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REVERSED-PHASE ION-PAIR SYSTEMS FOR THE PREDICTION OF *n*-OCTANOL-WATER PARTITION COEFFICIENTS OF BASIC COMPOUNDS BY HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY

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SUMMARY

The correlation between the retention of basic solutes in a reversed-phase ion-pair system, in which sodium dodecylsulphate is the pairing-ion, and their *n*-octanol-water partition coefficients ($\log P$) has been studied. The study comprises the influence on the correlation of the type of hydrophobic matrix (phenyl- and octadecylsilica) and of the pH and salt concentration in the mobile phase. The influence of the ion-pairing additives on the prediction of $\log P$ of neutral compounds from their retention has also been studied.

The results show that the correlation between $\log P$ and $\log k'$ for basic compounds is significantly better on phenylsilica than on octadecylsilica. The error in the prediction of $\log P$, ranging from 1 to 7, of basic compounds from their retentions in the ion-pair system on phenylsilica was found to be ± 0.1 log units at a 95% confidence limit. The correlation of $\log P$ values of neutral compounds with their k' values in the ion-pair system is not significantly worse than that found with a normal reversed-phase system. Moreover, it seems that the slopes of the linear regression of $\log P$ and $\log k'$ of neutral and basic compounds in the selected phase system are about equal. This permits the prediction of $\log P$ of neutral and basic compounds from their retention in the same phase system with an error in the prediction of ± 0.1 log units at a 95% confidence limit.

INTRODUCTION

It has been shown that *n*-octanol-water partition coefficients of compounds ($\log P$) can be predicted from their retentions on various types of reversed-phase liquid chromatographic columns¹⁻⁹. These methods have been found quite useful for neutral and acidic compounds but much less so for basic compounds. This can be attributed to the frequently strong interaction of basic compounds with the residual silanol groups on the surface of reversed-phase matrices¹⁰, which causes extra retention and asymmetric peak shapes. This strong interaction can be diminished by blocking the silanol sites with an amine, added to the mobile phase^{7,11,12}, or by masking the basic site of the solute molecules by ion-pair formation¹³⁻¹⁵. In this study, we

have examined whether the retention of basic compounds in reversed-phase ion-pair systems can be used for the prediction of their *n*-octanol–water partition coefficients. The study included the effect of the presence of ion-pair additives in the mobile phase on the prediction of log *P* values of neutral compounds.

EXPERIMENTAL

Apparatus

The liquid chromatograph was assembled from a high-pressure pump (Analytical LC pump, Kontron, Basel, Switzerland), a high-pressure injection valve (Model 7125, Rheodyne, Berkeley, CA, U.S.A.), equipped with a 20- μ l sample loop, and a UV detector (Model 481, Waters, Framingham, MA, U.S.A.). The columns were made of 316 stainless steel, 150 \times 4.6 mm I.D., were thermostatted at 22 \pm 0.1°C by means of a thermostating jacket, connected to a circulating water-bath (Model TP 42, Haake, Berlin, F.R.G.).

Materials

HPLC-grade methanol and water (J. T. Baker, Richardson, TX, U.S.A.) were used. The chemically modified silica gels were phenyl-Bondapak (10 μ m, Waters) and Hypersil ODS (5 μ m, Shandon, Runcorn, U.K.). The solutes were obtained from various commercial sources or kindly donated by colleagues from other departments. The test solutes and their log *P* values and molecular structures are given in Table I.

Procedures

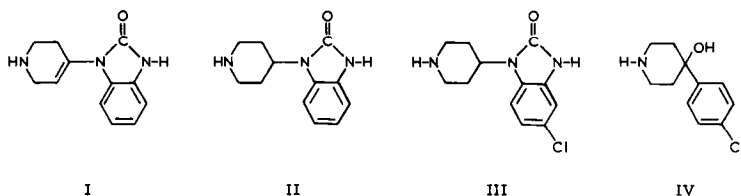
The columns were packed by a pressurized slurry technique, with tetrachloromethane as slurry liquid and methanol as the displacer liquid. The maximum filling pressure was 500 bar. The columns were washed with 100 ml of methanol and then equilibrated with the mobile phase until retention was constant. All capacity ratios, *k'*, used in the correlations were the mean of at least three measurements. Uracil was used as a non-retarded compound. The correlation between *k'* and the *n*-octanol–water partition coefficient *P*, was established by linear regression of log *P* and log *k'*.

RESULTS AND DISCUSSION

It has been demonstrated that the retention of basic compounds in reversed-phase systems significantly increases when an anionic surfactant is added to the mobile phase^{13–15}. The increase in retention has been the subject of many investigations of the retention mechanism^{13,15–18}. Thus some authors^{16,18} suppose that the basic solute and the anionic surfactant form an ion-pair in the mobile phase, which is then adsorbed on the hydrophobic stationary phase. Others have found evidence that the surfactant is adsorbed on the hydrophobic surface via its alkyl chain, leaving the anionic group available to act as an ion exchanger (dynamic ion exchange)^{13,15,17}. Whatever the true mechanism may be, it is found that the retention of basic solutes increases with increasing hydrophobicity of the solutes. This because the contribution of the pairing-ion (surfactant) to the hydrophobicity of the ion pairs can be considered to be constant. This means also that in ion-pair systems the hydrophobicity

TABLE I

SOLUTES USED IN THE CORRELATION STUDIES AND LITERATURE VALUES OF THEIR *n*-OCTANOL-WATER PARTITION COEFFICIENTS



<i>Neutral solutes</i>			<i>Basic solutes</i>		
<i>Code</i>	<i>Compound</i>	<i>log P</i>	<i>Code</i>	<i>Compound</i>	<i>log P</i>
1	Linurol	3.00	21	Clonidine	1.49
2	Acetophenone	1.63	22	4-Chloroaniline	1.64
3	Benzene	2.02	23	Baygon	1.52
4	Toluene	2.65	24	Atropine	1.80
5	Diuron	2.85	25	Droperidol	3.50
6	Dichlobenzil	2.65	26	Diphenylamine	3.37
7	Ethylbenzene	3.13	27	Diphenylhydramine	3.30
8	<i>p</i> -Xylene	3.18	28	Propanolol	3.56
9	Naphthalene	3.33	29	Halopemide	3.92
10	1,3-Dichlorobenzene	3.52	30	Haloperidol	4.31
11	Chloroxuron	4.00	31	Bromoperidol	4.72
12	Biphenyl	3.91	32	Pimozide	6.23
13	Diphenyl ether	4.20	33	Chlopimozide	7.05
14	Butylbenzene	4.28	34	Chlorpromazine	5.30
15	Phenanthrene	4.52	35	Indole	2.14
16	4,4'-Dichlorobiphenyl	5.28	36	I	0.85
17	Theobromine	-0.80	37	II	1.14
18	Aniline	0.90	38	III	1.59
19	Morphine	0.76	39	IV	1.98
20	Codeine	1.10			

of solutes is reflected in their retention. Consequently, it must be possible to predict $\log P$ values of basic compounds from their retention in ion-pair systems, as was previously indicated¹⁹. However, such a correlation can only be valid when the values of both parameters are determined under comparable states of ionization. For $\log P$ this should be the un-ionized state and for the capacity factor the values obtained with fully protonized solutes⁷⁻⁹. The ion-pair systems might also be used for the prediction of $\log P$ of neutral compounds, because it has been found that the presence of a small amount of surfactant hardly influences the retention of neutral and acidic compounds^{14,20}. In that case such a reversed-phase ion-pair system can serve as a universal phase system for the prediction of $\log P$ values of a large variety of compounds. In order to investigate the applicability of ion-pair systems for the prediction of $\log P$ values, we measured the retention of neutral and basic test solutes on phenyl- and octadecyl-modified silica using methanol-aqueous buffer mixtures, containing sodium dodecyl sulphate (SDS) as pairing-ion, as the mobile phase and correlated their capacity factors with their known $\log P$ values. The volume percentages of methanol used were 55% with phenylsilica and 65% with octadecylsilica. With these

percentages of methanol we previously found excellent correlations between $\log k'$ and $\log P$ of neutral compounds in plain reversed-phase systems⁶. Under these conditions, compounds with a $\log P$ of *ca.* 7 are eluted with a k' of *ca.* 50. Because we wanted to investigate the neutral compounds as well in the present ion-pair system, we decided to use the same percentages of methanol to avoid excessively large capacity factors for this group of compounds. The SDS concentration was adjusted to 0.1% (w/v) because, under these conditions, a large surface coverage with SDS occurs, which favours the equilibration time and reproducibility of the phase systems. However, with these mobile phase compositions the basic test solutes were eluted with an unfavourably large retention (*e.g.* a compound with a $\log P$ value of 5 was eluted with k' of *ca.* 100). The retention of the basic solutes can be decreased by adding to the mobile phase a cation that competes with the solute cation to form an ion-pair with the surfactant^{17,20}. Since sodium has been found useful for this purpose, we added 0.1 *M* sodium as sodium perchlorate to the mobile phase. The perchlorate salt was preferred to phosphate because the perchlorate ion is non-corrosive, dissolves very well in water-methanol mixtures and seems to improve significantly the peak shapes of basic solutes¹⁴. The addition of 0.1 *M* sodium perchlorate diminishes the capacity factors of the basic solutes by a factor of about three.

The results on the linear regressions of $\log P$ and $\log k'$ for the basic compounds, as obtained with various mobile phase compositions (effect of pH, SDS and sodium perchlorate addition) on phenyl- and octadecylsilica are collected in Tables II and III.

Table II shows the separate and combined effects of the addition of sodium perchlorate and SDS to the mobile phase on the correlation of $\log P$ and $\log k'$. As can be seen, the correlation is worse when only sodium perchlorate is present in the mobile phase. Although the perchlorate ion can form ion-pairs with very hydrophobic compounds, it is unlikely that this occurs with all compounds under investigation. Consequently the solutes are retained by a different mechanism and thus a good correlation between $\log P$ and $\log k'$ cannot be expected. When only SDS is added to the mobile phase, the correlation between $\log P$ and $\log k'$ improves remarkably. This is to be expected, because SDS is a very good pairing-ion which forms ion-pairs with all the test solutes, and thus they are all retained by the same mechanism. How-

TABLE II

LINEAR REGRESSION DATA FOR THE CORRELATION OF *n*-OCTANOL-WATER PARTITION COEFFICIENTS ($\log P$) AND CAPACITY RATIOS (k') OF BASIC COMPOUNDS ON PHENYLSILICA WITH VARIOUS MOBILE PHASE COMPOSITIONS

Calculated according to: $\log P = a \log k' + b$. r = regression coefficient; F = F -ratio; S.D. = standard deviation. Mobile phases: I = methanol-phosphate buffer (0.005 *M*, pH 4.0) (55:45, v/v) containing 0.1 *M* sodium perchlorate. II = methanol-phosphate buffer (0.005 *M*, pH 4.0) (55:45, v/v) containing 0.1% (w/v) SDS. III = methanol-phosphate buffer (0.005 *M*, pH 4.0) (55:45, v/v) containing 0.1 *M* sodium perchlorate and 0.1% (w/v) SDS.

Mobile phase	<i>a</i>	<i>b</i>	<i>r</i>	<i>F</i>	S.D.
I	3.14	2.73	0.846	33	1.21
II	3.17	0.74	0.991	733	0.30
III	3.57	1.46	0.996	1443	0.21

TABLE III

LINEAR REGRESSION DATA FOR THE CORRELATION OF *n*-OCTANOL-WATER PARTITION COEFFICIENTS AND CAPACITY RATIOS OF BASIC COMPOUNDS ON OCTADECYLSILICA AND PHENYLSILICA AT DIFFERENT pH VALUES OF THE MOBILE PHASE

Calculated according to: $\log P = a \log k' + b$. Mobile phases: IV = methanol-phosphate buffer (0.005 M) (65:35, v/v) containing 0.1 M sodium perchlorate and 0.1% (w/v) SDS. V = methanol-phosphate buffer (0.005 M) (55:45, v/v) containing 0.1 M sodium perchlorate and 0.1% (w/v) SDS.

Silica	Mobile phase	pH	<i>a</i>	<i>b</i>	<i>r</i>	<i>F</i>	<i>S.D.</i>
Octadecyl	IV	4.0	4.26	1.61	0.980	194	0.45
		5.0	4.18	1.43	0.983	235	0.41
		6.5	3.60	1.68	0.970	137	0.53
Phenyl	V	3.0	3.59	1.42	0.989	763	0.32
		4.0	3.57	1.46	0.996	1443	0.21
		6.5	5.55	1.08	0.940	6	0.77

ever, the correlation is the best when SDS and sodium perchlorate are both present in the mobile phase. This can partly be attributed to the better peak shape of some solutes in the presence of sodium perchlorate.

As mentioned before, the correlation of $\log P$ and $\log k'$ can be valid only if the parameters are determined under comparable ionization conditions. For the measurement of the capacity factor this is the fully protonized form of the solute. This depends on the pK_b of the solutes and on the pH of the mobile phase. The influence of the pH of the mobile phase on the correlation was investigated on phenylsilica as well as on octadecylsilica. The results of these measurements are collected in Table III. Two significant results can be noticed in this table: (1) the correlation of $\log P$ and $\log k'$ is significantly better, on both stationary phase, at low pH (3–5); (2) the correlation is significantly better on phenylsilica than that on octadecylsilica.

The effect of the pH agrees with the degree of protonization of the solutes on basis of their pK_b values. These pK_b values range from 4 to 9, and thus in the pH range 3–5 all solutes are fully protonated, which is necessary to ensure a similar retention mechanism for all basic solutes (*e.g.* ion-pairing). The better correlation on phenylsilica has previously been observed with neutral compounds in plain reversed-phase mobile phases⁶. The difference might be attributed to the difference in the percentage of methanol as used to measure the capacity factors on phenyl- and oc-

TABLE IV

LINEAR REGRESSION DATA FOR THE CORRELATION OF *n*-OCTANOL-WATER PARTITION COEFFICIENTS AND CAPACITY RATIOS OF NEUTRAL SOLUTES IN ION-PAIR SYSTEMS ON OCTADECYLSILICA AND PHENYLSILICA

Calculated according to: $\log P = a \log k' + b$. Mobile phases: with octadecylsilica, mobile phase IV, pH 4.0; with phenylsilica, mobile phase V, pH 4.0 (see Table III).

Silica	<i>a</i>	<i>b</i>	<i>r</i>	<i>F</i>	<i>S.D.</i>
Octadecyl	2.14	1.89	0.946	153	0.40
Phenyl	3.36	1.75	0.990	705	0.30

tadecylsilica. However, the results previously obtained with neutral compounds and plain mobile phase show a systematically better correlation on phenylsilica than on octadecylsilica when the same percentage of methanol is used. It is therefore unlikely that this will be different with the present ion-pair systems. However, this will be investigated in more detail in the near future.

Another advantage of the phenyl phase over the octadecyl phase is the smaller retention. This allows a more favourable speed of $\log P$ determination and detectability of solutes with large $\log P$ values.

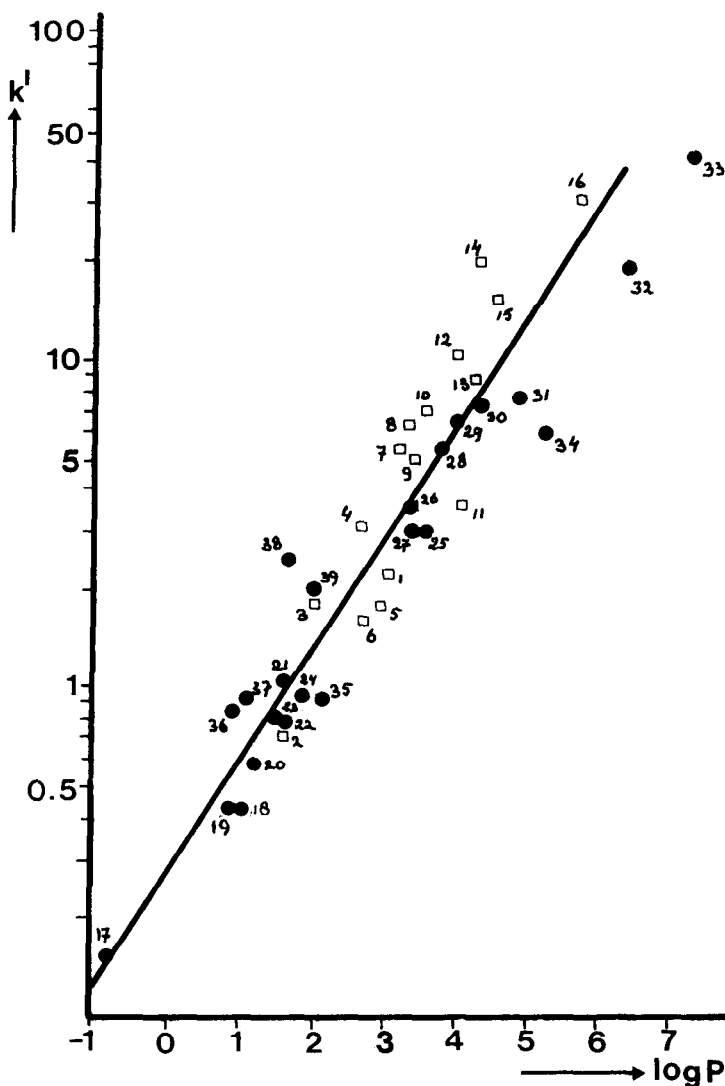


Fig. 1. Log-log relationship between *n*-octanol-water partition coefficients of neutral (\square) and basic (\bullet) solutes and their capacity ratios as measured in the ion-pair systems with octadecylsilica and phenylsilica as stationary phase. Mobile phase IV, pH 4.0 ($r = 0.940$; $F = 281$; S.D. = 0.60).

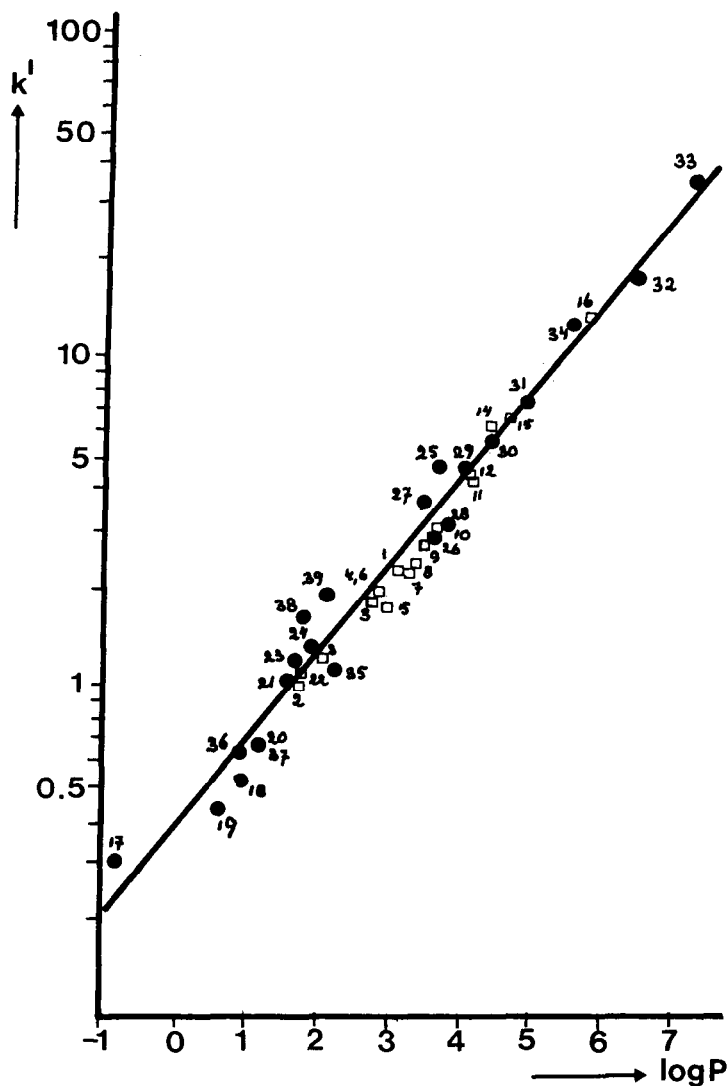


Fig. 2. Log-log relationship between *n*-octanol-water partition coefficients of neutral (\square) and basic (\bullet) test solutes and their capacity ratios as measured in the ion-pair systems with octadecylsilica and phenylsilica as stationary phase. Mobile phase V, pH 4.0 ($r = 0.987$; $F = 1392$; S.D. = 0.30).

On the basis of the results in Tables II and III it can be concluded that the phenylsilica stationary phase combined with a mobile phase of 0.05 *M* phosphate buffer-methanol (45:55, v/v), containing 0.1% SDS (w/v) and 0.1 *M* sodium perchlorate, and a pH of 3-4, is a good choice for the correlation of $\log P$ and $\log k'$ of the basic compounds under investigation. Under these conditions, the capacity factor of solutes with a $\log P$ value of 7 is *ca.* 40, which allows fast determination of $\log P$ in the range 1-7 with an error in the prediction of $\log P$ of ± 0.1 log units at a 95% confidence limit.

In a previous report⁶, we have shown that $\log P$ of neutral compounds can be accurately predicted from their retention on phenyl silica, and to a lesser extent on octadecylsilica with aqueous buffer-methanol mixtures. It was worth investigating whether the retention of neutral compounds in the present ion-pair system can also be used to predict their $\log P$ values. Thus the retentions of a number of neutral test solutes with $\log P$ values in the range 1-5 were measured on both silica phases and correlated with their known $\log P$ values; the results are summarized in Table IV. This table shows that the correlation on phenylsilica is significantly better than that on octadecylsilica, in agreement with earlier findings⁶. The results on the phenyl phase allow the prediction of $\log P$ of neutral compounds with an error of ± 0.15 log units at a 95% confidence limit. This is slightly worse than the results obtained with plain buffer-methanol mixtures as the mobile phase, when $\log P$ can be predicted with an error of ± 0.10 log units.

The results so far indicate that the ion-pair system is suitable for the prediction of $\log P$ of basic and neutral compounds separately. However, when $\log P$ and $\log k'$ are plotted together (see Figs. 1 and 2) the points apparently do not deviate much from a common regression line, even though the two groups of compounds are retained by different mechanisms. No explanation for this behaviour has yet been found. The correlation of both neutral and basic compounds is poor on octadecylsilica, but it is surprisingly good on the phenyl phase. On the latter, $\log P$ values of the neutral and basic compounds can be predicted from a common regression line with an error in the prediction of ± 0.10 log units at a 95% confidence limit. Because the retention of basic solutes can be changed, without significantly influencing the k' values of neutral solutes, by variation of the sodium perchlorate concentration, it is worth investigating this parameter to optimize the fit of neutral and basic solutes on the common regression line. Such a study has been undertaken with a larger set of test compounds, including acidic solutes.

CONCLUSIONS

The main conclusions of this study can be summarized as follows:

- (1) The retention of basic compounds in reversed-phase ion-pair systems can be used to predict their *n*-octanol-water partition coefficients ($\log P$).
- (2) The correlation between the retention of neutral compounds in reversed-phase ion-pair systems and their $\log P$ values is not significantly different from that obtained in other reversed-phase systems.
- (3) Phenylsilica seems to be a better stationary phase than octadecylsilica for the prediction of $\log P$ from retention data.
- (4) It seems possible to predict $\log P$ of both neutral and basic compounds from their retention in the same ion-pair system, despite differences in the retention mechanism, from a common regression line with an error in the prediction of $\log P$ of ± 0.10 log units at a 95% confidence limit.

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