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COMPARISON OF PHENYL- AND OCTADECYL-MODIFIED SILICA GEL AS STATIONARY PHASE FOR THE PREDICTION OF n-OCTANOL-WATER PARTITION COEFFICIENTS BY HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY

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SUMMARY

The correlation between the retention of 29 aromatic compounds on phenyl and octadecyl silica gel with their n-octanol-water partition coefficients has been studied. The work also covered the influence of mobile phase buffers and the effect on the correlation of using k' values extrapolated to 100% water. The results show that the retention on phenyl silica correlates significantly better than that on octadecyl silica. The error in the prediction of log P, ranging from 1 to 8, from retention data on phenyl silica at a 95% confidence limit was found to be ±0.04 log units at log P = 4, and ±0.07 log units at log P = 7 when extrapolated k' values were used. When k' values measured at one fixed mobile phase composition were used, the error was found to be ±0.10 and ±0.16 log units at log P = 4 and 7, respectively.

INTRODUCTION

The n-octanol-water partition coefficient (log P value) is generally accepted as a useful parameter in structure-activity relationship studies (QSAR) for the prediction of biological or pharmacological activity of compounds[1]. The conventional method of measuring log P values, the shake-flask method, has some disadvantages. The method is time-consuming and requires very pure compounds, and the precision of the measurement for compounds with log P > 4 is usually poor because of the errors involved when determining very low concentrations in the aqueous phase. If the structure of the compound is known, log P can be estimated from substituent constants, as proposed by Hansch[1,2]. However, for a number of compounds with complex structures, this approach is not yet reliable.

For these reasons many attempts have been made to use liquid chromatographic retention data for the prediction of n-octanol-water partition coefficients.

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This has definite advantages in terms of the speed of determination and of the requirements put on the purity of the compounds and the amount of material needed.

Thus thin-layer chromatography[3,4], column liquid chromatography using n-octanol or oleyl alcohol as stationary phase[4,5] and reversed-phase liquid chromatography on alkyl-modified silica gels[6-10] have been used to predict log P values. All these studies assume that the extent of retention reflects the hydrophobicity of a solute. However, some authors[7,11] found significant deviations attributed to the presence of residual silanol groups on the surface of the alkyl-modified silica gels. It is therefore worth investigating other hydrophobic stationary phases for the prediction of log P values.

In this study we measured the retention of solutes on phenyl-modified silica gel for the prediction of n-octanol–water partition coefficients. The results, obtained with 29 aromatic compounds with log P values in the range 1–8, were compared with those obtained on octadecyl-modified silica gel (ODS).

EXPERIMENTAL

Apparatus

The liquid chromatographic equipment consisted of a high-pressure pump (Kontron Analytical LC Pump, Kontron, Switzerland), a high-pressure injection valve (Rheodyne 7125, Berkeley, U.S.A.) equipped with a 20-μl sample loop, a UV detector (Waters 481, Waters Assoc., Milford, U.S.A.), and an integrator (Hewlett-Packard 3390 A, Avondale, U.S.A.). The columns were 316 SS (150 × 4.6 mm I.D.) and thermostatted at 22 ± 0.1°C using a circulating water-bath (Haake TP 42, Berlin, F.R.G.).

Materials

In all experiments HPLC-grade methanol and water (S. T. Baker, Richardson, U.S.A.) were used. The chemical modified silica gels were phenyl-Bondapak 10 μm (Waters Assoc.) and Hypersyl ODS 5 μm (Shandon, U.K.). The solutes were obtained from various commercial sources and are listed in Table I, together with their log P values as given in the literature.

Procedures

The columns were packed by a pressurized slurry technique, using tetrachloromethane as slurry liquid and methanol as displacer liquid, up to a maximum pressure of 500 bar. The columns were washed with 100 ml of methanol and then equilibrated with the mobile phase, which consisted of mixtures of methanol and water [55–75% (v/v) of methanol] or sodium phosphate buffered methanol–water mixtures (0.005 M Na_2HPO_4, pH 7.4).

All reported capacity factors, k', are the mean of at least three measurements. Potassium dichromate and uracil were used as the non-retarded compound.

The correlation between the k' values of the test solutes on phenyl- and alkyl-modified silica gel and their shake-flask partition coefficients, P, were made by linear regression analysis of log k' versus log P.

TABLE I

SOLUTES USED IN THE CORRELATION STUDIES AND LITERATURE VALUES OF THEIR
n-OCTANOL–WATER PARTITION COEFFICIENTS (log *P*)

Solute	Log P	Source (ref.)
1 Benzene	2.02	12
2 Toluene	2.65	12
3 *p*-Xylene	3.18	12
4 Naphthalene	3.31	12
5 Ethylbenzene	3.13	12
6 Biphenyl	3.91	12
7 Butylbenzene	4.18	12
8 Phenanthrene	4.52	12
9 Hexylbenzene	5.24	12
10 Octylbenzene	6.30	12
11 4,4'-Polychlorobenzene	5.28	12
12 2,2'-Polychlorobenzene	5.02	12
13 2,4,5-Polychlorobenzene	5.77	12
14 2,5,2',5'-Polychlorobenzene	6.26	12
15 2,4,5,2',5'-Polychlorobenzene	6.85	12
16 2,4,6,2',4',6'-Polychlorobenzene	7.12	12
17 2,3,4,5,6,2',5'-Polychlorobenzene	7.93	12
18 1,3-Dichlorobenzene	3.52	13
19 Phenol	1.47	13
20 Aniline	0.90	13
21 4-Chloroaniline	1.64	13
22 Acetophenone	1.63	13
23 Indole	2.14	13
24 Diuron	2.85	13
25 Linuron	3.00	13
26 Dichlobenil	2.65	13
27 Diphenylamine	3.37	13
28 Chloroxuron	4.00	13
29 Diphenyl ether	4.20	13

RESULTS AND DISCUSSION

As temperature influences the distribution of solutes and thus the capacity
ratio *k'*, the effect of the column temperature on the *k'* values of a number of solutes
was investigated. It was found that *k'* varies *ca.* 1% per degree centigrade. Although
this variation of *k'* with temperature is not very large (and even less in log *k'*, as used
in the correlation), it was decided to thermostat the columns at 22°C in all further
experiments.

The capacity ratio of the test solutes was measured on phenyl and octadecyl
silica gel with two mobile phase compositions: (i) pure water–methanol; (ii) the same
mixture, but containing a sodium phosphate buffer of pH 7.4. Buffering of the mobile
phase might be necessary to obtain reproducible retention of ionizable compounds.

Figs. 1 and 2 show the results of the correlation of log *k'* and log *P*. Two
significant results are apparent from these figures and from the linear regression data
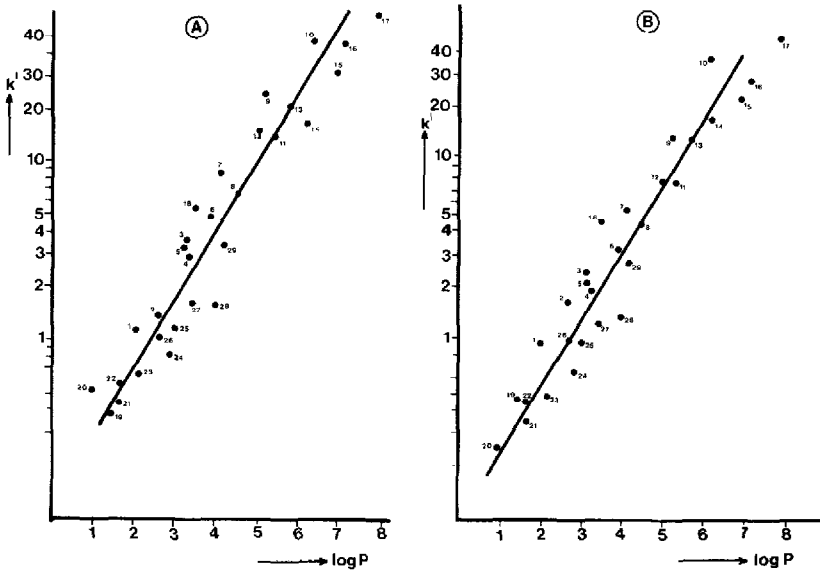(Table II): (i) the correlation on the phenyl phase is significantly better than that on

Fig. 1. Log–log relationship between *n*-octanol–water partition coefficients (log *P*) of 29 test solutes and their capacity ratios (log *k'*), as measured on Hypersil ODS at a fixed mobile phase compostion: (a) methanol–water 75:25 (v/v); (b) methanol–water 75:25 (v/v) containing 0.005 *M* Na$_2$HPO$_4$ (pH 7.4).

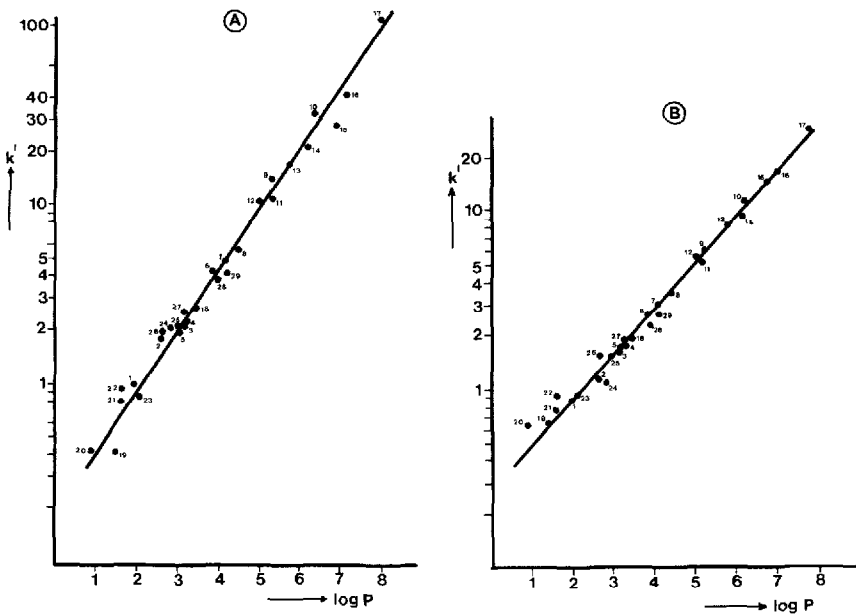Fig. 2. Log–log relationship between *n*-octanol–water partition coefficients (log *P*) of 29 test solutes and their capacity ratios (*k'*), as measured on phenyl-modified silica gel (Waters Assoc.): (a) mobile phase, methanol–water 65:35 (v/v); (b) mobile phase, methanol–water 65:35 (v/v) containing 0.005 *M* Na$_2$HPO$_4$ (pH 7.4).

TABLE II

LINEAR REGRESSION DATA OF THE CORRELATION OF *n*-OCTANOL–WATER PARTITION COEFFICIENTS (*P*) AND CAPACITY RATIO (*k'*) ON ODS AND PHENYL-MODIFIED SILICA GEL

Calculated according to log $P = a \log k' + b$.

Support	Conditions	a	b	r*	F*	S.D.*
ODS	Non-buffered mobile phase	2.61	2.38	0.9584	291	0.55
	Buffered mobile phase	2.72	2.69	0.9674	389	0.47
	Extrapolated to 100% water	0.76	3.47	0.9723	275	0.24
	Slope of log $k' = A - B\varphi$	0.66	1.20	0.9482	142	0.25
Phenyl	Non-buffered mobile phase	2.92	2.10	0.9882	1076	0.30
	Buffered mobile phase	3.98	2.29	0.9926	1796	0.23
	Extrapolated to 100% water	1.22	0.62	0.9894	917	0.07
	Slope of log $k' = A - B\varphi$	1.22	−0.19	0.9761	395	0.25

* r = regression coefficient; F = F test; S.D. = standard deviation.

the octadecyl phase; (ii) buffering of the mobile phase results in a better correlation and a decrease of *k'* on both stationary phases, but in particular on the phenyl phase.

A comparison of the correlations on the two mobile phases reveals that, on the phenyl phase (Fig. 2), only solutes with log *P* < 2 deviate from the calculated regression line. This is not surprising, as most of the test solutes with log *P* < 2 have acidic or basic properties and thus their retention behaviour is strongly dependent on the pH of the mobile phase.

On the octadecyl phase (Fig. 1) the points are scattered around the calculated regression line over the whole range of log *P*. As most of the solutes with log *P* < 2 have no acidic or basic properties this scattering cannot be attributed to the pH of the mobile phase. To determine whether this scattering is due to the type of ODS, we also investigated an ODS from another supplier (Waters Assoc.). The same scattering occurred on this material.

It is clear that the results obtained strongly depend on the precision of the shake-flask partition coefficients, which were taken from literature, and on the classes of compounds involved in the correlation. For instance, on the ODS phase the correlation of log *k'* and log *P* is much better if we correlate only polychlorinated biphenyls and polyaromatics or simple aromatics, (Fig. 3). However, correlations on the phenyl phase are better for a wide range of types of compound with log *P* values ranging from 1 to 8; we do not yet fully understand why. A better masking of the residual silanol groups by the phenyl moieties and/or the possibility of π–π interactions might be important factors. However, reliable answers to these questions can be obtained only if the study is extended to non-aromatic compounds and to measurements of the accessible silanol groups on the surface of the phenyl-modified silica gel.

The present results were obtained by correlating the *k'* values of solutes as measured at a fixed mobile phase composition. However, several authors[9,14] have proposed the use of retention data extrapolated to 100% water. The retention at
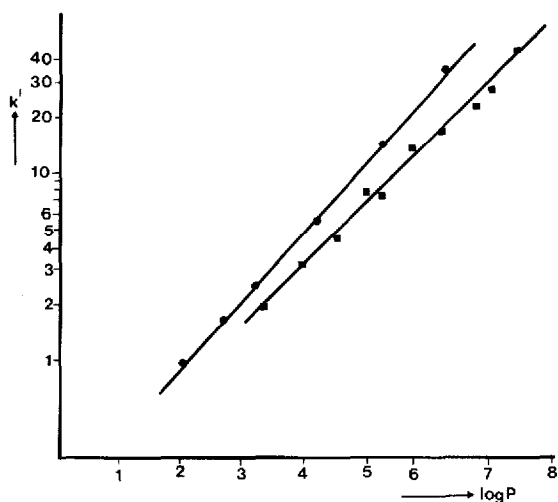
Fig. 3. Log–log relationship between *n*-octanol–water partition coefficients (log *P*) of aromatic (●) and polyaromatic (■) test solutes and their capacity ratios (log *k*′), as measured on Hypersil ODS silicagel at a fixed mobile phase composition: methanol–water 75:25 (v/v) containing 0.005 *M* Na$_2$HPO$_4$ (pH 7.4).

TABLE III

EXTRAPOLATED *k*′ VALUES ON ODS

Obtained from log *k*′ *versus* volume fraction of methanol correlations according to log $k' = A - B\varphi$ at three methanol concentrations.

Solute*	A (= log k'_0)	100 · B	r	log P
1	1.86	2.3	0.986	1.47
2	2.34	2.7	0.993	2.65
3	3.53	4.0	0.996	3.18
4	3.35	3.9	0.998	3.31
5	3.46	4.0	0.996	3.13
6	4.12	4.7	0.994	3.91
7	4.77	5.2	0.992	4.28
8	4.52	5.0	0.993	4.52
11	5.90	6.5	0.982	5.58
18	3.73	4.3	0.993	3.52
19	0.08	1.2	0.986	1.47
20	0.26	1.2	0.947	0.90
21	0.68	1.1	0.767	1.64
22	1.33	1.8	0.894	1.63
23	1.47	2.1	0.993	2.14
24	3.02	3.9	0.990	2.85
25	2.81	3.5	0.998	3.00
26	1.06	3.2	0.996	2.65
27	3.60	4.5	0.997	3.37
28	3.91	4.9	0.998	4.00
29	4.14	4.8	0.999	4.20

* See Table I.

100% water can be calculated from measurements at various concentrations of the organic modifier according to the equation

$$\log k' = A - B\varphi \tag{1}$$

where A and B are constants depending on the solute, and φ is the volume fraction of the organic modifier in the mobile phase. Eqn. 1 has been proven to be valid for methanol as organic modifier over a limited range[15]. For other organic modifiers quadratic relationships have to be used[15].

The arguments in favour of using extrapolated k' values are based on the observation that variations of log k' with the volume fraction of the organic modifier (the constant B) differ between solutes. Such differences may in some cases result in a cross-over of peaks (k'). Such cross-overs usually appear between solutes differing widely in nature but rarely with compounds belonging to the same class.

In order to investigate whether extrapolated k' values (at $\varphi = 0$) show a better correlation with log P than the measurements at a fixed mobile phase composition, the k' values of 21 test solutes were measured with three different methanol concentrations in the mobile phase. From these measurements k'_0 values (k' at $\varphi = 0$) were calculated from eqn. 1 (Tables III and IV). On the ODS phase, aniline, chloroaniline and acetophenone showed non-linear behaviour and so were excluded from the

TABLE IV

EXTRAPOLATED k' VALUES ON PHENYL SILICA GEL

Obtained from log k' *versus* volume fraction of methanol correlations according to log $k' = A - B\varphi$ at three methanol concentrations.

Solute*	A ($= \log k'_0$)	$100 \cdot B$	r	log P
1	1.13	1.8	0.997	2.02
2	1.52	2.1	0.999	2.65
3	2.03	2.7	0.999	3.18
4	2.18	2.8	0.999	3.31
5	2.04	2.7	0.999	3.13
6	2.74	3.4	0.998	3.91
7	3.21	4.0	0.997	4.28
8	3.12	3.5	0.999	4.52
11	3.76	4.3	0.996	5.58
18	2.18	2.9	0.999	3.52
19	0.44	1.1	0.993	1.47
20	0.26	0.9	0.975	0.90
21	0.94	1.6	0.998	1.64
22	0.95	1.6	0.998	1.63
23	1.21	1.9	0.972	2.14
24	1.83	2.6	0.999	2.85
25	2.09	2.9	0.999	3.00
26	1.80	2.6	0.999	2.65
27	2.39	3.1	0.999	3.37
28	2.81	3.6	0.998	4.00
29	2.88	3.6	0.996	4.20

* See Table I.

log k_0'–log P correlations. These three solutes did not show similar behaviour on the phenyl phase.

As can be seen from Table III, the log k_0' value on ODS is about equal to the value of log P in agreement with earlier findings[9,14]. On the phenyl phase log k_0' is about two thirds of log P, which reflects the different type of interaction (less hydrophobic) compared with the ODS phase.

Linear regression data for log k' and log k_0' with log P are collected in Table II. From these data it can be seen that the error in the prediction of log P from retention data is significantly smaller when using extrapolated k' values (k_0') than when using k' values obtained at one fixed mobile phase composition, at least with the solutes selected in this study. This result agrees with earlier findings[9]. If log k_0' correlates with log P, then B (the slope) of eqn. 1 should correlate as well. From Table II it can be seen that this is the case, but the correlation is significantly worse than that with log k_0'. Despite these results, it would be interesting to investigate further the k' dependence of a solute with the organic modifier content in relation to its structure and log P value, as this might provide additional information about substituent effects.

CONCLUSIONS

The main results of this study can be summarized as follows.

(1) Phenyl-modified silica gel seems to be a better stationary phase for the prediction of n-octanol–water partition coefficients of aromatic compounds from retention data than ODS.

(2) Buffering of the mobile phase at pH 7.4 has a favourable effect on the correlation between log k' and log P.

(3) The correlation of extrapolated k' values (at 100% water) and log P is significantly better than the correlation of k' as measured at one fixed mobile phase composition.

(4) The error in the prediction of log P values in the range 1–8 from extrapolated k' values on phenyl silica ranges between ±0.04 and ±0.07 log units.

(5) The error in the prediction of log P from a single k' measurement at 65% (v/v) organic modifier ranges between ±0.10 and ±0.16 log units. Under these conditions, solutes with log P $ca.$ 8 show a k' value of $ca.$ 30, which allows very fast determinations of log P in the range 1–8, although with a somewhat larger error.

REFERENCES

1 C. Hansch, in C. J. Cavallito (Editor), *Structure Activity Correlations*, Vol. 1, Pergamon Press, Oxford, 1973, p. 5.
2 R. F. Rekker, *The Hydrophobic Fragmental Constant*, Elsevier, Amsterdam, 1977.
3 E. Tomlinson, *J. Chromatogr.*, 113 (1975) 1.
4 A. Hulshoff and J. H. Perrin, *J. Chromatogr.*, 129 (1976) 263.
5 M. S. Mirlees, S. J. Moulton, C. T. Murphy and P. J. Taylor, *J. Med. Chem.*, 19 (1976) 615.
6 R. M. Carlson, R. E. Carlson and H. L. Kopperman, *J. Chromatogr.*, 107 (1975) 219.
7 J. M. McCall, *J. Med. Chem.*, 18 (1975) 549.
8 T. Yamana, A. Tsuji, E. Kiya and E. Miyamoto, *J. Pharm. Sci.*, 66 (1977) 747.
9 W. E. Hammers, G. J. Meurs and C. L. de Ligny, *J. Chromatogr.*, 247 (1982) 1.
10 T. L. Hafkenscheid and E. Tomlinson, *Int. J. Pharm.*, 16 (1983) 225.

11 S. H. Unger and G. H. Chiang, *J. Med. Chem.*, 24 (1981) 262.
12 W. A. Bruggeman, J. van der Steen and O. Hutzinger, *J. Chromatogr.*, 238 (1982) 335.
13 *Technical Report No. 7, Assessment of Reversed-Phase Chromatographic Methods for Determining Partition Coefficients*, Ecetoc, Brussels, 1983.
14 C. H. Lockmüller and D. R. Wilder, *J. Chromatogr. Sci.*, 17 (1979) 574.
15 P. J. Schoenmakers, H. A. H. Billiet and L. de Galan, *J. Chromatogr.*, 185 (1979) 179.