyl alcohol	2.08 [k]	1.52	-	-	-	-	-
40. 4-Bromobenzyl alcohol	2.15 [k]	1.63	-	0.76 [j]	1.19 [j]	0.44 [j]	0.49 [j]
41. 2-Iodobenzyl alcohol	2.43 [k]	1.68	-	-	-	-	-
42. 2,6-Difluorobenzyl alcohol	1.12 [k]	0.83	-	-	-	-	-
43. 2,6-Dichlorobenzyl alcohol	2.02	1.50	-	-	-	-	-
44. 2-Phenylethanol	1.36	-	1.93 [i]	0.63	0.99	0.52	0.39

(continued)

TABLE 1. *(Continued).*

Compound	log P_{oct} [a]	log k_w^{OS} [b]	log k_w^{ODPc}	$V_I/100$ [d]	π^* [e]	β [f]	α [g]
45. Phenol	1.46	1.33	1.81 [i]	0.54	0.72	0.33	0.61
46. 2-Fluorophenol	1.71	1.47	-	0.55 [l]	0.83 [l]	0.30 [l]	0.62 [l]
47. 4-Fluorophenol	1.77	1.48	-	0.57 [j]	0.75 [j]	0.28 [j]	0.69 [j]
48. 2-Chlorophenol	2.15	1.91	-	0.62 [l]	0.83 [l]	0.25 [l]	0.59 [l]
49. 4-Chlorophenol	2.39	2.02	-	0.63	0.72	0.23	0.67
50. 2-Bromophenol	2.35	2.07	-	0.66	0.89	0.25	0.59
51. 4-Bromophenol	2.59	2.20	-	0.67 [l]	0.79 [l]	0.23 [l]	0.67 [l]
52. 2-Iodophenol	2.65	2.32	-	-	-	-	-
53. 4-Iodophenol	2.91	2.51	-	0.72 [j]	0.94 [j]	0.35 [j]	0.71 [j]
54. 2,6-Difluorophenol	1.86 [k]	1.53	-	-	-	-	-
55. 2,6-Dichlorophenol	2.64	2.37	-	-	-	-	-
56. 2,6-Dibromophenol	3.12	2.66	-	-	-	-	-
57. 2,4,6-Trichlorophenol	3.69	3.09	-	-	-	-	-
58. Anisole	2.11	-	2.46 [i]	0.64	0.73	0.32	0.00
59. Phenyl Acetate	1.49	-	2.21 [i]	0.74	1.14	0.52	0.00
60. Methyl Benzoate	2.16	-	2.48 [i]	0.74	0.75	0.39	0.00
61. Benzoic Acid	1.87	1.81	-	0.64	0.74	0.40	0.59
62. 2-Fluorobenzoic Acid	1.77	1.91	-	-	-	-	-
63. 4-Fluorobenzoic Acid	2.07	1.99	-	0.68 [j]	0.74 [j]	0.36 [j]	0.67 [j]
64. 2-Chlorobenzoic Acid	2.05	2.12	-	-	-	-	-
65. 4-Chlorobenzoic Acid	2.65	2.49	-	0.74	0.74	0.36	0.63
66. 2-Bromobenzoic Acid	2.20	2.21	-	-	-	-	-

(continued)

TABLE 1. *(Continued).*

Compound	log P_{oct} [a]	log k_w^{OS} [b]	log k_w^{ODP} [c]	$V_I/100$ [d]	π^* [e]	β [f]	α [g]
67. 4-Bromobenzoic Acid	2.86	2.69	-	0.78	0.79	0.36	0.63
68. 2-Iodobenzoic Acid	2.40	2.49	-	-	-	-	-
69. 4-Iodobenzoic Acid	3.02	2.87	-	0.83[j]	0.79[j]	0.36[j]	0.63[j]
70. Benzaldehyde	1.48	-	1.74[i]	0.61	0.92	0.44	0.00
71. Benzonitrile	1.56	-	2.35[i]	0.59	0.90	0.37	0.00
72. Benzamide	0.64	0.51	1.24[i]	0.68[m]	0.90[m]	0.80[m]	0.49[m]
73. 2-Fluorobenzamide	0.64	0.59	-	-	-	-	-
74. 4-Fluorobenzamide	0.91	0.69	-	0.71[j]	1.00[j]	0.77[j]	0.57[j]
75. 2-Chlorobenzamide	0.63[k]	0.56	-	-	-	-	-
76. 4-Chlorobenzamide	1.55	1.22	-	0.77[j]	1.00[j]	0.77[j]	0.49[j]
77. 2-Bromobenzamide	0.70[k]	0.58	-	-	-	-	-
78. 4-Bromobenzamide	1.76	1.36	-	0.81[j]	1.05[j]	0.76[j]	0.59[j]
79. 2,6-Difluorobenzamide	0.23[k]	0.26	-	-	-	-	-
80. 2,6-Dichlorobenzamide	0.85[k]	0.55	-	-	-	-	-
81. Aniline	0.90	0.53	1.46[i]	0.56	0.73	0.50	0.26
82. 2-Fluoroaniline	1.26	0.78	-	0.59	0.83	0.45	0.28
83. 4-Fluoroaniline	1.15	0.69	-	0.59	0.73	0.45	0.28
84. 2-Chloroaniline	1.85[k]	1.20	-	0.65	0.83	0.40	0.25
85. 4-Chloroaniline	1.88[k]	1.23	-	0.65	0.73	0.40	0.31
86. 2-Bromoaniline	2.11	1.39	-	0.70	0.89	0.40	0.25
87. 4-Bromoaniline	2.26	1.45	-	0.66	0.79	0.40	0.31
88. 2-Iodoaniline	2.32	1.64	-	-	-	-	-

(continued)

TABLE 1. *(Continued).*

Compound	log P_{oct} [a]	log k_w^{OS} [b]	log k_w^{ODP} [c]	$V_I/100$ [d]	π^* [e]	β [f]	α [g]
89. 4-Iodoaniline	2.34	1.73	-	0.75 [j]	0.79 [j]	0.40 [j]	0.31 [j]
90. 2,6-Difluoroaniline	1.61 [k]	0.96	-	-	-	-	-
91. 2,6-Dichloroaniline	2.71	1.85	-	-	-	-	-
92. 2,4,6-Trifluoroaniline	1.81 [k]	1.06	-	-	-	-	-
93. 2,4,6-Trichloroaniline	3.52	2.64	-	-	-	-	-
94. N-Methylaniline	1.66	-	2.26 [i]	0.66	0.82	0.47	0.17
95. N,N-Dimethylaniline	2.28	-	2.87 [i]	0.75	0.90	0.43	0.00
96. Acetanilide	1.16	0.77	1.52 [i]	0.78 [m]	0.86 [m]	0.90 [m]	0.56 [m]
97. 2-Fluoroacetanilide	0.96	0.50	-	-	-	-	-
98. 4-Fluoroacetanilide	1.47	0.75	-	0.81 [j]	0.96 [j]	0.87 [j]	0.64 [j]
99. 2-Chloroacetanilide	1.35 [k]	0.78	-	-	-	-	-
100. 4-Chloroacetanilide	2.12	1.57	-	0.87 [j]	0.96 [j]	0.87 [j]	0.56 [j]
101. 4-Bromoacetanilide	2.29	1.62	-	0.91 [j]	1.01 [j]	0.86 [j]	0.66 [j]

[a] 1-Octanol/water partition coefficient. Values taken from the Pomona College Database. [b] Lipophilicity index measured by RP-HPLC on the OS stationary phase. [c] Lipophilicity index measured by RP-HPLC on the ODP stationary phase. [d] Cavity term. Values taken from ref [26]. [e] Dipolarity/polarizability term. Values taken from ref [26]. [f] Hydrogen-bond acceptor basicity. Values taken from [ref 26]. [g] Hydrogen-bond donor acidity. Values taken from ref [26]. [h] Not determined. [i] Taken from ref [31]. [j] Calculated according to ref [35]. [k] Measured by centrifugal partition chromatography (CPC). [l] Calculated according to ref [35], using the NH_2-OH replacement rule. [m] Taken from ref [13].

TABLE 2. Solvatochromic Analysis of Lipophilicity Data.

$XYZ = m(V_I/100) + s\pi^* + d\delta + b\beta + a\alpha + XYZ_0$ (95% confidence level in parentheses).

eq.	Data set	XYZ	*m*	*s*	*d*	*b*	*a*	XYZ_0	n	r^2	s.d.
(4)	Kamlet [a]	$\log P_{oct}$	5.35 (± 0.05)	-1.04 (± 0.04)	0.35 (± 0.03)	-3.84 (± 0.05)	0.10 (± 0.04)	0.32 (± 0.04)	245	0.992	0.13
(5)	OS [b]	$\log P_{oct}$	6.94 (± 0.74)	-0.85 (± 0.60)	0	-3.15 (± 0.32)	-0.08 (± 0.29)	-0.62 (± 0.48)	43	0.957	0.19
(6)	OS [b]	$\log k_w^{OS}$	6.21 (± 0.61)	-1.01 (± 0.50)	0	-3.10 (± 0.26)	0.85 (± 0.24)	-0.87 (± 0.39)	43	0.961	0.15
(7)	ODP [c]	$\log P_{oct}$	5.18 (± 0.78)	-1.00 (± 0.41)	0.51 (± 0.25)	-3.01 (± 0.44)	0.09 (± 0.37)	-0.08 (± 0.36)	40	0.976	0.20
(8)	ODP [c]	$\log k_w^{ODP}$	5.16 (± 0.86)	-0.17 (± 0.45)	0.65 (± 0.28)	-3.20 (± 0.49)	-0.27 (± 0.40)	-0.26 (± 0.40)	40	0.980	0.22

[a] Taken from ref. [26].

[b] Compounds n° 19, 24-27, 29-34, 36, 38, 40, 45-51, 53, 61, 63, 65, 67, 69, 72, 74, 76, 78, 81-87, 89, 96, 98, 100, 101 in Table 1.

[c] Compounds n° 1-14, 16-28, 34, 44, 45, 58-60, 70-72, 81, 94-96 in Table 1.

compounds, when the goal is not to predict partition coefficients but to unravel the underlying intermolecular forces.

Even if it is not a truly realistic interpretation of RP-HPLC retention mechanisms, the stationary phase can be compared to an organic solvent. Analysis of the regression terms *m*, *s*, *b* and *a* gives some interesting indications about the system investigated. The *m* term, which is related to the solvent cohesive energy density [37], is comparably large in all systems. This is due to the cohesive energy density of water being so large that a solute must prefer the organic over the aqueous environment, on account of the lower disruption of solvent structure in the former. The *s* term can be considered as a vector value resulting from a balance of solute-water, solute-solvent (stationary phase) dipole-dipole and induced dipole-induced dipole interactions. As a consequence, the *s* term is positive for solvents having dipole moments lower than or near that of water and negative for solvents with large dipole moments [37]. The *b* term is a measure of the balanced H-bond donor acidity of the two phases, a negative contribution meaning that water is a stronger H-bond donor than the solvent or stationary phase. The same applies to the *a* term, a measure of the balanced H-bond acceptor basicity of the two phases, a negative contribution meaning that water is a stronger H-bond acceptor than the solvent.

OS Stationary Phase

Using a silica-gel bonded phase (OS) for measuring lipophilicity, a global linear relation between log k_w^{OS} and log P_{oct} is obtained as follow:

$$\log P_{oct} = 1.09\ (\pm 0.09) \log k_w^{OS} + 0.30\ (\pm 0.16) \qquad (9)$$

$$n = 70;\ r^2 = 0.898;\ \text{s.d.} = 0.29$$

where n is the number of compounds, r^2 the correlation coefficient, s.d. the standard deviation and in parentheses the 95% confidence level.

A graphical illustration (figure 2) of such a relationship reveals that different linear relations exist for different chemical classes, expressing a difference of behaviour between the 1-octanol/water and the OS/water systems. Assuming that the halobenzenes (full line on fig. 2) do not interact in a special way with the stationary phase, all other compounds, which bear polar groups,

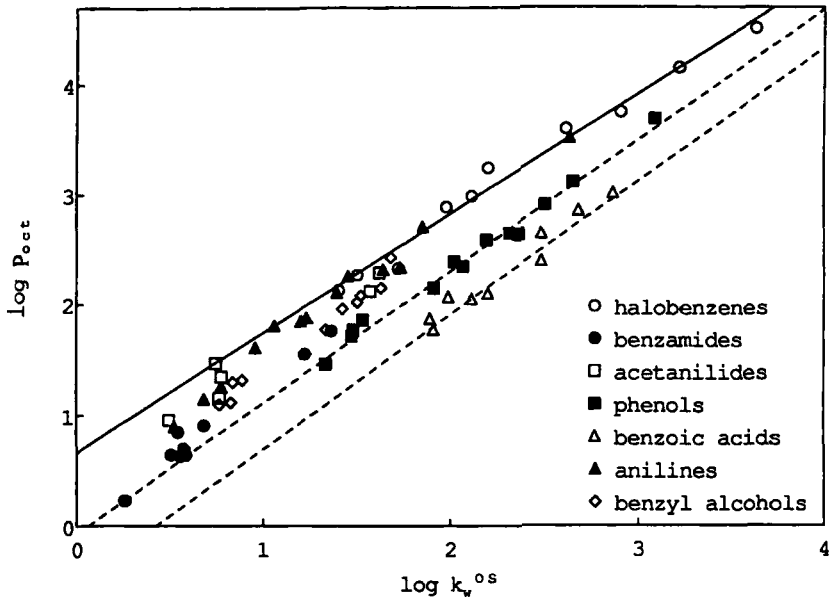


FIGURE 2. Plot of log P_{oct} versus log k_w (OS stationary phase) for solutes in Table 1. The full line refers to halobenzenes, while the two broken lines describe phenols and benzoic acids, respectively.

appear more lipophilic (i.e. are more retained) on the OS-stationary phase than in the 1-octanol/water system. The more retained compounds are, in decreasing order, benzoic acids and phenols (dashed lines), and benzamides, benzyl alcohols, anilines and acetanilides to a lesser extent.

Comparing the coefficients in equations 4, 5 and 6 (table 2) reveals some interesting trends: a) The coefficients of $V_I/100$ and β are comparable and in the three equations are the largest in absolute value. This means that the two molecular properties that most influence log P_{oct} and log k_w^{OS} are molecular size (i.e. hydrophobicity) and H-bond acceptor basicity. b) The *s* term is also comparable but small in the equations meaning that the contribution of π^* is modest. c) The *a* term, in contrast to the 1-octanol/water system where it is non-significant, is positive and of some importance (~ 10%, contribution given by the normalized equation, not shown) in explaining the retention on the OS

stationary phase. A positive contribution of the α parameter means that the stationary phase is a stronger H-bond acceptor than the aqueous mobile phase, thus enhancing the retention time and increasing the observed lipophilicity of strong H-bond donor solutes. Indeed, an inspection of the structure of OS-C8 (figure 1) shows the presence of strong H-bond acceptor groups (-Si-O-Si-). Brady et al. [38], examining the polar characteristics of silica-based GLC stationary phases, concluded that H-bond interactions occur between strong donor species and the silica gel matrix employed in RP-HPLC. Our results on the OS stationary phase are consistent with this conclusion.

The *a* term affords the only genuine difference between the 1-octanol/water and OS/water systems. Consequently, taking into account the H-bond donor acidity of the solutes (α) should improve the relationship between log P_{oct} and log k_w^{OS} (equation 10), as indeed found:

$$\log P_{oct} = 1.04\ (\pm 0.07) \log k_w^{OS} - 0.85\ (\pm 0.20)\ \alpha + 0.76\ (\pm 0.16) \qquad (10)$$

$$n = 43;\ r^2 = 0.966;\ \text{s.d.} = 0.16$$

This relation is limited to 43 solutes because the solvatochromic parameters are not available for the other compounds (see table 1).

ODP Stationary Phase

The ODP stationary phase has been shown to offer a promising alternative to silica-based packings for assessing lipophilicity [32]. In the present study, a good linear relationship between log k_w^{ODP} and log P_{oct} is found as follow:

$$\log P_{oct} = 0.83\ (\pm 0.05) \log k_w^{ODP} - 0.06\ (\pm 0.11) \qquad (11)$$

$$n = 40;\ r^2 = 0.965;\ \text{s.d.} = 0.23$$

Comparing the solvatochromic analysis of ODP retention (eq. 8), to that of partitioning in 1-octanol/water system (eq. 4 and 7) shows that: a) in both system, and in the OS/water system as well (eq. 6), the *m* and *b* terms are comparable and the largest, implying that here also solute molecular size ($V_I/100$) and H-bond acceptor strength (β) are the predominant factors governing both 1-octanol/water partitioning and ODP and OS retention; b) the *s* term, of poor contribution in eq. 4, becomes non-significant in eq. 8, meaning the ODP

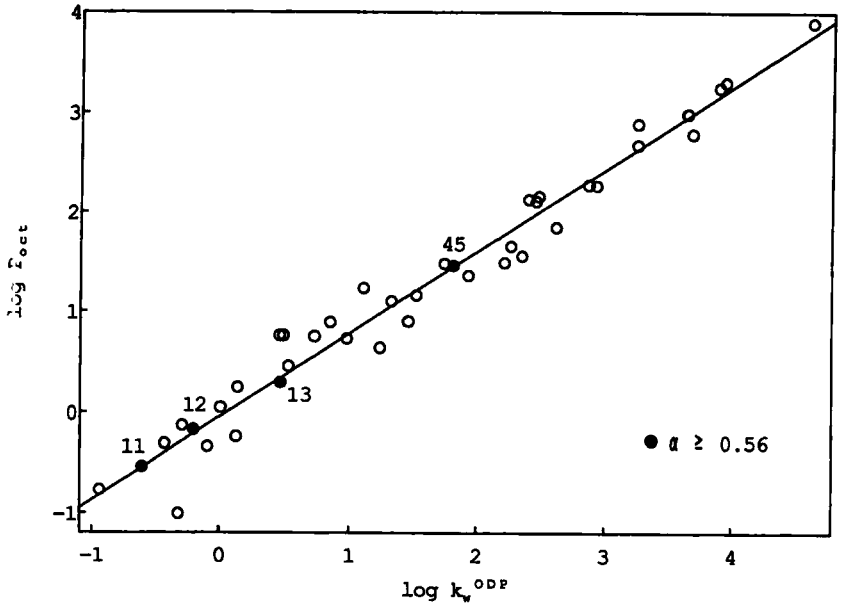


FIGURE 3. Plot of log P_{oct} versus log k_w (ODP stationary phase) for solutes in Table 1. Strong H-bond donating solutes ($\alpha \geq 0.56$) are not outliers. The line is described by equation 11.

stationary phase has a polarity cmparable to that of water; c) in both eq. 4 and 8, and in contrast to eq. 6, the *a* term is not significant, meaning that both 1-octanol and the ODP stationary phase have almost the same H-bond acceptor strength as water. Despite the fact that there are only few strong H-bond donor solutes in the ODP data set, these solutes (formic acid, acetic acid, propionic acid and phenol, $\alpha \geq 0.56$), do not deviate from the relation expressed by eq. 11 (figure 3), thus validating our conclusion. Of interest is the fact that the ODP stationary phase possesses H-bond acceptor groups (figure 1) which are not stronger H-bond acceptors than water, whereas the siloxane groups in the OS phase are. The explanation for such a difference can be electronic (greater electronegativity of C versus Si) or steric (inaccessibility of the ester groups).

Thus, the retention on the ODP stationary phase is essentially governed by molar volume and H-bond acceptor basicity of the solute. Removing non-significant terms from equation 8 yields:

$$\log k_w^{ODP} = 6.96\ (\pm 0.57)\ V_I/100 - 3.89\ (\pm 0.45)\ \beta - 0.75\ (\pm 0.38) \qquad (12)$$

$n = 40$; $r^2 = 0.964$; s.d. = 0.28

CONCLUSION

From the present and previous [29,30] findings, it can be concluded that:

a) The main factors accounting for solute retention, when aqueous mobile phases are used, are the size and H-bond acceptor strength of the solutes.

b) The silica-gel bonded stationary phases, in particular OS used in the present work, present an H-bond acceptor strength that increases retention and leads to an overestimated lipophilicity for strong H-bond donor solutes.

c) The ODP stationary phase is shown to resemble 1-octanol. It has the advantage of lacking SiOH groups (as it is not a silica-based phase), and of being usable with a 100% aqueous mobile phase, allowing direct determination of log k_w values.

d) When investigating a wide range of lipophilicity, one should prefer the log k_w index over isocratic ones, as it avoids the influence of cosolvents and renders the stationary phase/mobile phase system comparable to a biphasic organic phase/water system.

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