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Examination of some reversed-phase high-performance liquid chromatography systems for the determination of lipophilicity

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ABSTRACT

Three reversed-phase systems [based on the divinylbenzene-styrene copolymer (PRP-1), the C_{18}-derivatized divinylbenzene-styrene copolymer (ACT-l), and the Nucleosil C_8 columns] were studied for their suitability in lipophilicity determination. Acetonitrile-water was selected as the mobile phase. Correlation between log k' and log P_{cyc} for both the PRP-1 and Nucleosil C_8 systems was superior to the correlation between log k' and either log P_{oct} or log P_{cyc} (oct = octanol; cyc = cyclohexane) on the ACT-l column. On the PRP-1 and Nucleosil columns, correlation between log k' and log P_{oct} was much improved when test compounds were grouped into classifications of non-H bonding, single amphiprotics (alcohols, phenols, amides) or double amphiprotics. Although the PRP-1 system gave broad peaks with lipophilic substrates, there was good correlation between log k' values on the Nucleosil silica-based reversed-phase system and the polymer PRP-1 system, indicating that either is suitable for the determination of lipophilicity.

INTRODUCTION

While the lipophilicity index based on octanol-water partitioning (log P_{oct}) is well established in studies of quantitative structure-activity relationships [1,2], the lack of convenience and reliability of the traditional "shake-flask"method for log P_{oct} determination [3,4] has encouraged investigations into alternative methods. Since the mid-1970s, reversed-phase HPLC has been investigated for this purpose, and there have been several reviews of this area of research [5–11]. Generally, studies have evaluated the correlation between measured (or determined) lipophilicity and the logarithm of the capacity factor ***(k').***

The goal for many has been the development of a single HPLC system which will provide a direct measure of reliable log P_{oct} values for any given test compound. A wide variety of reversed-phase HPLC systems have been examined for this purpose. For one HPLC system to mimic partitioning in octanol-water it is necessary for the hydrophobic and hydrophilic interactions in the stationary and mobile phases to be similar to those interactions in (respectively) the octanol and aqueous bulk phases. Retention in reversed-phase packing materials is primarily attributed to hydrophobic interaction, whereas partitioning into the octanol is effected by both hydrophobic and hydrogen bonding interactions.

Methanol appears to be the preferred organic modifier for the determination of lipophilicity by reversed-phase HPLC [7]. It has been suggested that ODS systems eluted with methanol-water provide good log k'–log P_{oct} correlations as methanol coats the reversed phase, giving it the necessary hydrogen-bonding properties to act as an octanol mimic (for example refs. 12 and 13). A high proportion of methanol in the eluent seems to be required to obtain a good correlation between log k' and log P_{oct} [13,14], although this is not always the case (for ex-

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ample, ref. 15). Using either methanol or acetonitrile as the organic modifier, there have been many reports where log k' in reversed-phase HPLC correlates well with the log P_{oct} values of a set of congeners (for example, refs. 16–20). However, correlation can be poor when test compounds are non-congeners (for example, refs. 21-25). Thus, it would appear that good correlation between log k' and log P_{oct} (when non-congeners are examined) may be restricted to certain combinations of stationary phase-mobile phase.

In those cases where it is necessary to group congeners to achieve good correlation between log ***k'*** and log P_{oct} the classifications were typically non-H bonders, H-bond acceptors, and amphiprotics. Taking non-H bonders as the reference, then H-bond acceptors can show greater binding to ODS columns than might be expected from their log ***P*** values [26–29] although amine additives can, in part, correct this deviation by blocking free silanol groups [30–32]. Amphiprotics generally display log ***k'*** values which are lower than predicted from their log P_{oct} values [27,33–35], presumably resulting from the lower contribution of H-bonding to partitioning into the lipophilic stationary phase compared to that found in the octanol bulk phase.

Kaliszan [10] and Braumann [7] have suggested that the search for the perfect HPLC system for determining log P_{oct} values might be a futile exercise. For quantitative structure-activity relationships (QSARs), reversed-phase HPLC log ***k'*** values could be used directly, particularly as the interactions in "dynamic" reversed-phase HPLC might provide a better model of solute interations with biomembranes than "static" liquid-liquid partitioning [5,36,37]. There are now many examples of QSAR correlations which use log ***k'*** as the measure of lipophilicity [7,10,38], and correlations can be at least as good or better than provided by use of log P_{oct} values [39].

One potential problem to the use of log ***k'*** lipophilicity values is that, unlike log P_{oct}, the scale is not universal; *i.e.* any given log ***k'*** value is specific to one HPLC system, which cannot readily be reproduced elsewhere with certainty. Braumann [7] examined six ODS columns, and found that log k'_w values for any given test substrate were similar on all systems, indicating that log k'_w might be used as a universal indicator of lipophilicity. However. other studies have shown that log k'_w is not universal [40], which is not surprising given the diverse characteristics of ODS columns from different commercial sources [41]. Therefore, while log ***k'*** values may be used directly for QSARs studies, in reporting these data, it is still necessary to provide some data on the calibration of HPLC systems used in lipophilicity determination so that the log ***k'*** values and conclusions from the QSARs may be utilized by other researchers.

Our interest in lipophilicity determination stems from the necessity of developing structure–distribution relationships (SDRs) for the discovery of new ^{99m}Tc radiopharmaceuticals [42]. Problems of stability and purity with many ^{99m}Tc compounds indicate that an HPLC method for lipophilicity determination is preferable over the "shake-flask" method. In developing an HPLC method for the determination of lipophilicity of ^{99m}Tc radiopharmaceuticals, two groups independently selected the Hamilton PRP-1 column [43,44] calibrated to provide log ***P*** values. More recently, log ***k'*** values were used directly [45]. This column contained one of the first commercially available polymer-based reversed-phase packing materials. As it does not contain any uncapped silanol groups, the PRP-1 resin should not display any selective binding of basic compounds, as seen with most silica-based reversed-phase columns.

During the initial evaluation of the PRP-1 column with highly lipophilic compounds, Feld and Nunn [44] determined that use of aqueous methanol as mobile phase resulted in unacceptibly broad peaks and long retention times. Feld and Nunn found that acetonitrile was a superior organic modifier for use with this column, and selected a mobile phase of acetonitrile-ammonium acetate buffer (65:35) [44]. This system was calibrated using standard organic compounds and log ***P*** data from the MedChem database [46]. However, there are some reported disadvantages of the PRP- 1 packing material (low plate number and excessive resin swelling) [47], and π–π interactions with solutes may provide an additional retention mechanism [11]. As a result, we decided to investigate the potential of a other reversed-phase stationary phases (retaining the acetonitrile-ammonium acetate buffer mobile phase) for the determination of lipophilicity. We report here the results of studies using the divinylbenzene–

styrene copolymer (PRP-l), the C18-derivatized divinylbenzene-styrene copolymer (ACT-l), and the Nucleosil C8 columns for this purpose. Log *k'* values of test compounds obtained on these systems are compared to log P_{oct} and log P_{cyc} values for these compounds, obtained from the MedChem database.

EXPERIMENTAL

The following column-solvent combinations were examined (all systems tested used isocratic eluents):

(a) Interaction ACT-l 150 × 4.6 mm, 10 μm resin, eluted with acetonitrile–pH 4.6 0.1 *M* ammonium acetate buffer (70:30), at 0.75 ml/min.

(b) Nucleosil C_8 150 × 4.6 mm, 5 μm resin, eluted with acetonitrile–pH 4.6 0.1 *M* ammonium acetate buffer (60:40), at 1.5 ml/min.

(c) Hamilton PRP-1 150 × 4.1 mm, 10 μm resin, eluted with acetonitrile–pH 4.6 0.1 *M* ammonium acetate buffer (65:35), at 2.0 ml/min.

The HPLC system used consisted of two Rainin Rabbit HPX pumps, controlled by a personal computer operating Gilson 712 software. The system was fitted with a Kratos UV detector, operating at 210, 230 or 254 nm (as appropriate for the analyte). The system allowed the moment of sample injection to be detected by the software, and sample retention times were provided automatically by the software on data analysis. The retention time of sodium nitrate (detected at 210 nm) was used as the column dead-time. All retention times were determined in triplicate, and the mean used to determined log *k'*.

HPLC columns were obtained from Alltech. HPLC-grade acetonitrile was obtained from J.T. Baker. Water was obtained from a Milli-Q purification system. All solvents were filtered and degassed prior to use.

Ammonia solution and glacial acetic acid were obtained from Mallinckrodt. pH 4.6 0.1 A4 Ammonium acetate buffer was prepared by dissolving 6.75 ml of concentrated ammonium hydroxide solution and 11.5 ml of glacial acetic acid in 500 ml of water, and diluting to 2 1.

All test compounds were obtained from Aldrich.

Measured octanol-water partition coefficients were obtained from the MedChem database [46], as log *P** (the most reliable determined value of log P_{oct}) values. For three compounds (N,N-diethyl-*m*-toluamide, triphenylmethane and 2,6-diphenylphenol) log *P** values were unavailable, so cLog *P* (the value of log P_{oct} calculated by MedChem software) values were used instead. The values are listed in Table I.

Measured cyclohexane-water partition ratios were also obtained from the MedChem database, and are listed as log P_{cyc}. If several log P_{cyc} values were given in the MedChem database, the mean value was used (excluding any values which deviated substantially from the others). In some cases, log P_{cyc} values were calculated from given C_6–C_8 or C_{18} n-alkane-water partition ratios, using the conversions derived by Seiler [48].

RESULTS AND DISCUSSION

Determination of lipophilicity with the PRP-1 column

As indicated earlier, our original decison to use the PRP-1 column for lipophilicity determination was based upon a preference to have a packing material devoid of free silanol groups (which can provide mechanisms for the retention of amines in addition to partitioning). A polymer-based column appeared to be suitable for these studies [16,49], even though low plate number and resin swelling problems were reported for the PRP- 1 packing material. Good resolution between peaks was not a requirement of the study, and, provided that a single isocratic eluent was used, resin swelling would not be an issue *(i.e.* the void volume would remain constant). However, for the examination of lipophilic compounds (log $P_{oct} > 2$), a high proportion of an organic modifier was required, to elute such compounds in a reasonably short time. Feld and Nunn [44] elected to use acetonitrile-aqueous ammonium acetate (65:35) after evaluating a number of solvent combinations. This percentage of organic modifer is within the recommended guidelines (> 25% water) which followed a multi-center European study on the determination of log P_{oct} by HPLC [50], and appeared to provide reliable log P_{oct} values based on calibration curves containing relatively few example compounds [44]. Feld and Nunn [44] observed two calibration curves; one for compounds without hydroxyl groups, and one for compounds which contained an hydroxyl group.

TABLE I

LOG P_{oct} AND LOG P_{cyc} VALUES OF TEST COMPOUNDS USED IN THIS STUDY

n/a = Log P value not available

Compound	log P_{oct}	log P_{cyc}	Compound	log P_{oct}	log P_{cyc}
Acetanilide	1.16	− 1.51	Ethyl acetate	0.73	0.34
p-Anisidine	0.95	− 0.40	Ethylbenzene	3.15	2.76
Anisole	2.11	2.10	4-Ethylphenol	2.58	0.38
Benzaldehyde	1.48	1.24	Formamide	− 1.51	− 5.06
Benzamide	0.64	− 1.28	4-Hydroxybenzamide	0.33	n/a
Benzene	2.13	2.38	2-Hydroxybenzyl alcohol	0.73	n/a
Benzophenone	3.18	3.29	2,6-Lutidine	1.68	0.67
Benzyl alcohol	1.10	-0.70	Methylene chloride	1.25	n/a
4-Chloro-3-methylphenol	3.10	0.15	1-Naphthol	2.84	0.54
4-Chloroaniline	1.83	0.64	2-Naphthol	2.70	0.09
o-Dichlorobenzene	3.38	3.47	Napthalene	3.30	3.49
N,N-Diethyl-m-toluamide	2.31	n/a	Phenol	1.46	-0.81
1,5-Dihydroxynapthalene	1.82	− 2.23	N-Phenyl benzylamine	3.13	n/a
N,N-Dimethylbenzamide	0.62	n/a	2-Phenylphenol	3.09	1.71
1,2-Dichloroethane	1.48	1.67	Pyridine	0.65	-0.41
5,5-Diphenylhydantoin	2.47	− 2.34	Quinoline	2.03	1.26
Diphenylmethane	4.14	n/a	Resorcinol	0.80	− 3.79
Diphenylmethanol	2.67	n/a	Toluene	2.73	3.15
2,6-Diphenylphenol	5.25	n/a	Triphenylmethane	5.80	n/a
β-Estradiol	4.01	-0.02	Uracil	− 1.07	n/a

We have extended the calibration of the PRP- 1 system by measuring the log k' of more compounds, particularly compounds containing single hydroxyl and amide groups, and compounds with two amphiprotic substituents. The log k' values obtained are listed in Table II.

Linear regression analysis of the data was performed in plotting all log k' *values* against log P_{oct}, log P_{cyc} and log k' values of compounds separated into classes of non-H bonders, single amphiprotics (compounds with one hydroxyl or amide substituent), and double amphiprotics. Compounds with

TABLE II

LOG k' VALUES DETERMINED ON THE PRP-I COLUMN

Compound	log k'	Compound	log k'
Acetanilide	− 0.24	2,6-Diphenylphenol	1.30
Anisole	0.63	β-Estradiol	0.14
Benzamide	− 0.49	Ethyl acetate	− 0.05
Benzophenone	0.96	4-Ethylphenol	0.13
Benzyl alcohol	-0.15	Formamide	− 1.11
4-Chloroaniline	0.33	4-Hydroxybenzamide	-0.94
o-Dichlorobenzene	0.94	Methylene chloride	0.27
1,2-Dichloroethane	0.32	Naphthalene	1.15
1,5-Dihydroxynapthalene	-0.24	1-Naphthol	0.38
5,5-Diphenylhydantoin	-0.18	Phenol	− 0.06
Diphenylmethane	1.13	Toluene	0.76
Diphenylmethanol	0.37	Uracil	− 1.35

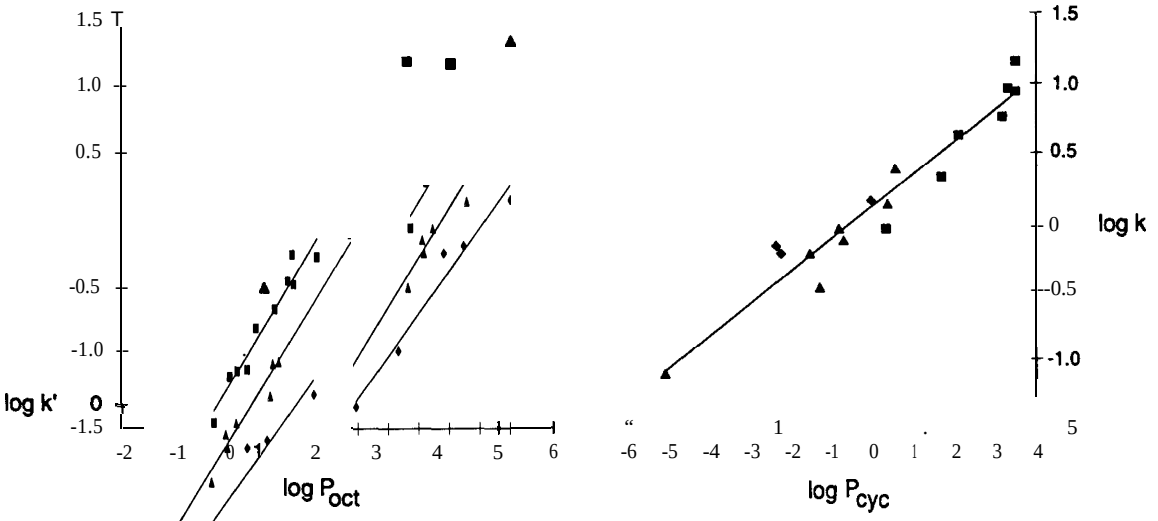

Fig. 1. Graphs of log ***k' vs. log P*** from data obtained on the PRP- 1 column. ■ = Non-hydrogen bonders; A = single amphiprotics; ● = double amphiprotics.

two amphiprotic substituents were selected such that minimal intramolecular H-bonding could occur. The results are as follows:

log ***k' vs.*** log P_{oct} (all compounds)
log ***k'*** = 0.392 log P_{oct} − 0.616; ***R*** = 0.879;
n = 24 (1)

log ***k' vs.*** log P_{cyc} (all compounds)
log ***k'*** = 0.236 log P_{cyc} + 0.106; ***R*** = 0.966;
n = 17 (2)

log ***k' vs.*** log P_{oct} (matched set')
log ***k'*** = 0.352 log P_{oct} + 0.525; ***R*** = 0.801;
n = 17 (3)

log ***k' vs.*** log P_{oct} (non-H bonders)
log ***k'*** = 0.364 log P_{oct} − 0.236; ***R*** = 0.971;
n = 10 (4)

log ***k' vs.*** log P_{oct} (all amphiprotics)
log ***k'*** = 0.347 log P_{oct} − 0.764; ***R*** = 0.937;
n = 14 (5)

log ***k' vs.*** log P_{oct} (single amphiprotics)
log *k'* = 0.356 log P_{oct} − 0.625; ***R*** = 0.993;
n = 9 (6)

[a] This set contains only those compounds which appear in the log ***k' vs.*** log P_{cyc} (all) set. The matched set was separated so that the linear regression analyses between log P_{oct} and log P_{cyc} can be compared directly.

log ***k' vs.*** log P_{oct} (double amphiprotics)
log ***k'*** = 0.306 log P_{oct} − 0.977; ***R*** = 0.966;
n = 5 (7)

The overall correlation between log ***k' vs.*** log P_{cyc} is far better than the correlation for log ***k' vs.*** log P_{oct} (eqns. 2 and 3). This is to be expected since hydrophobicity is the predominant factor influencing partitioning into both the stationary phase of this HPLC system, and the cyclohexane bulk phase, whereas partitioning into octanol, as described earlier, is influenced by both hydrophobic and H-bonding interactions between solute and solvent. When separated into amphiprotic (hydroxyl or primary-secondary amide) and non-H-bonding compounds, correlations between log ***k'*** and log ***P*** were improved, but it was clear that compounds containing two amphiprotic groups fell into a separate class to those containing a single amphiprotic substituent. Fig. 1 shows the log k'-log ***P*** data graphed, with the separate linear regression lines for the three classes of compounds examined, using log P_{oct} as the independent variable.

Determination of lipophilicity with the ACT-l column

ACT- 1 packing material is a C_{18} derivatised divinylbenzene-styrene copolymer which is claimed by the manufacturers to provide better resolution and

TABLE III

LOG k' VALUES DETERMINED ON THE ACT-l COLUMN

Compound	log k'	Compound	log k'
Acetanilide	-0.45	Ethyl acetate	-0.35
Anisole	0.21	4-Ethylphenol	− 0.05
Benzamide	-0.68	Formamide	− 1.15
Benzene	0.29	2-Hydroxybenzyl alcohol	− 0.52
Benzophenone	0.48	2,6-Lutidine	-0.38
Benzyl alcohol	-0.41	Phenol	− 0.27
4-Chloro-3-methylphenol	0.11	2-Phenylphenol	0.27
4-Chloroaniline	-0.04	Quinoline	0.27
o-Dichlorobenzene	0.63	Resorcinol	− 0.52
N,N-Dimethylbenzamide	-0.48	Toluene	0.43
Diphenylmethanol	0.04	Uracil	− 1.24

peak shapes than underivatized divinylbenzene–styrene copolymer (e.g. PRP-1). Lambert and co-workers [5 1,521 have studied the use of this packing for lipophilicity determinations in a 5-cm column (which is not commercially available), and found that, with a methanol-water eluent, log **k'** values were highly correlated to the log P_{alkane} coefficients of a diverse set of test samples. Our results using the ACT- 1 column are shown in Table III.

A disadvantage of the ACT-l column is the flow-rate restriction for acetonitrile-water mixtures of 0.75 ml/min. At this flow-rate, sample retention times are generally much longer on the 15-cm ACT-I column than on the 15-cm PRP- 1 column running at 2 ml/min. Therefore, while the claims for ACT-l (better peak shape than PRP-1 columns) may be justified at equivalent flow-rates, the low flow restriction is undesirable when dealing with highly lipophilic compounds. The results of linear regression analysis were as follows:

log **k'** *vs.* log P_{oct} (all compounds)
log **k'** = 0.361 log P_{oct} − 0.750; **R** = 0.942; n = 22 (8)

log **k' vs.** log P_{cyc} (all compounds)
log **k'** = 0.187 log P_{cyc} − **0.155; R** = 0.925; n = 19 (9)

log **k' vs.** log P_{oct} (matched set)
log **k'** = 0.357 log P_{oct} − 0.728; **R** = 0.925; n = 19 (10)

log **k' vs.** log P_{oct} (non-H bonders)
log **k'** = 0.379 log P_{oct} − 0.633; **R** = 0.986; n = 7 (11)

log **k' vs.** log P_{oct} (non-H bonders + H-bond acceptors)
log **k'** = 0.401 log P_{oct} − 0.712; **R** = 0.929; n = 10 (12)

log **k' vs.** log P_{oct} (all amphiprotics)
log k' = 0.310 log P_{oct} − 0.786; **R** = 0.988; n = 12 (13)

log **k' vs.** log P_{oct} (single amphiprotics)
log **k'** = 0.295 log P_{oct} − 0.756; **R** = 0.988; n = 9 (14)

log k' vs. log P_{oct} (double amphiprotics)
log **k'** = 0.395 log P_{oct} − 0.817; **R** = 0.999; n = 3 (15)

Graphs of log **k'** *vs.* log P_{oct} of individual compound classes are shown in Fig. 2. Two distinct classes can be seen, non-H bonders (a group which includes H-bond acceptors such as ethers and esters) and amphiprotics. There appears to be no distinction between compounds having either one or two amphiprotic groups.

Including all compounds for which log P_{oct} or log P_{cyc} data are available, correlation between log **k'** and either of these parameters is poor. Considering that an excellent correlation between log **k'** and log P_{alkane} was reported by Lambert and co-workers for the ACT-I packing using a mobile phase of aqueous methanol [51,52], acetonitrile is clearly inferior to methanol as mobile phase modifier in conjunction with this packing material for the measurement of lipophilicity.

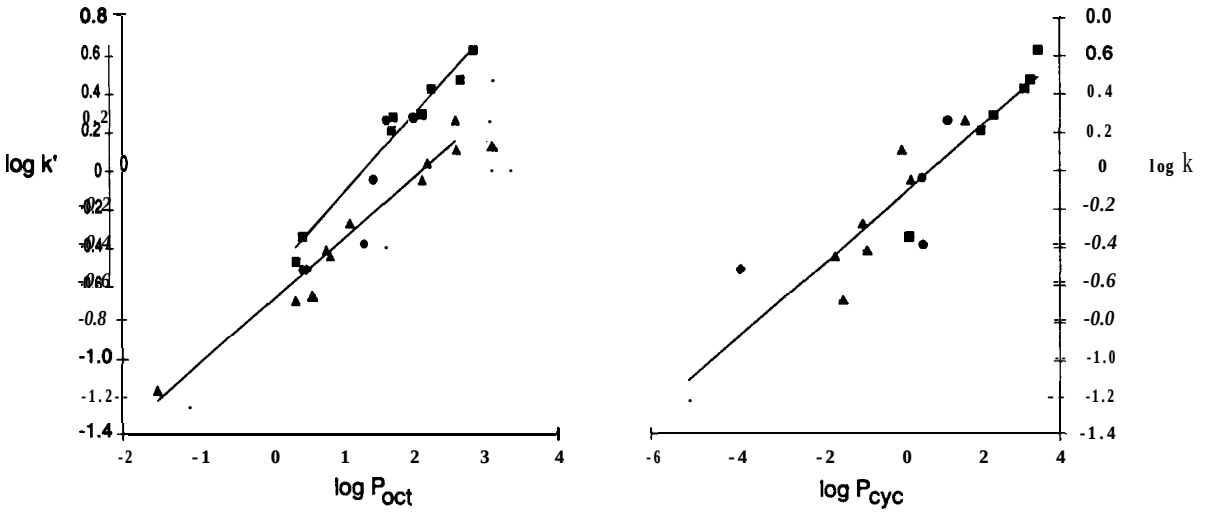

Fig. 2. Graphs of log *k' vs. log P* from data obtained on the ACT-l column. ■ = Non-hydrogen bonders; A = single amphiprotics; ● = double amphiprotics; ● = H-bond acceptors.

Determination of lipophilicity with the Nucleosil C8 column

The silica-based Nucleosil C8 column was included in this study for comparison with the polymer-based columns. We have used this column previously [53] and found no evidence that interaction with free silanols played an appreciable part in the retention of solutes. By comparison with the PRP-1 column, the higher plate number and lower lipophilicity of the stationary phase lead to sharper peaks and shorter retention times. After investigating several possible acetonitrile-buffer solvent ratios (not reported), an eluent containing acetonitrile–ammonium acetate buffer (60:40) was selected such that test compounds with a wide range of lipophilicities could be studied using a single solvent ratio. The

TABLE IV

LOG *k'* VALUES DETERMINED ON THE NUCLEOSIL C_8 COLUMN

Compound	log *k'*	Compound	log *k'*
Acetanilide	-0.12	β-Estradiol	0.17
p-Anisidine	-0.12	Ethyl acetate	− 0.09
Anisole	0.17	Ethylbenzene	0.36
Benzamide	-0.28	Formamide	-0.63
Benzophenone	0.33	4-Hydroxybenzamide	-0.52
Benzyl alcohol	-0.15	2,6-Lutidine	0.25
4-Chloro-3-methylphenol	0.07	Naphthalene	0.36
4-Chloroaniline	0.08	2-Naphthol	0.06
o-Dichlorobenzene	0.38	1-Naphthol	0.10
1,5-Dihydroxynaphthlene	-0.19	Phenol	-0.12
N,N-Dimethyl-m-toluamide	0.16	N-Phenyl benzylamine	0.32
N,N-Dimethylbenzamide	-0.10	Pyridine	0.07
5,5-Diphenylhydantoin	-0.13	Quinoline	0.23
Diphenylmethanol	0.13	Toluene	0.27
2,6-Diphenylphenol	0.52	Triphenylmethane	0.71
		Uracil	− 0.74

retention times of solutes using this system are listed in Table IV. Results of linear regression analyses on *log k'*–log *P* sets are given below:

log *k'* *vs.* log P_{oct} (all compounds)
log *k'* = 0.180 log P_{oct} − 0.325; *R* = 0.892; *n* = 31 (16)

log *k'* *vs.* log P_{cyc} (all compounds)
log *k'* = *0.111* log P_{cyc} + *0.006; R* = 0.955; *n* = 22 (17)

log *k'* *vs.* log P_{oct} (matched set)
log *k'* = 0.164 log P_{oct} + 0.267; *R* = 0.840; *n* = 22 (18)

log *k'* *vs.* log P_{oct} (non-H bonders)
log *k'* = 0.161 log P_{oct} − 0.186; *R* = 0.995; *n* = 10 (19)

log *k'* *vs.* log P_{oct} (all amphiprotics)
log *k'* = 0.175 log P_{oct} − 0.437; *R* = 0.962; *n* = 15 (20)

log *k'* *vs.* log P_{oct} (single amphiprotics)
log *k'* = 0.164 log P_{oct} − 0.360; *R* = 0.992; *n* = IO (21)

log *k'* *vs.* log P_{oct} (double amphiprotics)
log *k'* = 0.180 log P_{oct} − 0.556; *R* = 0.997; *n* = 5 (22)

The results were similar to those found on the PRP-1 system. The overall correlation between log *k'* and log P_{cyc} was better than that between log *k'* and log P_{oct} (eqns. 17 and 18). Correlations between log *k'* and log P_{oct} were much improved when test substances were separated into individual classes (non-H bonders and amphiprotics), and, as was the case with the PRP-1 system, the correlation between log *k'* and log P_{oct} of amphiprotics could be improved further by separating these compounds into two classes; those containing a single amphiprotic substituent, and those containing two amphiprotic substituents. Several compounds with H-bond acceptor functional groups were also included in this study, but little correlation was found between log *k'* and log P_{oct} or log P_{cyc}. Those with non-ionisable functional groups fell on (or close to) the non-H-bonder trend line. The remainder of the H-bond acceptors were bases; although weak bases were selected for study, the pH of the buffer used (pH 4.6) was probably inappropiate to ensure minimal ionization.

Comparison of the PRP-I and Nucleosil C_8 columns

One purpose for this study was to examine alternative systems to the PRP-1 HPLC method for the determination of lipophilicity because of the concerns about resolution and peak shape (highly

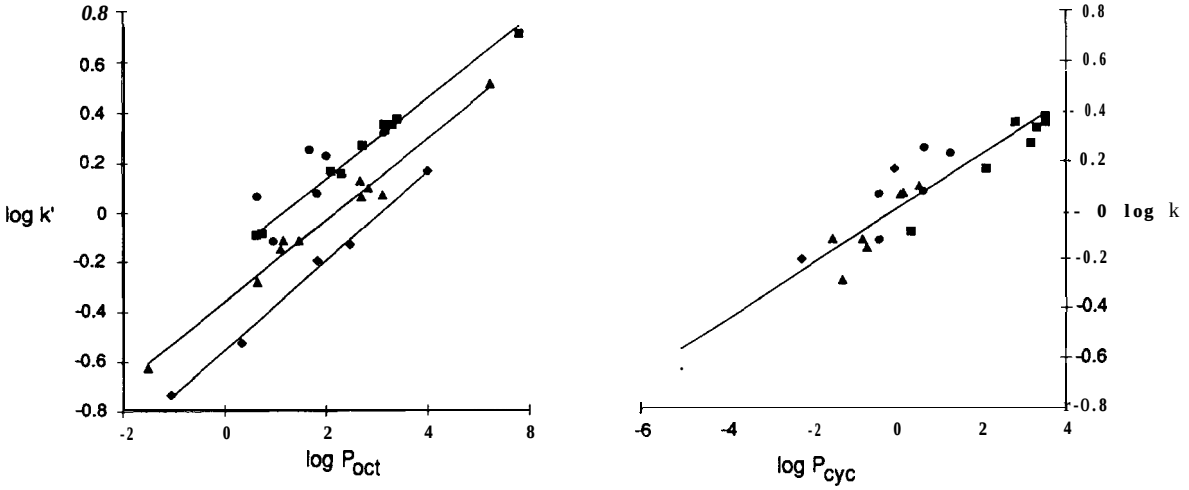

Fig. 3. Graphs of log ***k' vs.*** log ***P*** from data obtained on the Nucleosil C_8 column. ■ = Non-hydrogen bonders; A = single amphiprotics; ◆ = double amphiprotics; ● = H-bond acceptors.

lipophilic compounds gave broad peaks)' on the PRP-1 system. While the Nucleosil C8 system provided sharper peaks and shorter retention times than were observed on the PRP-1 system, the graphs shown in Figs. 1 and 3 suggest that both systems provided similar data. To confirm this, log k' values obtained on the PRP-1 system were compared with those on the Nucleosil C8 system.

A total of 20 compounds were examined on both PRP-1 (PRP) and Nucleosil (Nuc)C_8 systems. Linear regression analysis of log ***k'*** values obtained on both systems gave the following result:

$$\log k'_{Nuc} = 0.454 \log k'_{PRP} - 0.082. \; R = 0.995; \; n = 19 \quad (23)$$

One compound was excluded from this comparison. β-Estradiol had a shorter retention time on the PRP-1 system than might be expected from its log P_{oct}, although its log ***k'*** on the Nucleosil C_8 system is close to the trend line for compounds with two amphiprotic groups. The remaining compounds represent a mixture of non-H bonders, and compounds with one or two amphiprotic substituents. As there is a highly correlated linear relationship between the log ***k'*** values obtained on either system, and both show good correlation between either log P_{cyc} or log P_{oct} (for congeners), either system appears to be suitable for the determination of lipophilicity. As the silica-based column may present anomalies with certain classes of compounds due to silanol interaction, the PRP-1 system might be superior, despite giving broad peaks with highly lipophilic compounds.

The influence of hydrogen bonding on log k'

In several previous studies, it was noted that compounds with amphiprotic substituents formed a separate group from compounds with no hydrogen bonding substiuents when correlating log ***k'*** and log P_{oct} [for example 27,28,33–35]. This is not unreasonable when partitioning into the stationary phase has a high hydrophobic and low H-bonding components, such that log ***k'*** is more closely related to log P_{alkane} than log P_{oct}. Seiler [48] and others [54–56] have noted that interconversion of log P_{oct} and log P_{alkane} for any compound involves a term which sums all intermolecular hydrogen-bonding interactions experienced by that molecule. Thus, all amphiprotic compounds should not appear in a single series (one highly correlated log k'-log P_{oct} plot) in HPLC systems which show good correlation between log ***k'*** and log P_{alkane}. Instead, the deviation of any compound from the log k'–log P_{oct} regression line should be a function of its intermolecular H-bonding capacity.

As the log ***k'*** values on the PRP-1 and Nucleosil C_8 systems used in this study demonstrated better correlation with log P_{cyc} than log P_{oct} ***we*** decided to evaluate whether there was an additive H-bonding effect on log ***k'.*** A series of compounds having two amphiprotic substituents were selected for evaluation. Compounds were selected on the basis that little or no intramolecular hydrogen bonding was possible. When examined on the PRP-1 and Nucleosil C_8 systems, these compounds clearly formed a separate group from compounds with only one amphiprotic substituent. As expected, the log k'–log P_{oct} regression line of the double amphiprotics deviated further from the regression line of non-H bonders than did the single amphiprotics, as would be expected from the known relationship between log P_{oct} and log P_{alkane}.

The validity of dividing the compounds into the groups non-hydrogen bonders, single amphiprotics and double amphiprotics in log ***k' vs.*** log P_{oct} plots was tested by two statistical methods. Using analysis of covariance, the slopes of the three sets of data for each column (PRP-1 and Nucleosil C_8) were found not to be different, whereas the intercepts were significantly different at $p < 0.05$. The Bonferroni multiple comparison procedure was used to ascertain the pairwise relationship between sets of data for each column. These were found to be different atp < 0.001.

CONCLUSIONS

The specific interations experienced by test substrates in shake-flask determinations of lipophilicity are unlikely to be duplicated exactly in the HPLC experiment. It is therefore doubtful that any HPLC system can provide a scale of lipophilicity values which correlates perfectly with those obtained in the shake-flask experiment. Instead, HPLC can provide a scale of lipophilicity which is dependent upon the column-solvent combination, and which, as shown by others, can be equally valid in structure-activity correlations as the log P lipophilicity

scale. However, HPLC systems do need to be calibrated with substances of known (shake-flask) lipophilicity, so that lipophilicities (log *k'* values) obtained on one HPLC system can be compared to those obtained on another system.

In this study, the polymer-based PRP-1 and ACT-l columns, and the silica-based C_8 systems were compared. The highly retentive nature of the PRP-1 column towards lipophilic compounds necessitated the use of acetonitrile as the organic modifier for this column, and all three columns were eluted with isocratic acetonitrile-aqueous ammonium acetate. The results from this study indicate that an acetonitrileebuffer mixture is unsuitable as an eluent for lipophilicity determination on the ACT- 1 column. On the PRP-1 and Nucleosil C_8 columns with acetonitrile-buffer as eluent, there was far better correlation between log *k'* and log P_{cyc} than log *k'* and log P_{oct} (*R* = 0.966 and 0.801, respectively, on the PRP-1 column; 0.955 and 0.840, respectively, on the Nucleosil C_8, from data using matched sets of compounds). However, when test substrates were divided into individual classes of non-H bonders, and compounds with either one or two amphiprotic substituents, correlation between log *k'* and log P_{oct} was notably improved. In plotting log *k' vs.* log P_{oct}, compounds with either one or two amphiprotic substituents formed two distinct groups.

There was high correlation between log *k'* values obtained on the PRP-1 and Nucleosil C_8 systems. The lipophilicity scale provided by these systems should place series of compounds in a similar rank order to that obtained with log P_{cyc} measurement, but the rank order of lipophilicity will only be comparable to log P_{oct} in series of compounds with similar hydrogen-bonding properties.

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