Chem. Pharm. Bull.
34(11)4787—4796(1986)

Method for Determination of Partition Coefficients by High-Performance Liquid Chromatography on an Octadecylsilane Column. Examination of Its Applicability

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(Received June 12, 1986)

The high-performance liquid chromatography (HPLC) of various compounds on an ODS (octadecylsilane) stationary phase was carried out with mobile phases containing various amounts of organic solvents, and the retention behavior was analyzed in terms of the partition coefficient between octanol and water P_{oct}. It was found that the capacity factor k' increased linearly with P_{oct}. However, this relation was dependent on the hydrogen-bonding ability of the compounds. The retention behaviors of non-hydrogen bonders, hydrogen acceptors and amphiprotic compounds were different.

It was shown that from the results of HPLC on an ODS column, values of P_{oct} of various compounds can be determined accurately and simply, by a single equation, when the hydrogen-bonding property of the compounds is taken into account. The accuracy of the estimated P_{oct} value increased with decrease in the concentration of the organic solvent in the mobile phase.

Keywords——partition coefficient; hydrogen-bonding ability; non-hydrogen bonder; hydrogen acceptor; amphiprotic compound; capacity factor; octadecylsilane; HPLC

The partition coefficient, expressed as the ratio of the concentration of a bioactive compound in a water-immiscible organic solvent to that in water, is known to be one of the most important physical parameters relating to the activity of the compound.[1] However, the partition coefficients of most potent bioactive compounds are very high and the compounds are hardly soluble in water. Thus, the determination of their partition coefficients by the conventional shaking-flask method is very difficult.[2] High-performance liquid chromatography (HPLC) has been used as a convenient method for determination of the partition coefficient.[3-6] We recently found that glyceryl-coated controlled-pore glass beads (gly-CPG) are especially useful as a stationary phase for determination of high partition coefficients.[7] The capacity factor (k') of a wide variety of compounds in HPLC on this column is well correlated with the partition coefficient between octanol and water (P_{oct}) up to $\log P_{\mathrm{oct}}$ of 6. In this case, values of $\log k'$ of miscellaneous compounds show a single correlation with the $\log P_{\mathrm{oct}}$ values.

The octadecylsilane (ODS) column is the most commonly used stationary phase in reversed-phase HPLC, and attempts have been made to determine partition coefficients with this column. However, the linear relation between $\log k'$ and $\log P_{\mathrm{oct}}$ seemed in general to be restricted to compounds in a homologous series,[6,8,9] and only a few systematic examinations have been carried out on the relation between partition coefficient and k'.

Thus, in this study, we tried to clarify the factors which govern the value of k' in terms of chemical structure and partition coefficient in chromatography on an ODS column. We also tried to establish a method to determine the partition coefficient of a wide variety of compounds.

Experimental

The chemicals used in this study were phenols, benzoic acids, alkylbenzenes, halogenobenzenes, and other aromatic compounds. They were obtained from commercial sources. These compounds were dissolved in methanol at about 0.5 mg/ml without further purification, and a 0.2—4 μl aliquot was injected onto the column.

HPLC was carried out at 30 °C with a TRIROTAR-II (JASCO, Tokyo) equipped with a ultraviolet (UV) detector UVIDEC-III (JASCO). The stationary phase was Finepak SIL C_{18}-T (JASCO, 25 cm × 4.6 mm i.d.), and the mobile phase was a mixture of water and an organic solvent, such as methanol, acetonitrile or tetrahydrofuran (THF). The concentration of organic solvents was expressed in terms of v/v %. The pH of the mobile phase was adjusted to 2.0 with phosphoric acid to avoid the effect of acid dissociation of the sample compounds. The flow rate was 1—2 ml/min. The values of k' that we determined were those of the neutral form of compounds.

The retention time was independent of the sample size and flow rates examined. Thus, under such chromatographic conditions, the capacity factor, k' of the sample compounds could be expressed in terms of the retention times of the sample compound (t_R) and of the unretained reference compound potassium iodide (t_0) according to Eq. 1.[10]

$$k' = (t_R - t_0)/t_0 \qquad (1)$$

Potassium iodide was confirmed not to be retained on the stationary phase, and showed an identical retention time with all mobile phases. Furthermore, the optical absorption of potassium iodide at 254 nm is very high. Thus, it is suitable for use as a reference compound.

Values of $\log P_{oct}$ used in this study were obtained from ref. 11, unless otherwise noted.

Results

In this study we used three types of compounds with respect to hydrogen-bonding ability: non-hydrogen bonders (non-H-bonders) such as alkyl- and halogenobenzenes, hydrogen acceptors (H-acceptors) such as cyanobenzene and anisole, and amphiprotic compounds such as phenols and benzoic acids. The compounds used in this study are summarized in Table I, together with values of $\log P_{oct}$ and parameters obtained from HPLC, as described below. The HPLC on an ODS-type column was carried out with a mobile phase consisting of aqueous phosphoric acid solution, pH 2.0, and an organic solvent. Methanol, acetonitrile and THF

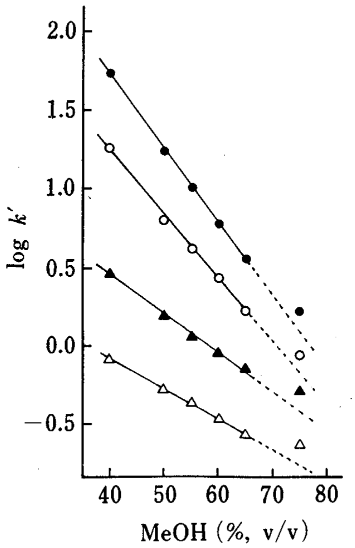

Fig. 1. Relationship between $\log k'$ and the Concentration of Methanol in the Mobile Phase

●, diphenyl; ○, *n*-propylbenzoate; ▲, nitrobenzene; △, phenol.

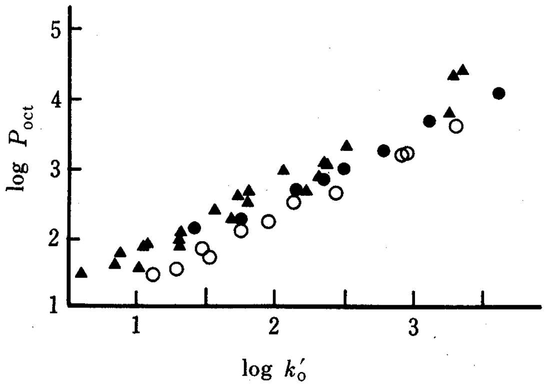

Fig. 2. Relationship between $\log P_{oct}$ and $\log k'_0$ Determined with Methanol-Containing Mobile Phase

●, non-H-bonders; ○, H-acceptors; ▲, amphiprotic compounds.

TABLE I. The Values of $\log k'_0$ and m in Eq. 2 and $\log P_{oct}$

Compounds	$\log P_{oct}$[b]	Methanol[a]		Acetonitrile[a]		THF[a]	
		$\log k'_0$	$m \cdot 10^2$	$\log k'_0$	$m \cdot 10^2$	$\log k'_0$	$m \cdot 10^2$
Non-H-bonders							
Benzene	2.13	1.430	2.189	1.321	2.502	1.925	3.372
F–Benzene	2.27	1.697	2.538	1.421	2.814	1.897	3.272
Cl–Benzene	2.84	2.356	3.331	2.035	3.657	2.511	4.444
Br–Benzene	2.99	2.510	3.488	2.183	3.854	2.575	4.456
I–Benzene	3.25	2.795	3.788	2.414	4.125	2.851	5.008
Toluene	2.69	2.166	3.048	1.877	3.301	2.358	4.274
Cumene	3.66	3.124	4.075	2.818	4.627	3.047	5.208
Diphenyl	4.04	3.633	4.766	3.140	5.350	3.341	5.884
H-Acceptors							
NO_2–Benzene	1.85	1.486	2.550	1.331	2.801	1.649	3.050
CN–Benzene	1.56	1.301	2.578	0.981	2.247	1.103	2.242
Benzaldehyde	1.48	1.134	2.209	0.866	2.058	0.928	1.926
Anisole	2.10	1.767	2.847	1.423	2.829	1.715	3.114
Ethoxybenzene	2.51	2.146	3.200	1.818	3.368	2.161	3.774
Acetophenone	1.73	1.540	2.844	1.048	2.389	0.965	2.038
Benzophenone	3.18	2.922	4.378	2.374	4.382	2.389	4.376
Methylbenzoate	2.23	1.966	3.239	1.448	2.935	1.600	3.130
Ethylbenzoate	2.64	2.450	3.748	1.930	3.655	1.922	3.520
n-Propylbenzoate	3.20	2.961	4.264	2.410	4.286	2.544	4.636
Phenylbenzoate	3.59	3.312	4.710	2.824	5.069	2.991	5.436
Amphiprotic compounds							
Benzoic acid (BA)	1.87	1.051	2.259	0.650	2.015	1.178	2.576
4-F–BA	2.07	1.460	2.779	0.875	2.498	1.553	3.062
4-Cl–BA	2.65	1.829	3.002	1.352	3.143	2.178	4.322
4-Br–BA	2.86	2.312	4.045	1.509	3.391	2.175	4.152
4-I–BA	3.02	2.387	3.777	1.705	3.622	2.403	4.586
4-Methyl–BA	2.27	1.750	3.173	1.057	2.661	1.538	3.164
4-Ethyl–BA	2.65	2.246	3.694	1.500	3.301	1.940	3.736
4-Phenyl–BA	3.75[c]	3.251	4.838	2.362	4.690	2.710	5.178
4-NO_2–BA	1.89	1.319	2.646	0.872	2.444	1.775	3.394
4-CN–BA	1.56	1.031	2.449	0.564	2.027	1.211	2.548
N-Phenylanthranilic acid	4.36	3.351	4.876	3.349	6.674	3.218	5.862
Phenol	1.48	0.613	1.767	0.426	1.614	1.051	2.144
4-F–Phenol	1.77	0.888	2.120	0.630	1.937	1.447	2.846
4-Cl–Phenol	2.39	1.576	2.921	1.227	2.908	2.054	3.882
4-Br–Phenol	2.59	1.751	3.107	1.376	3.135	2.241	4.254
4-I–Phenol	2.93	2.066	3.491	1.576	3.367	2.555	4.838
2,3,4,6-Cl–Phenol	4.26	3.280	4.423	2.767	4.984	3.492	5.917
4-Methylphenol	1.94	1.076	2.263	0.818	2.208	1.431	2.770
4-Ethylphenol	2.26	1.700	3.146	1.337	3.002	1.820	3.526
Thymol	3.30	2.530	3.870	2.309	4.490	2.917	5.136
4-NO_2–Phenol	1.91	1.081	2.381	0.816	2.321	1.515	3.042
4-CN–Phenol	1.60	0.858	2.345	0.528	1.950	1.253	2.704
Methylparaben	1.96	1.316	2.832	0.646	2.071	1.244	2.734
Ethylparaben	2.47	1.823	3.423	1.175	2.961	1.748	3.618
Propylparaben	3.04	2.378	4.058	1.632	3.578	2.235	4.394

a) Organic solvent in the mobile phase. *b*) From ref. 11. *c*) Determined in this study by the conventional shaking-flask method.

were used as organic solvents to examine their effects on the chromatography. The organic solvent in the mobile phase is necessary to increase the solubility of the test compounds and to adjust the retention time to be in a suitable range.

Figure 1 shows the dependence of $\log k'$ on the concentration of methanol. In all cases, $\log k'$ decreased almost linearly with increase in the concentration of methanol up to 60%. At more than 60%, the change in $\log k'$ became gradual. Similar relations were also observed with the mobile phases containing acetonitrile and THF. The linear relation between $\log k'$ and the concentration of organic solvent, [solv], is expressed by Eq. 2:

$$\log k' = \log k_0' - m\,[\mathrm{solv}] \qquad (2)$$

where k_0' is the capacity factor of the test compound in the absence of organic solvent, and m is the slope of the straight line.

We determined $\log k_0'$ and m with various compounds by the use of Eq. 2. These values are summarized in Table I. The concentration range of organic solvent in the mobile phase was 40%—60% for methanol, 30%—50% for acetonitrile and 30%—45% for THF. The correlation coefficients were always more than 0.97.

As shown in Fig. 2, values of $\log k_0'$ with the methanol-containing mobile phase increased almost linearly with $\log P_{\mathrm{oct}}$ values as expressed by Eq. 3. Such a linearity has sometimes been reported,[8,9] but

$$\log P_{\mathrm{oct}} = a_1 + b_1 \log k_0' \qquad (3)$$

this linear relationship depends on the chemical class of compounds,[12-14] due to differences in hydrogen-bonding ability.

Compounds used in this study were grouped into three classes with regard to hydrogen-bonding ability, and values of a_1 and b_1 determined by the least-squares calculation are summarized in Table II. Values obtained with acetonitrile and THF in the mobile phase are also included in Table II.

TABLE II. Values of a_1[a] and b_1[a] in Eq. 3: $\log P_{\mathrm{oct}} = a_1 + b_1 \log k_0'$

Solvent[b]	Chemicals	a_1	b_1	r[c]	s[d]	F[e]	n[f]	Eq. No.
Methanol	Non-H-bonders	0.77 (0.15)	0.90 (0.06)	0.997	0.055	974.4	8	4
	H-Acceptors	0.34 (0.13)	0.97 (0.06)	0.996	0.064	1265.7	11	5
	Amphiprotics	0.73 (0.17)	1.00 (0.09)	0.979	0.165	530.5	25	6
	All compounds	0.71 (0.19)	0.93 (0.09)	0.957	0.223	459.3	44	7
Acetonitrile	Non-H-bonders	0.76 (0.07)	1.03 (0.03)	0.999	0.027	4040.4	8	8
	H-Acceptors	0.52 (0.13)	1.10 (0.07)	0.996	0.068	1116.8	11	9
	Amphiprotics	1.12 (0.12)	1.05 (0.09)	0.983	0.149	651.3	25	10
	All compounds	1.07 (0.18)	0.96 (0.10)	0.943	0.257	336.6	44	11
THF	Non-H-bonders	−0.22 (0.30)	2.60 (0.24)	0.994	0.080	464.7	8	12
	H-Acceptors	0.49 (0.39)	1.04 (0.20)	0.966	0.195	125.4	11	13
	Amphiprotics	0.26 (0.28)	1.15 (0.14)	0.961	0.223	278.1	24	14
	All compounds	0.36 (0.23)	1.09 (0.11)	0.962	0.213	510.9	44	15

a) Values in parentheses are 95% confidence limits. *b*) Organic solvent in the mobile phase. *c*) Correlation coefficient. *d*) Standard deviation. *e*) Standard variance ratio statistics. *f*) Number of compounds.

It is shown in Table II that non-H-bonders, H-acceptors and amphiprotics are each accomodated very well by Eq. 3 with high statistical significance; Eqs. 4—6 are for methanol-containing eluent, Eqs. 8—10 are for acetonitrile-containing eluent, and Eqs. 12—14 are for THF-containing eluent. However, the correlation with all compounds for the case of methanol (Eq. 7) was less than those with Eqs. 4—6. In each solvent, the values of a_1 for the three classes of compounds were different from each other, though values of b_1 were almost the same, except for non-H-bonders in THF. From the finding that values of a_1 for amphiprotic compounds, non-H-bonders and H-acceptors are different, the amphiprotics are expected to act as hydrogen donors in HPLC. This was supported by the fact that addition of hydrogen-accepting and hydrogen-donating terms in the analysis gave improved correlations, as described below with methanol and acetonitrile.

We analyzed these relations by multiple regression analysis taking into account the hydrogen-bonding capability of compounds according to Eq. 16,

$$\log P_{\mathrm{oct}} = a_2 + b_2 \log k'_{\mathrm{o}} + c_2\mathrm{HA} + d_2\mathrm{HD} \qquad (16)$$

where HA is the hydrogen-accepting indicator variable (HA = 1 for H-acceptors and HA = 0 for non-H-bonders and amphiprotics) and HD is the hydrogen-donating indicator variable (HD = 1 for amphiprotic compounds and HD = 0 for non-H-bonders and H-acceptors).

The results of the analysis are shown in Table III. It is noteworthy that all the $\log k'_{\mathrm{o}}$ values of compounds with different hydrogen-bonding abilities fall in a single relation as a result of the addition of the HA and HD terms with methanol and acetonitrile, as expressed

Table III. Values of a_2, b_2, c_2 and d_2 in Eq. 16 for All Compounds Listed in Table I[a)]

$$\log P_{\mathrm{oct}} = a_2 + b_2 \log k'_{\mathrm{o}} + c_2\mathrm{HA} + d_2\mathrm{HD} \qquad (16)$$

Solvent	a_2	b_2	c_2	d_2	r	s	F	n	Eq. No.
Methanol	0.58	0.98	−0.25	0.18	0.986	0.133	451.2	44	17
	(0.17)	(0.06)	(0.13)	(0.12)					
Acetonitrile	0.70	1.06	−0.11	0.41	0.989	0.119	574.5	44	18
	(0.14)	(0.05)	(0.11)	(0.11)					
THF	0.07	1.13	0.23	0.22	0.967	0.200	193.1	44	19
	(0.29)	(0.10)	(0.20)	(0.17)					

a) For r, s, F and n, see Table II.

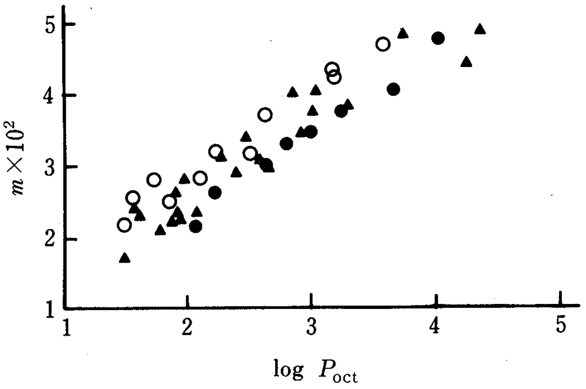

Fig. 3. Relationship between $\log P_{\mathrm{oct}}$ and Slope m in Eq. 20

●, non-H-bonders; ○, H-acceptors; ▲, amphiprotic compounds.

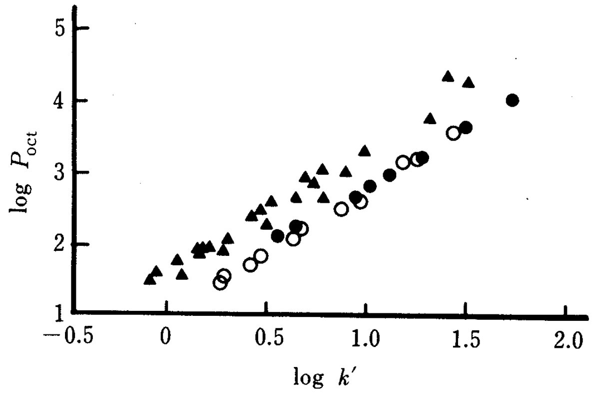

Fig. 4. Relationship between $\log P_{\mathrm{oct}}$ and $\log k'$ Determined with 40% Methanol-Containing Mobile Phase

●, non-H-bonders; ○, H-acceptors; ▲, amphiprotic compounds.

by Eqs. 17 and 18, respectively. The addition of these two terms was statistically significant in both cases. However, with THF, the addition of indicator variables did not improve the correlation (see Eqs. 15 and 19).

The results in Tables II and III show that the partition coefficient is dependent on the ability of compounds to form hydrogen-bonds, and thus we must take into consideration the hydrogen-bonding ability when we evaluate P_{oct} by the HPLC method.

As shown in Fig. 1, with methanol-containing eluent the slope m seemed to become greater with increase in the hydrophobicity of the compound. Thus, we next plotted m against $\log P_{\text{oct}}$ of the compounds as shown in Fig. 3. Apparently, m increases linearly with increase in $\log P_{\text{oct}}$, as expressed by Eq. 20.

$$\log P_{\text{oct}} = a_3 + b_3 m \tag{20}$$

In contrast to the case of the relation between $\log k_0'$ and $\log P_{\text{oct}}$, the addition of HA and HD terms to Eq. 20 did not improve the correlation. Such relationships were also observed on chromatography in the presence of acetonitrile and THF in the mobile phase. The slope m has been reported to be linearly dependent on $\log k_0'$.[5] This is also expected in this study from the result that $\log k_0'$ of each class of compounds is a linear function of $\log P_{\text{oct}}$, as summarized in Table II.

Next, the relation between P_{oct} and k' obtained at a certain organic solvent concentration in the linear region in Fig. 1, [solv], was examined. From Eqs. 2, 3 and 20, we obtained Eq. 21 for each class of compounds.

$$\log P_{\text{oct}} = \frac{1}{b_3 - b_1\ [\text{solv}]}(a_1 b_3 - a_3 b_1\ [\text{solv}] + b_1 b_3 \log k') \tag{21}$$

When [solv] is constant, Eq. 21 becomes Eq. 22.

$$\log P_{\text{oct}} = a_4 + b_4 \log k' \tag{22}$$

Equation 22 indicates that $\log k'$ determined with a mobile phase containing a certain concentration of organic solvent should be linearly dependent on the partition coefficient P_{oct}.

Figures 4 to 6 show some representative results on the linear relation between $\log k'$ and $\log P_{\text{oct}}$. In these figures, k' values with 40% methanol (Fig. 4), with 30% acetonitrile (Fig. 5), and with 30% THF (Fig. 6) are shown. As in the results in Fig. 3, $\log k'$ increased linearly with $\log P_{\text{oct}}$, as expected from Eq. 21, but this linear relation was also dependent on the

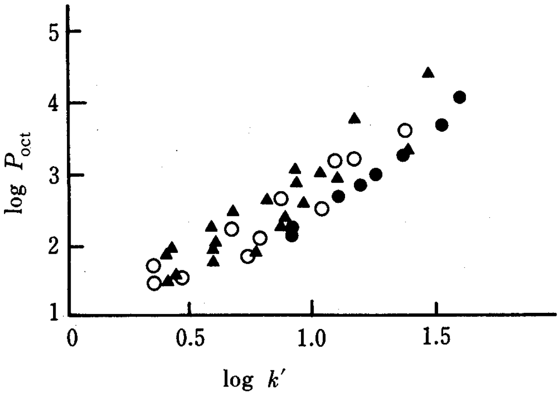

Fig. 5. Relationship between $\log P_{\text{oct}}$ and $\log k'$ Determined with 30% Acetonitrile-Containing Mobile Phase

●, non-H-bonders; ○, H-acceptors; ▲, amphiprotic compounds.

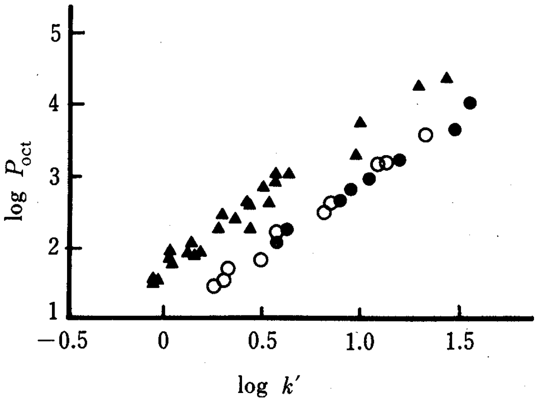

Fig. 6. Relationship between $\log P_{\text{oct}}$ and $\log k'$ Determined with 30% THF-Containing Mobile Phase

●, non-H-bonders; ○, H-acceptors; ▲, amphiprotic compounds.

hydrogen-bonding ability of compounds, as in the case of $\log k_0'$. From Eqs. 2, 16 and 20, we obtain Eq. 23 for all compounds in the three classes.

$$\log P_{\text{oct}} = \frac{1}{b_3 - b_2\,[\text{solv}]}\,(a_2b_3 - a_3b_2\,[\text{solv}] + b_2b_3\log k' + b_3c_2\,\text{HA} + b_3d_2\,\text{HD}) \qquad (23)$$

TABLE IV. Values of a_4 and b_4 in Eq. 22[a]: $\log P_{\text{oct}} = a_4 + b_4 \log k'$

Methanol (%)	Chemicals	a_4	b_4	r	s	F	n	Eq. No.
40	Non-H-bonders	1.17	1.64	0.999	0.031	3113.2	8	25
		(0.07)	(0.06)					
	Acceptors	1.00	1.77	0.998	0.049	2112.9	11	26
		(0.07)	(0.09)					
	Amphiprotics	1.56	1.75	0.985	0.141	726.3	25	27
		(0.09)	(0.13)					
	All compounds	1.50	1.52	0.935	0.273	293.9	44	28
		(0.15)	(0.18)					
50	Non-H-bonders	1.35	2.15	0.998	0.048	1303.1	8	29
		(0.09)	(0.12)					
	Acceptors	1.50	2.18	0.993	0.090	624.2	11	30
		(0.10)	(0.19)					
	Amphiprotics	2.15	2.10	0.978	0.164	497.9	25	31
		(0.07)	(0.19)					
	All compounds	1.99	1.69	0.902	0.333	163.9	44	32
		(0.13)	(0.25)					
55	Non-H-bonders	1.55	2.44	0.999	0.036	2309.8	8	33
		(0.06)	(0.10)					
	Acceptors	1.75	2.48	0.988	0.118	355.4	11	34
		(0.11)	(0.29)					
	Amphiprotics	2.43	2.36	0.974	0.182	425.7	25	35
		(0.07)	(0.23)					
	All compounds	2.21	1.85	0.888	0.355	156.4	44	36
		(0.12)	(0.30)					
60	Non-H-bonders	1.80	2.78	0.998	0.044	1556.5	8	37
		(0.07)	(0.14)					
	Acceptors	2.03	2.89	0.981	0.148	224.5	11	38
		(0.11)	(0.42)					
	Amphiprotics	2.76	2.68	0.970	0.196	368.9	25	39
		(0.08)	(0.28)					
	All compounds	2.451	2.023	0.870	0.381	130.6	44	40
		(0.12)	(0.35)					
65	Non-H-bonders	2.08	3.27	0.998	0.045	1439.9	8	41
		(0.06)	(0.17)					
	Acceptors	2.43	3.34	0.973	0.173	163.0	11	42
		(0.12)	(0.58)					
	Amphiprotics	3.20	3.00	0.962	0.220	285.8	25	43
		(0.12)	(0.34)					
	All compounds	2.74	2.13	0.838	0.421	98.9	44	44
		(0.13)	(0.43)					
75	Non-H-bonders	2.93	4.57	0.954	0.211	60.9	8	45
		(0.15)	(1.17)					
	Acceptors	3.51	5.00	0.951	0.234	85.3	11	46
		(0.31)	(1.20)					
	Amphiprotics	4.29	3.97	0.949	0.254	208.6	25	47
		(0.27)	(0.55)					
	All compounds	3.29	2.35	0.752	0.509	54.6	44	48
		(0.25)	(0.64)					

[a] For r, s, F and n, see Table II.

TABLE V. Values of Coefficients in Eq. 24[a)]

$\log P_{\text{oct}} = a_5 + b_5 \log k' + c_5\text{HA} + d_5\text{HD}$

Methanol (%)	a_5	b_5	c_5	d_5	r	s	F	n	Eq. No.
40	1.06 (0.12)	1.74 (0.08)	−0.04 (0.11)	0.51 (0.10)	0.990	0.111	652.6	44	49
50	1.37 (0.14)	2.12 (0.12)	0.15 (0.14)	0.77 (0.13)	0.985	0.137	428.7	44	50
55	1.57 (0.14)	2.40 (0.15)	0.20 (0.15)	0.86 (0.15)	0.982	0.151	352.0	44	51
60	1.82 (0.14)	2.73 (0.19)	0.23 (0.17)	0.94 (0.17)	0.978	0.166	288.3	44	52
65	2.13 (0.15)	3.10 (0.25)	0.30 (0.19)	1.10 (0.20)	0.971	0.190	217.2	44	53
75	2.93 (0.17)	4.20 (0.45)	0.40 (0.25)	1.46 (0.29)	0.950	0.246	123.9	44	54

a) For *r*, *s*, *F* and *n*, see Table II.

When [solv] is constant, we obtain Eq. 24 from Eq. 23.

$$\log P_{\text{oct}} = a_5 + b_5 \log k' + c_5\text{HA} + d_5\text{HD} \tag{24}$$

Each coefficient in Eqs. 22 and 24 was determined at various concentrations of organic solvents, as summarized for the case of methanol in Tables IV and V, respectively.

The results in Table IV indicate that the partition coefficient can be determined accurately by using Eq. 22 from k' at any concentration of organic solvent. However, since values of a_4 in Eq. 22 are dependent on H-bonding abilities at each methanol concentration, the HA and HD terms shown in Eq. 24 were found to be necessary to obtain a single relation covering all compounds, as summarized in Table V. The correlations in Tables IV and V are at almost the same level as those in Tables II and III, respectively. However, it should be noted that the correlation of Eqs. 22 and 24 became lower as the concentration of organic solvent became greater. It is also apparent that all the coefficient values in Tables IV and V, as would be expected from Eqs. 21 and 23, became greater with increase in the concentration of methanol in the mobile phase. Similar results were obtained with acetonitrile. However, in the case of THF, addition of HA and HD terms did not improve the correlation, as in the case of the relation between $\log k'_0$ and $\log P_{\text{oct}}$.

Discussion

In this study we measured the capacity factor k' with non-H-bonders, H-acceptors and amphiprotic compounds at various concentrations of organic solvent in the mobile phase on an ODS column as the stationary phase. Values of k' decreased almost linearly with increase in the concentration of organic solvent, as observed with penicillins[8)] and phenols.[9)] From the linear relationship between $\log k'$ and the concentration of organic solvent, the value of the capacity factor in the absence of organic solvent, k'_0, was determined. Both $\log k'$ at a certain concentration of organic solvent and $\log k'_0$ were found to be well correlated with $\log P_{\text{oct}}$ values. From these relationships, the value of $\log P_{\text{oct}}$ can be determined.

The direct determination of k'_0 of hydrophobic bioactive compounds with an eluent not containing an organic solvent is almost impossible because they are only sparingly soluble in water and their retention times are too great to determine their exact values. Furthermore, it is tedious to determine k'_0 from several k' values with eluents containing various amounts of

Table VI. Values of $\log P_{oct}$ and $\log k'_0$ from Ref. 17 and Calculated $\log P_{oct}$ Values from Eqs. 55 and 56

Compounds	HA[a]	HD[b]	$\log k'_0$	$\log P_{oct}$ Observed[c]	$\log P_{oct}$ Eq. 55	$\log P_{oct}$ Eq. 56
Non-H-bonders						
Benzene (Benz)	0	0	2.11	2.14	2.07	2.13
CH_3–Benz	0	0	2.74	2.74	2.72	2.72
1,2-$(CH_3)_2$–Benz	0	0	3.19	3.12	3.19	3.15
1,3-$(CH_3)_2$–Benz	0	0	3.30	3.20	3.30	3.26
1,4-$(CH_3)_2$–Benz	0	0	3.29	3.15	3.29	3.25
F–Benz	0	0	2.28	2.27	2.24	2.29
Cl–Benz	0	0	2.80	2.84	2.78	2.78
Br–Benz	0	0	2.90	2.99	2.89	2.88
H-Acceptors						
Benz–CHO	1	0	1.74	1.45	1.68	1.52
Benz–CN	1	0	1.83	1.56	1.78	1.61
Benz–NO_2	1	0	1.91	1.84	1.84	1.68
Benz–$COCH_3$	1	0	2.02	1.66	1.97	1.79
Benz–OCH_3	1	0	2.23	2.08	2.19	1.99
Benz–$COOCH_3$	1	0	2.44	2.18	2.41	2.18
Benz–$N(CH_3)_2$	1	0	2.57	2.30	2.54	2.31
Amphiprotic compounds						
Phenol (Phe)	0	1	1.27	1.48	1.19	1.50
2-Cl–Phe	0	1	2.02	2.16	1.97	2.21
3-Cl–Phe	0	1	2.29	2.50	2.25	2.47
4-Cl–Phe	0	1	2.27	2.40	2.23	2.45
2,4-$(Cl)_2$–Phe	0	1	2.90	3.06	2.89	3.05
2,5-$(Cl)_2$–Phe	0	1	2.90	3.20	2.89	3.05
2,4,5-$(Cl)_3$–Phe	0	1	3.67	3.72	3.69	3.78

a) Indicator variable for hydrogen acceptors. *b*) Indicator variable for hydrogen donors. *c*) Taken from ref. 17.

organic solvents. As shown in Fig. 1, the decrease of $\log k'$ with the concentration of organic solvent in the mobile phase is not always linear, but the change becomes quadratic at above a certain concentration of organic solvent.[15,16] Thus the determination of the true value of k'_0 is very difficult. We will deal with this problem in the subsequent paper. For practical purposes, it is more convenient to determine $\log P_{oct}$ from the $\log k'$ at a certain concentration of organic solvent in the mobile phase. However, the correlation increased with decrease in the concentration of organic solvent in the mobile phase, as shown in Table V.

It was shown in this study that these relations were dependent on the hydrogen-bonding ability of compounds. Equation 24 is very useful for the analysis of experimental data. Hammers *et al.*[17] determined $\log k'_0$ values of various compounds with methanol-containing mobile phases on an ODS column. We collected experimental values of 22 compounds from their paper, so as to have similar numbers of compounds in three classes; 8 non-H-bonders, 7 H-acceptors and 7 amphiprotic compounds (Table VI). The data were analyzed and Eqs. 55 and 56 were obtained, where n is the number of data, r is the correlation coefficient and s is the standard deviation. Numbers in parentheses are 95% confidence limits.

$$\log P_{oct} = \underset{(0.35)}{-0.12} + \underset{(0.14)}{1.04} \log k'_0 \qquad (55)$$

$$n=22, \quad r=0.958, \quad s=0.189$$

$$\log P_{\mathrm{oct}} = \underset{(0.20)}{-0.12} + \underset{(0.07)}{0.95} \log k'_{\mathrm{o}} - \underset{(0.10)}{0.26}\,\mathrm{HA} + \underset{(0.09)}{0.17}\,\mathrm{HD} \qquad (56)$$

$$n = 22, \quad r = 0.993, \quad s = 0.082$$

Here, the HA and HD terms were also shown to be necessary to obtain a high correlation between $\log P_{\mathrm{oct}}$ and $\log k'$, regardless of the hydrogen-bonding ability of chemical compounds.

The above results show that the HPLC method is useful for the determination of P_{oct} on an ODS column and that the hydrogen-bonding ability of the test compounds should be considered. For performing HPLC, methanol and acetonitrile were useful organic solvents as a component of the mobile phase, but THF was not. The reason for this is not clear at present.

The amphiprotic compounds were found to act as hydrogen donors in chromatography. It is noteworthy that for compounds with the same $\log P_{\mathrm{oct}}$ values, k' became greater in the order of H-donors, non-H-bonders and H-acceptors. This result seems strange, because the surface of ODS is covered with alkyl groups, and should be insensitive to hydrogen bonding of the test compounds. However, the retention behavior on an ODS column is complex. It is possible that the organic solvent in the mobile phase is adsorbed on the surface of the ODS and the adsorbed organic solvent governs the chromatographic behavior. Further study is necessary to clarify the mechanism of retention of organic compounds in the chromatography.

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