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Deactivated hydrocarbonaceous silica and immobilized artificial membrane stationary phases in high-performance liquid chromatographic determination of hydrophobicities of organic bases: relationship to log *P* and CLOGP

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Abstract: Retention parameters for a series of 29 organic base drugs (including 17 phenothiazine derivatives) were measured by reversed-phase high-performance liquid chromatography (HPLC) employing new columns of distinctive partition properties. One column was a deactivated alkyl-bonded silica and two others were packed with lecithin-bonded propylamino-silica, i.e. the immobilized artificial membrane (IAM) columns; one of the IAM stationary phases had the unreacted propylamine moieties additionally end-capped with methylglycolate. The highly deactivated hydrocarbonaceous silica column showed regular rectilinear relationships between logarithms of chromatographic capacity factors and the content of organic modifier in aqueous eluent; it is suitable for generating a chromatographic scale of hydrophobicity. Such a scale (hydrocarbonaceous) is different from that provided by measurement of partitioning of solutes between *n*-octanol and water (alkanol log *P* scale). The relative hydrophobicity parameters determined by HPLC on the IAM columns were different from both log *P* scale and from the hydrocarbonaceous chromatographic hydrophobicity scale. The hydrophobicity parameter, CLOGP, theoretically calculated by the fragmental methods, correlated better than log *P* with chromatographic hydrophobicity parameters. It has been postulated that each hydrophobicity measuring system reveals some specific aspects of the hydrophobicity phenomenon and that the nature of hydrophobic binding sites on receptors and plasma proteins may require different hydrophobicity models than drug permeation through biological membranes. By means of HPLC, diverse hydrophobicity measures can readily be determined, among which those most suitable for specific QSAR applications can be identified.

Keywords: *Hydrophobicity; log* P; *CLOGP; chromatographic hydrophobicity parameters; deactivated hydrocarbonaceous silica stationary phase; immobilized artificial membrane stationary phases.*

Introduction

Hydrophobicity expresses the property of rejecting, or not preferring, the aqueous environment, it is the complex net effect of fundamental intermolecular interactions which determine the state of all matter. While these fundamental physicochemical interactions (orientation, inductive, dispersive, hydrogen bonding, charge transfer) can be related to the properties of the solutes themselves, what is observed as hydrophobicity appears to depend in part on the properties of the solvent [1, 2]. Indeed, the quantification or scaling of hydrophobicity is realized by measuring the tendency of two (or more) solute molecules to aggregate in aqueous solution [3].

The hydrophobic effect is assumed to be the 'driving force' in the case of liquid–liquid partitioning, passive diffusion transport through biological membranes and some aspects of drug–receptor binding. Since hydrophobicity depends strongly on the environment, separate (however related) hydrophobicity scales are best suited for the prediction of solute behaviour in individual systems.

The *n*-octanol–water liquid–liquid partition system is the common reference system providing the most popular scale of hydrophobicity, i.e. logarithm of partition coefficient, log *P* [4]. The log *P* is a unique, continuous scale for comparing hydrophobicity of chemical compounds. Although the *n*-octanol–water system

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cannot be assumed the best possible model for all biological permeation barriers and receptor binding sites, the large compilations of log *P* data [5] form the basis for many satisfactory bioactivity predictions.

The problems associated with the determination of log *P* resulted in the development of thin-layer chromatographic [6, 7] and high-performance liquid chromatographic [8, 9] methods of hydrophobicity parameterization. Chromatographic methods and procedures of hydrophobicity evaluation can be divided into three main classes:

(i) approaches aimed at mimicking the standard 'shake-flask' *n*-octanol–water partition system;

(ii) attempts to establish a convenient, reliable, universal, continuous chromatographic scale of hydrophobicity;

(iii) a search for bio-mimicking, pharmacologically distinguished hydrophobicity scales.

Reports on 'octanol-like' HPLC systems are reviewed elsewhere and severe limitations are indicated which make the approach impractical [2]. However, recent progress in the technology of reversed-phase materials has raised the possibility of designing an HPLC system which is more convenient and even more versatile than the classical octanol–water partition system [10].

For years the octadecyl-bonded silica (ODS) stationary phases were employed in hydrophobicity studies. Yet, the retention data obtained with nominally the same type of HPLC columns under identical mobile phase conditions are hardly comparable [11]. To get an inter-laboratory comparable, universal hydrophobicity scale like that of log *P*, the classical alkyl-bonded silica materials are unsuitable even if special precautions are undertaken to reduce the specific effects of the stationary phase. These specific effects are especially disturbing in the case of basic organic solutes. Recently, HPLC columns have become available which are loaded with a specially deactivated hydrocarbonaceous silica materials and which are reported to be devoid of the former limitations [12]. The performance of such a column in the determination of hydrophobicity is investigated here.

If the hydrophobicity measuring system is to model a given biological phenomenon then the close similarity of the component entities is a prerequisite. Thus, the partitioning system expected to model the transport through biological membranes should be composed of an aqueous phase and an organized phospholipid layer (bilayer). Miyake *et al.* [13] derived HPLC hydrophobicity parameters employing a column of silica gel coated physically with dipalmitoyl phosphatidylcholine (DPPC). Leaving aside inconveniences regarding their preparation and stability, the systems with DPPC adsorbed onto silica most probably do not emulate the lipid dynamics of biological membranes, because the adsorbed lipids are not organized in a manner similar to natural (or artificial) membranes.

The immobilized artificial membranes (IAM), recently introduced as chromatographic packing materials, appear to be more reliable and convenient models of natural membranes [14–16]. The IAM surfaces are synthesized by covalent binding of the membrane forming phospholipids to solid silica surfaces. They are confluent monolayers of immobilized membrane lipids, wherein each lipid molecule is covalently bound to the surface. Membrane lipids possess a polar headgroup and two nonpolar alkyl chains. One of the alkyl chains is linked to the solid surface. The immobilized lipid headgroups protrude away from the stationary phase surface and are the first contact site between solutes and IAM [16].

Preliminary studies [15, 16] have demonstrated a good correlation between retention data determined on IAM and human skin permeation for short series of alcohols and steroids. The same biological data showed poor correlation with chromatographic retention parameters determined on a regular hydrocarbonaceous reversed-phase column. The stable HPLC columns of the IAM type appear especially interesting from the viewpoint of determining the biologically most relevant hydrophobicity parameters. Their performance is tested here for a representative group of drugs of the organic base type.

Materials and Methods

Materials

The solutes studied are listed in Table 1. 2-Acetylphenothiazine, 2-methoxyphenothiazine and 2-(trifluoromethyl) phenothiazine were obtained from Chemical Dynamics Corporation (South Plainfield, NJ, USA). The remaining solutes were purchased from Sigma

Table 1
Solutes analysed and their hydrophobicity parameters

No.	Compound	pK_a*	log P*	CLOGP*	log k'_w†	log k'_{80}‡ IAM	log k'_{75}§ IAM MG
1	Fluphenazine	3.9 8.1	4.36	5.90	4.79	1.654	1.401
2	Propiomazine	6.6 9.10		5.00	3.94	1.412	1.265
3	Ethopropazine	9.6	3.48‖	5.54	3.64	1.242	1.154
4	Promethazine	9.1		4.65	3.66	1.325	1.195
5	2-Acetylphenothiazine				3.80	1.041	1.126
6	Triflupromazine	9.2	5.19	5.53	4.37	1.674	1.474
7	2-Trifluoromethylphenothiazine				5.22	1.582	1.646
8	Promazine	9.4	4.55	4.28	3.37	1.353	1.171
9	Phenothiazine	2.52	4.15	4.06	3.86	1.020	1.134
10	Trimeprazine	9.00		4.95	3.54	1.319	1.168
11	2-Methoxyphenothiazine				3.98	1.094	1.164
12	Chlorpromazine	9.30	5.35	5.20	4.02	1.624	1.440
13	Trifluoperazine	8.1	5.03	6.48	4.86	2.066	1.754
14	Perphenazine	7.8	4.20	5.57	4.12	1.612	1.373
15	Thioridazine	9.50	5.90	6.42	4.18	2.042	1.747
16	Procholorperazine	3.78 8.1		6.15	4.66	2.050	1.782
17	Acetopromazine				3.20	1.270	1.037
18	Carbamazepine		2.45	1.98	2.15	0.097	0.178
19	*cis*-thiothixene	7.67 7.97	3.78	4.80	3.70	1.713	1.450
20	Chlorprothixene	7.60	5.18	5.30	4.22	1.634	1.494
21	Imipramine	9.5	4.80	4.41	3.15	1.267	1.092
22	Clomipramine	9.38	5.19	5.30	3.87	1.577	0.995
23	Desipramine	10.4	4.9	4.09	2.00	1.174	0.995
24	Triprolidine	6.5 9.50	3.92	3.47	2.42	1.003	0.824
25	Pyrilamine	4.02 8.92	3.27	2.77	2.18	0.809	0.554
26	Pheniramine	4.2 9.3		2.02	1.60	0.649	0.364
27	Diphenhydramine	9.0	3.27	3.36	2.37	0.800	0.604
28	Chlorpheniramine	9.16	3.39	2.73	2.57	0.967	0.744
29	Tripelenamine	4.2 8.71		2.85	2.19	0.765	0.557

* pK_a values, logarithms of experimental (log P) and theoretical (CLOGP) n-octanol–water partition coefficients were taken from ref. 5, except where noted.

† Logarithm of HPLC capacity factor from deactivated hydrocarbonaceous silica column, normalized to 0% organic modifier in mobile phase.

‡ Logarithm of HPLC capacity factor determined on immobilized artificial membrane (IAM) column (3 cm length, non-end-capped) with acetonitrile–pH 7.00 buffer (20:80, v/v).

§ Logarithm of HPLC capacity factor determined on IAM column (15 cm length, end-capped with methyl glycolate) with acetonitrile–pH 7.00 buffer (25:75, v/v).

‖ log P determined at pH 7.8.

Chemical Co. (St Louis, MO, USA) and used as received.

Deactivated reversed-phase column Suplex™ pKb-100 (15 cm × 4.6 mm i.d.) was purchased from Supelco, Inc. (Bellefonte, PA, USA).

1-Myristoyl-2-[(13-carboxyl)-tridecoyl]-*sn*-3-glycerophosphocholine (lecithin-COOH) bonded to silica-propylamine (IAM.PC) column and the IAM.PC column with the unreacted propylamine moieties end-capped with methylglycolate (IAM.PC.MG), were kindly provided by Regis Chemical Company (Morton Grove, IL, USA). The IAM.PC column was 3 cm × 4.6 mm (i.d.); particle diameter 12 μm; pore diameter 300 Å. The IAM.PC.MG column was 15 cm × 4.6 mm (i.d.) with other characteristics as for the IAM.PC column.

Apparatus

The chromatographic system consisted of a Spectroflow 400 pump, 480 injector module equipped with 20-μl loop, a 783 programmable absorbance detector obtained from ABI Analytical (Ramsey, NJ, USA) and a Shimadzu C-R6A integrator (Shimadzu Corporation, Kyoto, Japan).

Chromatographic conditions

The chromatographic experiments were carried out at ambient temperature using a flow rate of 1 ml min^{-1}.

Hydrocarbonaceous silica column Suplex™ pKb-100. Chromatography was carried out under gradient conditions using methanol–buffer eluents in the following proportions (v/v): 90:10, 80:20, 70:30, 60:40 and 50:50. The pH 7.00 buffer was prepared by adding 0.1 M NaOH to a solution of 0.02 M CH_3COOH, 0.02 M H_3PO_4 and 0.02 M H_3BO_3.

Capacity factors were calculated assuming constant dead volume of the column. The dead volume was determined by measuring signals of deuteromethanol (CD_3OD) or deuterium oxide (D_2O) chromatographed with pure methanol (CH_3OH) or water (H_2O) eluents, respectively [17].

Logarithms of capacity factors (log k') for individual solutes were regressed against volume fraction of methanol in the eluent. Excellent linearity of the relationships over the whole eluent composition range studied (correlation coefficient $r > 0.995$ for all solutes, except desipramine for which $r = 0.983$) allowed for extrapolation of log k' to 0% methanol (100% buffer). The retention parameters normalized to pure buffer, log k'_w, were subjected to further analysis.

Immobilized artificial membrane columns IAM.PC and IAM.PC.MG. Chromatography was carried out isocratically using eluents in proportions selected to provide good differentiation among the solutes with reasonable retention times. In the case of the short, non-end-capped IAM.PC column, the eluent was acetonitrile–pH 7.00 buffer (20:80, v/v), while comparable retention times in the case of longer, end-capped IAM.PC.MG column were provided by acetonitrile–buffer (25:75, v/v). A 0.1 M sodium phosphate buffer of pH 7.00 was used.

Capacity factors were calculated assuming as the dead volume of the column the mean of solvent disturbance signals given by acetonitrile and water.

Retention properties of the IAM.PC column with regard to the markedly hydrophobic solutes were observed to increase with time of column operation. Retention time increased approximately 3% per 24 h in the case of unsubstituted phenothiazine. During the *ca* 10 h required to complete the isocratic experiment, the change in retention time was about 1%, which is acceptable. However, the operation time of several days which would be required to complete experiments under gradient conditions aimed at determining the capacity factor normalized to pure buffer (log k'_w) precluded such an attempt.

Reference hydrophobicity parameters

The logarithms of *n*-octanol–water partition coefficients (log *P*) were determined experimentally (log *P*). The reference hydrophobicity parameters were also calculated according to the Pomona College MedChem program (CLOGP). The CLOGP values for 25 of the compounds were found in a recent compilation [5]. The pK_a values were also taken from the same compilation [5].

Results and Discussion

The solutes used in this study are listed in Table 1 together with their pK_a values and hydrophobicity parameters (where known). The first 17 of the 29 solutes studied are phenothiazine derivatives; the remaining solutes being other aromatic bases of psychotropic and antihistaminic classes.

The new, highly deactivated hydrocarbon-bound silica stationary phase, Suplex™ pKb-100 provided excellent rectilinearity in the dependence of log k' on organic modifier concentration in mobile phase over the whole eluent composition range studied (90–50% of methanol). These findings fully confirm the observations reported for other chemical classes of solutes [12–18]. The good rectilinearity of log k' vs methanol concentration in the eluent in the case of basic organic solutes proves that there has been effective suppression of the silanophilic interactions typical of regular ODS reversed-phase materials. This rectilinearity makes it possible to normalize the measured chromatographic hydrophobicity parameters to the value of log k'_w extrapolated to pure water (buffer). Thus, the new reversed-phase material appears promising from the viewpoint of constructing a standard, reference chromatographic scale of hydrophobicity which should lack many of the disadvantages of the classical log *P* scale [19]. Certainly, it cannot be expected simply to parallel the log *P* scale. Hydrocarbonaceous silica is different from n-octanol and log k'_w will comprise

different information on the structure of solutes other than log P, although both parameters should reflect the relative 'phobicity' of solutes towards aqueous environment.

The efforts to specify an approach which would precisely correlate the reversed-phase HPLC parameters with log P for a larger series of non-cogeneric solutes appear to be futile and unnecessary. However, the log P hydrophobicity scale has received such a degree of recognition that for the majority of authors, the only indispensable and sufficient condition for using a chromatographic measure of hydrophobicity the extent to which it conforms with the log P scale. For example, when recommending the Suplex™ pKb-100 stationary phase for use in hydrophobicity determinations, the inventors claimed a good correlation of log k' with log P [12]. However, to arrive at this apparently 'good' correlation, they had to exclude three solutes out of the 10 studied. The actual correlation for the complete set of solutes was evidently poor. In addition, when a series of benzodiazepine derivatives was chromatographed on the Suplex™ pKb-100 stationary phase, the correlation between log k'_w and CLOGP was $r = 0.75$ [18].

Correlation between the log k'_w data determined in this study and the experimental log P data was also low. For the subset of 19 solutes for which log P data were available, the correlation coefficient was $r = 0.63$ (Table 2), and for a set of closely congeneric phenothiazine derivatives there was practically no correlation at all ($r = 0.34$).

Table 2
Linear relationships between the hydrophobicity measures investigated

Dependent vs independent variable*	Number of data points	Intercept	Slope	Correlation coefficient
CLOGP vs log P (all solutes)	19	−0.080	1.077	0.756
CLOGP vs log P (phenothiazines)	9	3.308	0.455	0.402
log P vs log k'_w (all solutes)	19	2.251	0.612	0.634
log P vs log k'_w (phenothiazines)	9	2.574	0.511	0.339
CLOGP vs log k'_w (all solutes)	25	0.185	1.266	0.920
CLOGP vs log k'_w (phenothiazines)	13	0.332	1.233	0.773
log P vs log k'_{80} (all solutes)	19	2.298	1.527	0.814
log P vs log k'_{80} (phenothiazines)	9	2.165	1.589	0.737
CLOGP vs log k'_{80} (all solutes)	25	1.097	2.597	0.938
CLOGP vs log k'_{80} (phenothiazines)	13	2.143	2.052	0.888
log P vs log k'_{75} (all solutes)	19	2.405	1.698	0.786
log P vs log k'_{75} (phenothiazines)	9	1.185	2.492	0.786
CLOGP vs log k'_{75} (all solutes)	25	1.294	2.881	0.944
log P vs log k'_{75} (phenothiazines)	9	0.130	0.234	0.838
log k'_{75} vs log k'_{80} (all solutes)	29	−0.000	0.869	0.946
log k'_{75} vs log k'_{80} (phenothiazines)	17	0.332	0.685	0.931
log k'_{75} vs log k'_w (all solutes)	29	−0.228	0.389	0.901
log k'_{75} vs log k'_w (phenothiazines)	17	−0.131	0.365	0.817
log k'_{80} vs log k'_w (all solutes)	29	−0.034	0.382	0.814
log k'_{80} vs log k'_w (phenothiazines)	17	−0.106	0.393	0.647

*For definition of variables see footnotes in Table 1.

These results are at variance with those found when CLOGP values were compared to log k'_w. In this case, a significant correlation, $r = 0.92$, was found between the values. However, when the comparison was restricted to phenothiazines, there was a drop in the observed correlation to $r = 0.77$.

In the present paper, the CLOGP calculations are performed assuming an 'extended' form of the solute molecule. This form might be considered to prevail in the hydro-organic chromatographic mobile phase, whereas some molecular 'folding' might be considered to affect log *P* measurements in 1-octanol–water systems.

These observations call into question the opinion expressed by Eadsforth [20] and Leo [21] that CLOGP parameters can be more reliable predictors of log *P* than the reversed-phase HPLC retention parameters. Actually, neither of the two appears to be suitable for the prediction of log *P* values for the solutes used in this study. This does not disqualify chromatographic hydrophobicity scales. On the contrary, such a scale (hydrocarbonaceous) could provide information on properties of drug solutes supplementary to the information contained in the log *P* scale (alkanolic). With the highly deactivated hydrocarbon-bonded silica HPLC columns now available, the respective retention data can readily be generated.

The structure of IAM stationary phases implies that the partition mechanism is different from that used in determining log *P*. Retention on IAM involves partitioning of a solute into the organized layer of the hydrocarbon region and its interactions with the polar headgroups which contain both anion and cation exchange sites. Additionally, in the case of the non-end-capped phase IAM.PC, polar interactions with the uncapped primary amine (propylamine) must be taken into consideration. The presumed mechanism of retention on IAM stationary phases should mimic biological membrane permeation processes and not log *P*.

As is evident from Table 2, correlations between log k' data determined on both IAM type columns and experimental log *P* are not high. In the case of a column that is not end-capped the logarithm of the capacity factor determined with eluent composed of 80% (v/v) sodium phosphate buffer of pH 7.00 and 20% (v/v) acetonitrile, log k'_{80}, correlates to log *P* with the coefficient $r = 0.81$. In the case of the column that is end-capped with methylglycolate (IAM.PC.MG) operated with the eluent composition buffer–acetonitrile (75:25, v/v) the respective correlation was $r = 0.76$. Again, the correlations did not improve if determined for a subseries of phenothiazines (Table 2). Instead, significant correlations appeared between CLOGP and log k' data from both IAM columns. For the pairs of comparisons CLOGP vs log k'_{20} and CLOGP vs log k'_{25}, the corresponding correlation coefficients were $r = 0.938$ and 0.944, respectively.

The hydrophobicity parameters determined on the non-end-capped and on the methylglycolate-end-capped IAM columns differ (intercorrelation $r = 0.946$). Even in this case, however, phenothiazines show only a slightly decreased correlation ($r = 0.931$).

There is a rather weak correlation between log k' on the IAM column and log k'_w determined on the deactivated hydrocarbonaceous silica column, with phenothiazines decreasing this correlation even more (Table 2). Retention data determined on IAM columns contain information on properties of solutes which are distinctive from those provided by hydrocarbonaceous silica reversed-phase columns and by the *n*-octanol–water slow equilibrium systems.

The IAM columns are easy to operate, although there is a problem with column stability. For the evaluation of the comparative hydrophobicity of a series of agents which are available at the same time, this causes no problem. It would be difficult, however, to compare retention data determined at different times. Evidently, under the experimental conditions the polar phosphatidylcholine moieties are systematically stripped off the stationary phase. This manifests itself as an increasing retention of hydrophobic solutes due to increased interactions with 'bare' aliphatic chains bound to propylaminosilica. The recommendation by the producer to avoid alcohols as mobile phase components also causes some inconvenience.

Conclusions

Although the aim of this report was to demonstrate the specific properties of IAM columns which might be of value for characterization of solute hydrophobicity, the observed weak correlations of the chromatographic

measures of hydrophobicity and standard log P (CLOGP) parameters is open to dicussion. It can be suggested that the poor correlations of log k'_w with the literature 1-octanol–water partition data in the present work may have been due to the use of log P and CLOGP values uncorrected for ionization at pH 7.00. Using the pK_a data from Table 1, log P and CLOGP parameters were corrected by the factor log $(1 + 10^{pH-pK_a})$. The resulting log D and CLOGD parameters correlated better with log k'_w: (1) with log D the correlation coefficient was $r = 0.880$ (18 data points) and $r = 0.565$ (nine phenothiazines); (2) with CLOGD $r = 0.946$ (24 solutes) and $r = 0.863$ (13 phenothiazines).

However, the use of ionization-corrected log D and CLOGD parameters instead of log P and CLOGP for prediction of retention on the IAM columns may not yield any additional advantage. When the relationship log D vs log k'_{80} was considered, the calculated correlation coefficients were $r = 0.704$ (18 solutes) and $r = 0.187$ (nine phenothiazines); while log D vs log k'_{75} gave r values of 0.810 and 0.370, respectively. Similarly, CLOGD vs log k'_{80} yielded correlation coefficients of $r = 0.844$ (18 solutes) and $r = 0.627$ (nine phenothiazines), while CLOGD vs log k'_{75} yielded correlation coefficients of $r = 0.883$ (24 solutes) and $r = 0.684$ (13 phenothiazines), respectively.

It can be assumed that the IAM columns have hydrophobicity characteristics that are suitable for modelling membrane transport phenomena and pharmacokinetics of drugs. This kind of hydrophobicity scale need not be the most appropriate for modelling hydrophobic interactions with pharmacological receptors and plasma proteins. The authors advocate the view that diverse hydrophobicity parameters, originating in several partition systems, contain more versatile information on the structure of solutes, than does any single hydrophobicity scale [22]. Systematic information extracted from sets of intercorrelated chromatographic hydrophobicity data by factorial methods of data analysis has been demonstrated to reflect the pharmacological classification of chemically related drugs [23].

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