Lipophilicity Measurement by Reversed-Phase High-Performance Liquid Chromatography (RP-HPLC): A Comparison of Two Stationary Phases Based on Retention Mechanisms

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The mechanisms of retention of two recent stationary phases of interest in lipophilicity measurements, namely of the silica-based *Discovery-RP-Amide-C16* phase and the polymer-based *ODP-50-4B* phase, were assessed and compared. A set of 41 model solutes and drugs with well-defined solvatochromic parameters were selected to allow a broad distribution of property spaces. The chromatographic results showed that, under the conditions of study, the lipophilicity index $\log k_w$ obtained with the former stationary phase was more closely related to experimental $\log P_{oct}$ values than was $\log k_w$ obtained with the *ODP-50-4B* phase. Linear solvation/free-energy relationship (LSER) analyses showed that the retention mechanisms of the two stationary phases are different, retention on the *Discovery-RP-Amide-C16* phase and partitioning in octan-1-ol/H_2O being controlled by the same balance of intermolecular forces (*Van der Waals* volume V_w, H-bond acceptor basicity β, and dipolarity/polarizability π^*).

Introduction. – The lipophilicity of solutes, traditionally expressed by their partition coefficients in the octan-1-ol/H_2O system (noted $\log P_{oct}$), is of high significance from both a physicochemical and a pharmacological viewpoint [1][2]. The partitioning process expresses the combined effects of a number of intermolecular forces between a solute and its environment, here the solvents between which the solute partitions. These intermolecular forces are of particular importance in pharmacology since they also control the partitioning of solutes into biomembranes. Numerous studies have reported a relationship between $\log P_{oct}$ and absorption or permeability in cell cultures and tissue preparations used as models of, *e.g.*, the gastrointestinal tract or the blood-brain barrier [3–7].

The reference procedure to measure $\log P_{oct}$ is the shake-flask method which, however, is time-consuming and limited in range (*ca.* $-3 < \log P < 4$). Beyond these limits, $\log P_{oct}$ values measured by the shake-flask method become unreliable.

The reversed-phase HPLC method is a promising alternative to the shake-flask method, having such advantages as a higher throughput, an insensitivity to impurities or degradation products, and a broader lipophilicity range. In reversed-phase HPLC, lipophilicity indices are derived from the capacity factor $\log k$, which is calculated by *Eqn. 1*, where t_R and t_0 are the retention times of the solute and of an unretained compound, respectively. Some workers have used isocratic $\log k$ values measured in an appropriate mobile phase as a lipophilicity parameter [8–10]. However, many more investigators use capacity factors extrapolated to 100% H_2O ($\log k_w$) to eliminate organic-solvent effects [11–15], and they have indeed demonstrated the usefulness of

the log k_w parameter when investigating series of solutes covering a broad lipophilicity range. Generally, the extrapolation to 100% H_2O is based on a quadratic relationship between the isocratic capacity factor log k and the volume fraction of organic solvent in the mobile phase, ϕ [16]. When MeOH is used as the organic modifier, a linear relationship, *Eqn. 2*, is often obtained for neutral solutes [17][18], where S is the slope and log k_w the intercept of the regression curve.

$$k = (t_R - t_0)/t_0 \tag{1}$$

$$\log k = -S\phi + \log k_w \tag{2}$$

Until recently, most lipophilicity studies were based on reversed-phase HPLC octadecyl silica (ODS) stationary phases. The correlations between log P_{oct} and log k_w or log k so obtained are good mostly for structurally related solutes [19][20]. The decrease in correlation between capacity factors and log P_{oct} with increasing structural diversity of solutes is believed to result from specific interactions of the compounds with the residual silanol groups in such stationary phases [21].

Measures have been taken to decrease the effects of free silanol groups. A masking agent such as decylamine was added to the mobile phase [22][23]. Great progress has been achieved with silica-based stationary phases exhibiting a high level of silanol deactivation, of which the *Discovery-RP-Amide-C16* phase is a good example. In this stationary phase, the alkyl chains contain an amide group that electrostatically shields silanols from highly polar analytes.

The polymer-based octadecylpolyvinyl (ODP) stationary phase is devoid of reactive silanol groups and is regarded as promising for assessing lipophilicity. Unlike other polymer-based stationary phases, it does not undergo swelling or shrinkage and offers the possibility of having reasonable flow rates without undesirable pressure increases at the column inlet [24–26].

A highly informative interpretation of retention mechanisms on reversed-phase HPLC stationary phases can be obtained by linear solvation/free-energy relationships (LSERs) based on the solvatochromic parameters [27–32]. This method has also been used to evaluate partitioning mechanisms of solutes in various organic/aqueous biphasic systems [33–36]. LSERs can be expressed by *Eqn. 3*, where S_p is a given molecular property of a neutral organic solute, here log k_w or log P_{oct}. The four structural parameters are the *Van der Waals* volume V_w, which accounts for hydrophobic and dispersive forces, and polar terms known as solvatochromic parameters (dipolarity/polarizability π^*, H-bond donor acidity α, and H-bond acceptor basicity β), which account for polar interactions between solute and solvents. The regression coefficients v, p, a, and b reflect the relative contribution of each solute parameter to S_p.

$$S_p = v \cdot V_w + p \cdot \pi^* + a \cdot \alpha + b \cdot \beta + c \tag{3}$$

The objective of this study was to assess and compare lipophilicity values measured with the silica-based *Discovery-RP-Amide-C16* and the polymer-based *ODP-50-4B* stationary phases. A set of solutes with well-defined structural parameters (V_w, π^*, β, and α) were selected (*Table 1*). This set of solutes included simple monofunctional

Table 1. *Investigated Solutes*[a]) *and Their Physicochemical Parameters*

						Discovery RP Amide C16		*ODP-50-4B*	
	V_w [b][c])	π^* [b][d])	β [b][e])	α [b][f])	log P_{oct} [b])	log k_w [g])	S [h])	log k_w [i])	S [j])
Model solutes									
Bases:									
Acridine	174.9	1.57	0.52	0.00	3.40	2.74	4.40	3.29	4.00
$PhNH_2$	98.0	0.94	0.41	0.06	0.90	0.11	0.96	1.41	2.04
PhNHEt	133.0	0.78	0.45	0.03	2.16	1.52	2.70	2.46	3.00
2-Cl-C_6H_4-NH_2	111.8	1.06	0.41	0.06	1.91	1.48	2.80	2.55	3.00
2-NH_2-C_6H_4-Ph	173.9	1.55	0.41	0.18	2.84	2.53	4.00	3.49	3.90
Neutrals:									
$PhCH_2CN$	121.5	1.22	0.45	0.00	1.56	1.16	2.70	2.28	2.90
PhCOMe	122.3	1.12	0.51	0.00	1.58	1.11	2.50	1.91	2.60
MeCOOBu	123.0	0.55	0.45	0.00	1.82	1.33	2.50	2.10	3.20
$PhNO_2$	107.6	1.01	0.28	0.00	1.85	1.50	2.80	2.51	2.90
2-Cl-C_6H_4-NO_2	122.0	1.13	0.28	0.00	2.24	2.10	3.50	3.04	3.50
$Ph(CH_2)_2Ph$	196.9	0.99	0.20	0.00	4.80	4.27	5.40	4.70	4.90
$PhCH_2OH$	111.6	0.84	0.58	0.33	1.08	0.70	2.10	1.37	2.50
4-Cl-C_6H_4-CH_2OH	126.3	0.96	0.58	0.33	1.96	1.62	3.00	2.10	3.00
Acids:									
3-Cl-C_6H_4-OH	107.8	0.84	0.16	0.69	2.49	2.36	3.80	2.88	3.60
3-NO_2-C_6H_4-OH	112.9	1.54	0.23	0.79	2.00	1.75	3.10	2.78	3.50
$Ph(CH_2)_2COOH$	146.0	1.12	0.45	0.60	1.89	1.59	2.90	2.18	3.10
$Ph(CH_2)_3COOH$	162.4	1.12	0.45	0.60	2.42	2.08	3.40	2.64	3.60
$Ph(CH_2)_4COOH$	179.8	1.12	0.45	0.60	2.85	2.53	3.80	3.06	4.00
$Ph(CH_2)_7COOH$	230.6	1.12	0.45	0.60	4.09	4.43	5.80	4.34	5.10
$C_9H_{19}COOH$	188.7	0.55	0.45	0.60	4.09	4.37	5.70	3.99	4.90
PhCOOH	111.8	0.74	0.40	0.59	1.96	1.50	3.00	1.83	2.70
4-Br-C_6H_4-COOH	133.8	0.94	0.40	0.59	2.86	2.48	3.70	2.94	3.60
3-Cl-C_6H_4-COOH	126.2	0.86	0.30	0.59	2.71	2.29	3.50	2.75	3.50
4-Cl-C_6H_4-COOH	126.5	0.86	0.27	0.59	2.06	2.28	3.50	2.76	3.50
4-I-C_6H_4-COOH	141.6	0.96	0.42	0.59	3.13	2.51	3.60	2.92	3.40
1-Naphthoic acid	158.5	1.05	0.40	0.59	3.10	2.47	3.80	2.95	3.50
Drugs:									
Flurbiprofen	223.1	1.78	0.49	0.60	3.81	3.54	4.90	3.93	4.60
Ibuprofen	197.0	1.14	0.49	0.60	3.87	3.62	4.90	3.84	4.80
Indomethacin	283.5	1.86	1.29	0.60	3.18	3.67	5.00	4.41	4.70
Ketoprofen	239.1	2.12	0.99	0.60	2.77	2.54	4.00	3.23	4.10
Naproxen	216.5	1.64	0.79	0.60	3.06	2.72	4.10	3.39	4.00
Phenobarbital	204.5	0.59	1.26	0.20	1.44	0.96	2.40	2.04	3.00
Phenytoin	228.3	1.45	1.02	0.60	2.68	1.83	3.40	2.87	4.00
Sulfabenzamide	233.6	2.48	1.25	0.33	1.46	0.88	2.40	2.62	3.60
Sulfacetamide	174.8	2.58	1.25	0.33	–0.16	–0.66	0.93	0.54	1.45
Sulfamethazine	237.5	2.72	1.90	0.33	0.25	–0.32	1.34	0.76	1.40
Sulfamethizole	211.7	2.25	1.46	0.36	0.55	0.07	1.85	1.37	2.20
Sulfamethoxazole	207.5	2.59	1.64	0.36	0.72	0.30	1.78	2.04	2.85
Sulfamethoxypyridazine	229.6	2.93	2.38	0.33	0.35	–0.18	1.40	1.17	1.80
Sulfanilamide	139.1	1.89	1.26	0.60	–0.69	–1.30	0.00	0.47	1.25
Sulfapyridine	209.4	2.76	1.78	0.33	0.02	–0.33	2.04	0.81	1.50

[a]) The structures of the complex compounds are shown in *Fig. 1*. [b]) Taken from [36]. [c]) *Van der Waals* volume. [d]) Dipolarity/polarizability. [e]) H-Bond acceptor basicity. [f]) H-Bond donor acidity. [g]) $0.01 \leq$ s.d. ≤ 0.15. [h]) $0.01 \leq$ s.d. ≤ 0.29. [i]) $0.01 \leq$ s.d. ≤ 0.26. [j]) $0.01 \leq$ s.d. ≤ 0.35.

compounds and complex drugs (*Fig. 1*) covering a broad range of structural parameters (*Fig. 2*) and log P_{oct} values. The relationship between log k_w and log P_{oct}, as well as the relationship between log k_w and the slope S, were investigated. The LSERs approach was applied to unravel the retention mechanisms of the solutes on the two stationary phases and to compare them with the partitioning mechanism in octan-1-ol/H_2O.

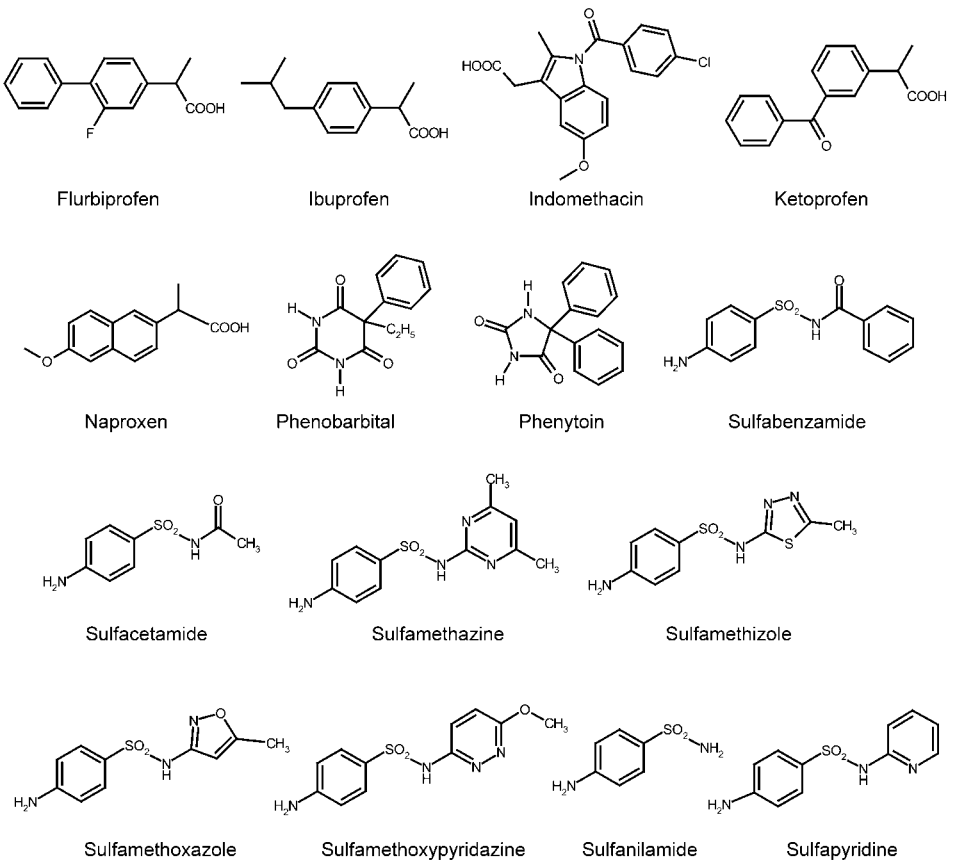

Fig. 1. *Structures of the drugs under study*

Results and Discussion. – *Relationship between log* k *and* ϕ. With both the *Discovery-RP-Amide-C16* and *ODP-50-4B* phases, a linear relationship between log k and ϕ was found for all compounds. In all cases, the squared correlation coefficient was higher than 0.99, except for aniline and sulfapyridine ($r^2 = 0.98$) on the *Discovery-RP-Amide-C16* phase and sulfamethazine ($r^2 = 0.98$) on the *ODP-50-4B* phase. The log k_w and S values of the 41 compounds on both phases were calculated by *Eqn. 2* and are presented in *Table 1*.

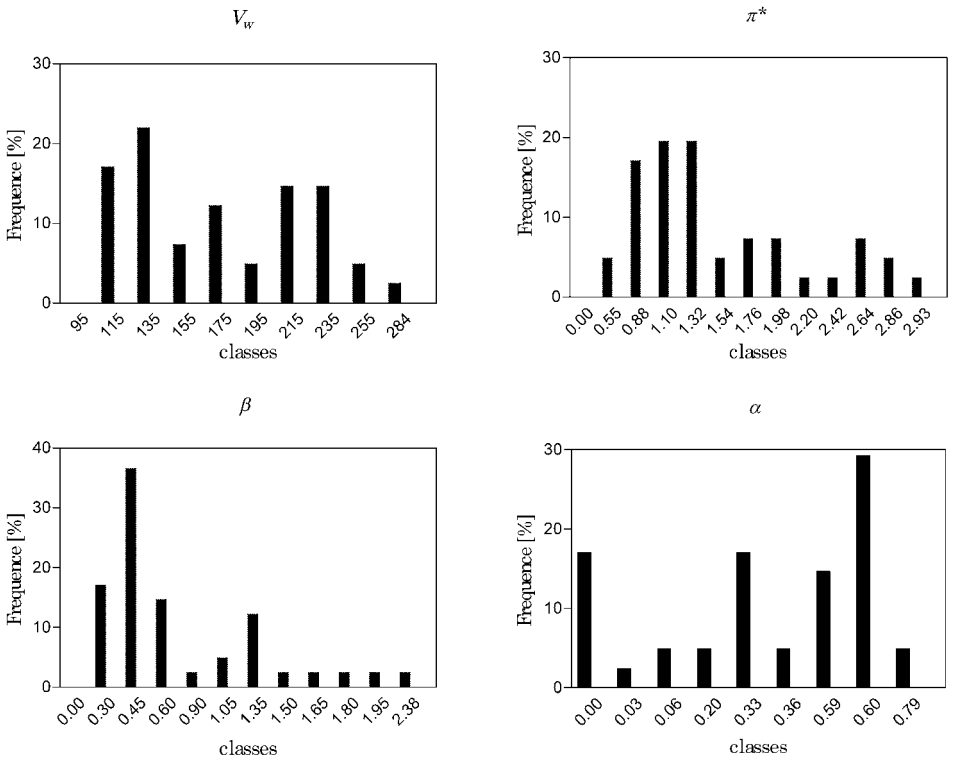

Fig. 2. *Distribution of the investigated compounds in the parameter space of the* Van der Waals *volume* V_w, *dipolarity/polarizability* π^*, *H-bond acceptor basicity* β *and the H-bond donor acidity* α

Correlation between log k_w *and Slope* S. The correlations between log k_w and slope S are described by *Eqns. 4* and *5* and are shown in *Fig. 3*, *i.e.* by *Eqn. 4* for the *Discovery-RP-Amide-C16* phase and by *Eqn. 5* for the *ODP-50-4B* phase.

$$S = 0.94\ (\pm 0.05)\ \log k_w + 1.50\ (\pm 0.11) \tag{4}$$

$$n = 41,\ q^2 = 0.97,\ r^2 = 0.97,\ s = 0.22,\ F = 1407$$

$$S = 0.93\ (\pm 0.07)\ \log k_w + 0.90\ (\pm 0.18) \tag{5}$$

$$n = 41,\ q^2 = 0.95,\ r^2 = 0.96,\ s = 0.21,\ F = 839$$

In these and the following equations, 95% confidence limits are in parentheses, n is the number of the compounds, q^2 the cross-validated correlation coefficient, r^2 the squared correlation coefficient, s the standard deviation, and F the *Fisher's* test.

The linear correlations between log k_w and S are significant for both phases and for the complete set of compounds. The correlation is slightly better for the *Discovery-RP-Amide-C16* than for the *ODP-50-4B* phase. However, and as shown in *Fig. 3a*, there are two outliers on the *Discovery-RP-Amide-C16* phase, namely sulfapyridine and aniline

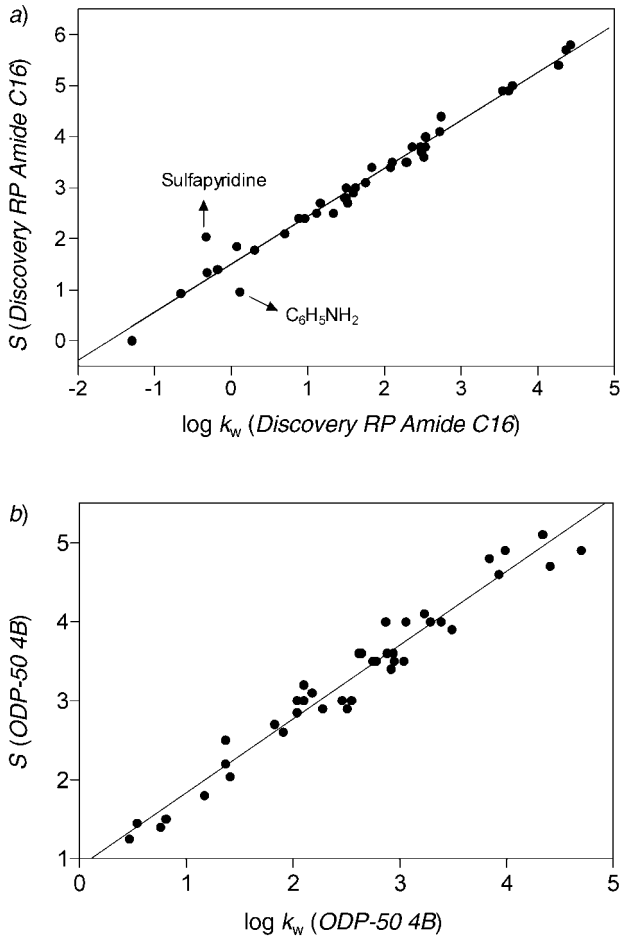

Fig. 3. *Relationship between log* k_w *and slope* S: a*) on the* Discovery-RP-Amide-C16 *phase and* b*) on the* ODP-50-4B *phase*

probably due to the lower quality of the linear regression between log k *vs.* ϕ. After omission of the two outliers, the correlation between log k_w and S becomes excellent ($r^2 = 0.99$).

In previous studies, good correlations between log k_w and S were obtained only for simple or closely related compounds [13][16][17]. Here, significant correlations were obtained for a structurally diverse set of analytes including model compounds and drugs. These significant correlations imply that log k_w and S are controlled by the same factors under the present conditions. In agreement with previous results [20], the slope of the correlation between log k_w and S is nearly 1 on each of the two stationary phases.

Correlation between log P_{oct} *and log* k_w. *Eqns. 6* and *7* and *Fig. 4* show the correlations between log P_{oct} and log k_w values, *i.e.*, *Eqn. 6* for the *Discovery-RP-Amide-C16* phase and *Eqn. 7* for the *ODP-50-4B* phase.

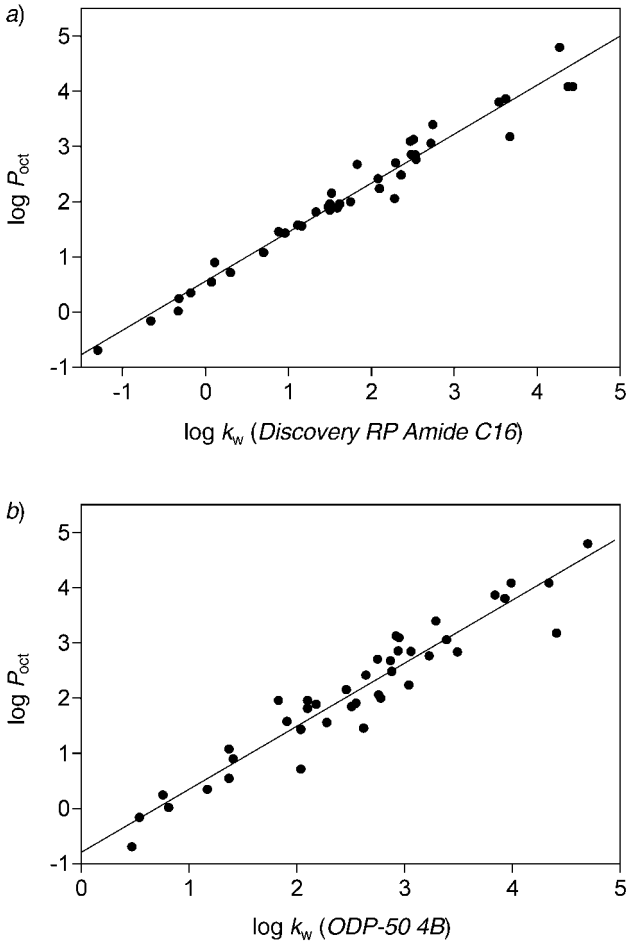

Fig. 4. *Relationship between log* P_{oct} *and log* k_w*:* a) *on the* Discovery-RP-Amide-C16 *phase and* b) *on the* ODP-50-4B *phase*

$$\log P_{oct} = 0.89\ (\pm 0.06)\ \log k_w + 0.56\ (\pm 0.12) \tag{6}$$

$$n = 41,\ q^2 = 0.96,\ r^2 = 0.96,\ s = 0.24,\ F = 1054$$

$$\log P_{oct} = 1.14\ (\pm 0.12)\ \log k_w - 0.80\ (\pm 0.32) \tag{7}$$

They clearly show that log k_w obtained with the *Discovery-RP-Amide-C16* phase is better correlated with log P_{oct} than log k_w obtained with the *ODP-50-4B* phase. In other words, there is a greater similarity between the partitioning mechanism in octan-1-ol/H_2O and the chromatographic retention mechanism on the *Discovery-RP-Amide-C16* phase than on the *ODP-50-4B* phase. This is probably due to the polar amido groups embedded in the former phase. Also, the throughput of the *Discovery-RP-Amide-C16*

phase to derive $\log k_w$ is higher than that of the *ODP-50-4B* phase due to a more-limited flow rate of the latter and its larger lipophilicity scale.

The correlation between $\log P_{oct}$ and S was found to be of lower quality than that between $\log P_{oct}$ and $\log k_w$, implying that the $\log k_w$ parameter is better suited than S for $\log P_{oct}$ approximations by reversed-phase HPLC.

We conclude from the above results that the silica-based *Discovery-RP-Amide-C16* phase is a better choice than the polymer-based *ODP-50-4B* phase to derive a lipophilicity index ($\log k_w$) correlated with $\log P_{oct}$ under the mobile-phase conditions used here.

Comparison between Retention Mechanisms on the Two Stationary Phases and Partitioning Mechanism in Octan-1-ol/H_2O by LSERs Analysis. The $\log k_w$ values obtained with the two stationary phases were analyzed by linear solvation/free-energy relationships (LSERs), yielding statistically significant equations describing the structural properties governing retention mechanisms, *i.e.* for the *Discovery-RP-Amide-C16* phase, *Eqn. 8* and, after removal of the insignificant variable, *Eqn. 8a*, and for the *ODP-50-4B* phase, *Eqn. 9* and, after removal of the insignificant variables, *Eqn. 9a*. *Eqn. 8a* shows that the main factors governing retention on the *Discovery-RP-Amide-C16* phase are the solute's molecular volume (V_w) and H-bond acceptor basicity (β), while the importance of dipolarity/polarizability (π^*) is smaller and the H-bond donor acidity (α) is not significant. *Eqn. 9a* reflects the different balance of structural parameters controlling $\log k_w$ on the *ODP-50-4B* phase, for which V_w and β are important parameters, whereas π^* and α are not significant.

$$\log k_w = 2.65 \cdot 10^{-2}(\pm 0.46 \cdot 10^{-2}) \cdot V_w - 0.49(\pm 0.45) \cdot \pi^* - 2.58(\pm 0.58) \cdot \beta + 0.30(\pm 0.64) \cdot \alpha - 0.28(\pm 0.64) \quad (8)$$

$$n = 41,\ q^2 = 0.86,\ r^2 = 0.88,\ s = 0.50,\ F = 66$$

$$\log k_w = 2.72 \cdot 10^{-2}(\pm 0.44 \cdot 10^{-2}) \cdot V_w - 0.48(\pm 0.44) \cdot \pi^* - 2.62(\pm 0.57) \cdot \beta - 0.24(\pm 0.63) \quad (8a)$$

$$n = 41,\ q^2 = 0.87,\ r^2 = 0.88,\ s = 0.50,\ F = 87$$

$$\log k_w = 2.18 \cdot 10^{-2}(\pm 0.38 \cdot 10^{-2}) \cdot V_w - 0.12(\pm 0.36) \cdot \pi^* - 2.18(\pm 0.47) \cdot \beta - 0.16(\pm 0.55)\ \cdot \alpha + 0.69(\pm 0.52) \quad (9)$$

$$n = 41,\ q^2 = 0.84,\ r^2 = 0.86,\ s = 0.52,\ F = 56$$

$$\log k_w = 2.12 \cdot 10^{-2}(\pm 0.34 \cdot 10^{-2}) \cdot V_w - 2.27(\pm 0.32) \cdot \beta + 0.63(\pm 0.46) \quad (9a)$$

$$n = 41,\ q^2 = 0.85,\ r^2 = 0.86,\ s = 0.40,\ F = 114$$

To allow a better comparison, the $\log P_{oct}$ values were also analyzed by LSERs, yielding *Eqn. 10*. After removal of the insignificant variable, *Eqn. 10a* is obtained.

$$\log P_{oct} = 2.40 \cdot 10^{-2}(\pm 0.42 \cdot 10^{-2}) \cdot V_w - 0.42(\pm 0.40) \cdot \pi^* - 2.41(\pm 0.52) \cdot \beta + 0.01(\pm 0.64) \cdot \alpha + 0.41(\pm 0.57) \quad (10)$$
$$n = 41,\ q^2 = 0.87,\ r^2 = 0.88,\ s = 0.45,\ F = 67$$

$$\log P_{oct} = 2.41 \cdot 10^{-2}(\pm 0.38 \cdot 10^{-2}) \cdot V_w - 0.42(\pm 0.40) \cdot \pi^* - 2.41(\pm 0.51) \cdot \beta + 0.41(\pm 0.56) \quad (10a)$$
$$n = 41,\ q^2 = 0.87,\ r^2 = 0.88,\ s = 0.45,\ F = 92$$

One can see from *Eqns. 10* and *10a* that V_w and β are the two main structural properties governing the partitioning mechanism in octan-1-ol/H_2O, whereas π^* is of lesser significance and α is devoid of any significance. The ratios of the normalized regression coefficients in *Eqns. 8a* and *10a* are nearly identical (details not shown), meaning that the same balance of intermolecular forces is encoded by $\log P_{oct}$ and $\log k_w$ measured on the *Discovery-RP-Amide-C16* phase. This finding confirms the highly significant correlation between these two parameters as shown in *Eqn. 6*.

A comparison between *Eqns. 9a* and *10a* indicates that the balance of forces encoded by $\log k_w$ measured on the *ODP-50-4B* phase is different from that encoded by $\log P_{oct}$, explaining the lower correlation between these two parameters (*Eqn. 7*). When the structural descriptors were included in *Eqn. 7*, the correlation quality becomes higher as shown by *Eqn. 11* and, after removal of the insignificant term, *Eqn. 11a*. The correlation coefficient in *Eqn. 11a* is better than that in *Eqn. 7*, but it remains lower than that between $\log P_{oct}$ and $\log k_w$ on the *Discovery-RP-Amide-C16* phase (*Eqn. 6*).

$$\log P_{oct} = 0.80(\pm 0.26)\ \log k_w + 0.65 \cdot 10^{-2}(\pm 0.64 \cdot 10^{-2}) \cdot V_w - 0.32(\pm 0.28) \cdot \pi^* - 0.66(\pm 0.67) \cdot \beta + 0.14(\pm 0.43) \cdot \alpha - 0.14(\pm 0.44) \quad (11)$$
$$n = 41,\ q^2 = 0.94,\ r^2 = 0.94,\ s = 0.32,\ F = 118$$

$$\log P_{oct} = 0.80(\pm 0.26)\ \log k_w + 0.70 \cdot 10^{-2}(\pm 0.62 \cdot 10^{-2}) \cdot V_w - 0.32(\pm 0.28) \cdot \pi^* - 0.70(\pm 0.66) \cdot \beta - 0.12(\pm 0.42) \quad (11a)$$
$$n = 41,\ q^2 = 0.94,\ r^2 = 0.94,\ s = 0.31,\ F = 150$$

Conclusions. – For the two stationary phases investigated, linear relationships were found between isocratic $\log k$ and the volume fraction of MeOH in the eluent. The significant correlation between the derived parameters $\log k_w$ and slope S (*Eqn. 2*) implies that these two parameters encode the same information under the experimental conditions of the present study.

By using a wide range of solutes (including drugs) and eluents enriched in octan-1-ol, the silica-based *Discovery-RP-Amide-C16* phase yielded a lipophilicity index $\log k_w$ which was better correlated with $\log P_{oct}$ than the $\log k_w$ index obtained with the

polymer-based *ODP-50-4B* phase. A LSER analysis showed that retention on the *Discovery-RP-Amide-C16* phase and partitioning in octan-1-ol/H_2O are controlled by the same balance of structural properties, namely *Van der Waals* volume (V_w), H-bond acceptor basicity (β), and dipolarity/polarizability (π^*). In contrast, the retention mechanism on the *ODP-50-4B* phase is governed by a different balance of structural properties.

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Experimental Part

Solutes and Reagents. All compounds were obtained from commercial sources (*Wako*, Osaka, Japan; *TCI*, Tokyo, Japan; *Sigma-Aldrich*, Tokyo, Japan, and *Steinheim*, Germany; *ICN*, Aurora, USA; *Merck*, *Schuchardt*, Germany) and in the highest available purity. Dist. H_2O, HPLC-grade MeOH, and octan-1-ol (*Sigma-Aldrich*, Steinheim, Germany) were used throughout.

Selection of the solutes. A set of 41 compounds with exper. log P_{oct} values ranging from -0.69 to 4.80 were selected. This set consists of model compounds and drugs having a relatively rigid structure and well-defined parameters (V_w, π^*, β, and α) [36]. The investigated compounds and their physicochemical parameters are shown in *Table 1*. The broad range of parameter spaces (V_w, π^*, β, and α) is demonstrated in *Fig. 2*.

Measurement of Capacity Factors. The capacity factors were measured with a liquid chromatograph equipped with a *880-PU*-HPLC pump, a *875*-UV/Vs detector (both from *Jasco*, Tokyo, Japan), a *655A-40* autosampler, and a *D-2000* chromato-integrator (both from *Hitachi*, Tokyo, Japan). The *Supelcosil Discovery-RP-Amide-C16* column (5 cm × 4.6 mm i.d., 5 μm) was from *Supelco* (Bellefonte, PA, USA) and the *Asahipak ODP-50-4B* column (5 cm × 4.6 mm i.d., 5 μm) from *Asahi Chemicals* (Kawasaki, Japan). The mobile phase consisted of 0.02M phosphate buffer and MeOH in varying proportions (80 → 10% *v*/*v*). The phosphate buffer was adjusted to pH 7 for all nonionizable compounds and to a pH value (pH 3, 4, or 7) where the neutral form was in large excess for the ionizable compounds. To increase the similarity with octan-1-ol/buffer partitioning [15][22], a 0.25% (*v*/*v*) amount of octan-1-ol was added to MeOH, and octan-1-ol-sat. H_2O was used to prepare the buffer. The phosphate buffer was filtered under vacuum through a 0.45-μm *HA-Millipore* filter (*Millipore*, Milford, MA, USA) before being mixed with MeOH. The retention times t_R were measured at r.t. by the UV/Vs detector at wavelength λ_{max} of the analytes. The solns. to be injected (10^{-4} M to 10^{-3} M) were prepared by dissolving the solutes in the mobile phase; the injection volume was 10 μl. Uracil was used as the unretained compound. On the *Discovery-RP-Amide-C16* phase, the measurements were carried out at a flow rate of 1.0 ml/min for compounds with a log P_{oct} value higher than 1, and 0.5 ml/min for compounds with log P_{oct} below 1. Since the highest pressure limit of the *ODP-50-4B* column is much lower (*ca.* 730 psi) than that of silica-based columns (4000 psi), a low flow rate (0.5 ml/min) was used for the *ODP-50-4B* phase to prolong its life. In all cases, three different MeOH concentrations were used for extrapolation to log k_w. MeOH concentrations were adapted to the log P_{oct} values of the solutes as described in *Table 2*. The capacity factor log *k* was calculated by *Eqn. 1*. All log *k* values were the average of three measurements. The log *k* values were then extrapolated to 100% H_2O by using *Eqn. 2*.

Table 2. *Concentrations of Organic Modifier (MeOH) Used with the Two Stationary Phases*

log P_{oct} of solutes	% MeOH	
	Discovery-RP-Amide-C16	*ODP-50-4B*
> 3	60, 65, 70	70, 75, 80
1 – 3	40, 45, 50	60, 65, 70
< 1	10, 20, 25	20, 30, 40

Statistical Analysis. All regression analyses were performed *via* the JMP statistical software package (version 5.1.1, Japanese Edition, *SAS Institute Inc*).

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