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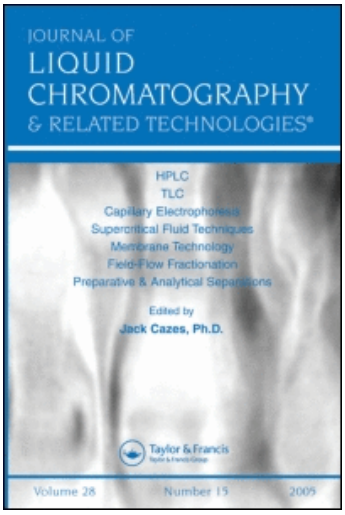

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Alessandra Pagliara[a]; Essam Khamis[a]; An Trinh[a]; Pierre-Alain Carrupt[a]; Ruey-Shiuan Tsai[a]; Bernard Testa[a]

[a] Institut de Chimie Thérapeutique Section de Pharmacie, Université de Lausanne, Lausanne, Switzerland

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STRUCTURAL PROPERTIES GOVERNING RETENTION MECHANISMS ON RP-HPLC STATIONARY PHASES USED FOR LIPOPHILICITY MEASUREMENTS

ALESSANDRA PAGLIARA, ESSAM KHAMIS, AN TRINH, PIERRE-ALAIN CARRUPT, RUEY-SHIUAN TSAI, AND BERNARD TESTA*

Institut de Chimie Thérapeutique
Section de Pharmacie
Université de Lausanne
CH-1015 Lausanne, Switzerland

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ABSTRACT

In this study, the retention mechanisms on the Supelcosil LC-ABZ stationary phase were analyzed by linear solvation free-energy relationships (LSERs). In a first phase, a set of 60 compounds was selected by cluster analysis from a large set (253) compounds of known van der Waals volume (V_W), polarity/polarizability (π^*), H-bond donating acidity (α) and H-bond acceptor basicity (β). The capacity factors log k' at 40% methanol and the log k_W values were shown to be highly correlated with octanol/water partition coefficients (log P_{oct}) and to contain pratically the same structural information, as assessed by LSERs. Test sets of model compounds and dipeptides were used to

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validate the log k_w/log P and log k'/log P relations. For anions and zwitterions, specific interactions with the stationary phase occur which produce systematic deviations in the log k/log P relations.

INTRODUCTION

Lipophilicity is a molecular property of drugs and other xenobiotics of significance mainly because of its intimate relation with biological activity, as unambiguously demonstrated in many quantitative structure-activity relationship (QSAR) studies [1]. Lipophilicity is conventionally expressed as partition coefficients in immiscible organic/aqueous biphasic systems such as octanol/water (log P_{oct}), alkane/water or chloroform/water.

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The shake-flask or centrifugal partition chromatography methods generally used to measure partition coefficients reveal their limitations mainly for highly lipophilic drugs and environmental toxins. Several studies have demonstrated that capacity factors derived from reversed-phase HPLC (RP-HPLC) (either isocratic log k' using methanol/water as mobile phase or log k_w as extrapolated to 100% water) offer a good alternative for determining the lipophilicity of compounds with log $P_{oct} > 3$ [2-5].

The stationary phases most frequently used to simulate the octanol/water-saturated phase are lipophilic alkylsilanes (e.g. octadecylsilane, ODS) bound to silica. However, two main sources of problems exist with alkylsilanes, namely silanophilic interactions which cause peak tailing and overestimation of lipophilicity, and breakdown of stationary phase at pH above 7.5.

Being devoid of reactive silanol groups and stable over a wide range of pH, the polymer-based lipophilic stationary phases such as octadecylpolyvinyl-alcohol copolymer (ODP) and polystyrene-divinylbenzene copolymer (PLRP-S) offer an alternative to assess molecular lipophilicity [6-8]. However, the relatively longer retention times in the PLRP-S column relative to the ODS column make it less interesting from a practical point of view. Furthermore, the more flexible polymeric support implies some limitations on the hydrodynamic pressure applied to the polymer-based columns.

In RP-HPLC, both partitioning and adsorption mechanisms are implicated in the retention on ODS and ODP phases. For the resemblance of these phases to octanol/water systems where solute adsorption is lacking, the principal retention mechanism in RP-HPLC must be a partitioning mechanism reflecting not only hydrophobic or solvophobic interactions between small nonpolar solutes and the mobile phase, but also polar interactions of solutes with the solvated layer(s) of stationary phases [9,10]. To unravel the structural determinants governing the retention of solutes in RP-HPLC, a powerful approach uses linear solvation free-energy relationships (LSERs) based on the solvatochromic parameters.

Indeed, LSERs were extensively used by Taft, Kamlet, Abraham and co-workers [11-14] to factorize some given molecular properties (S_p) of neutral organic solutes in terms of structural parameters such as the calculated molecular volume (V/100) and the so-called solvatochromic parameters (dipolarity/polarizability π^*, hydrogen-bond donor acidity α and hydrogen-bond acceptor basicity β). The linear equation 1 reflects a

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solvation model constructed with two factors, namely the endoergic creation of a cavity in the solvent (as reflected by the V/100 term accounting for solvophobic/hydrophobic and dispersive forces) and the introduction of the solute in the cavity which leads to exoergic polar interactions (as reflected by the π^*, α and β terms).

$$S_p = v \bullet {}^{V}\!/_{100} + p \bullet \pi^* + a \bullet \alpha + b \bullet \beta + c \qquad 1$$

In this equation, v, p, a and b are the regression coefficients which reflect the relative contribution of each solute parameter to S_p. This approach has been applied to evaluate and identify the intermolecular interaction forces underlying the partitioning mechanisms of solutes in various organic/aqueous biphasic systems as described by the equations in Table 1 [12,15].

To be similar to solvent/water partition coefficients [16], RP-HPLC retention indices must correlate with log P values, while the LSER equations must reflect a comparable balance (comparable coefficients) between the structural parameters. However, stationary phases in RP-HPLC are of a complex nature, being composed of nonpolar alkyl chains, unreacted residual polar groups, significant amounts of organic modifier (only methanol is concerned in this study), and adsorbed water. Therefore, the very nature of the stationary phase and hence the retention behavior of solutes will change with the composition of methanol/water mobile phases, leading to different LSER equations [8,17]. The equations in Table 2 allow to select the RP-HPLC indices most similar to those of log P_{oct}. However, quantitative comparison is rendered difficult because the sets of compounds used to generate the equations in Tables 1 and 2 are limited and not always comparable.

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TABLE 1.

Solvatochromic analysis of partition coefficients in organic solvent/water biphasic systems.

S_p[a)]	v	p	a	b	c[b)]	n	r^2	s	F	Ref
log P_{oct}	5.35 ± 0.05	-1.04 ± 0.04	0.10 ± 0.04	-3.84 ± 0.05	0.32 ± 0.04	245	0.99	0.13	-	[12]
log P_{oct}	5.83 ± 0.53	-0.74 ± 0.31	-0.15 ± 0.23	-3.51 ± 0.38	-0.02 ± 0.34	78	0.92	0.30	249	[15]
log P_{dee}	5.54 ± 0.81	0.02 ± 0.45	-0.20 ± 0.41	-3.83 ± 0.82	-0.34 ± 0.57	44	0.90	0.33	76	[15]
log P_{ba}	6.34 ± 0.80	0.91 ± 0.60	0.30 ± 0.53	-3.87 ± 1.82	-1.52 ± 1.12	26	0.97	0.26	122	[15]
log P_{chf}	6.00 ± 0.69	-0.14 ± 0.40	-2.99 ± 0.27	-3.17 ± 0.49	-0.18 ± 0.43	60	0.95	0.30	221	[15]
log P_{hep}	6.78 ± 0.69	-1.02 ± 0.39	-3.54 ± 0.30	-5.35 ± 0.50	-0.06 ± 0.43	75	0.95	0.36	438	[15]

a) Organic solvent: oct = 1-octanol; dee = diethylether; ba = n-butyl acetate; chf = chloroform; hep = heptane.

b) The regression coefficients are those of equation 1; 95 % confidence limits are given.

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TABLE 2.

Solvatochromic analysis of RP-HPLC capacity factors.

S_p[a)]	v	p	a	b	c[b)]	n	r^2	s	F	Ref
$\log k_w^{DB}$	5.58 ± 0.88	-0.56 ± 0.51	-0.53 ± 0.30	-2.28 ± 0.52	-1.27 ± 0.47	71	0.86	0.34	79	[17]
$\log k_w^{ODS}$	4.34 ± 1.77	-0.33 ± 0.96	-0.34 ± 0.59	-2.96 ± 0.95	0.59 ± 0.93	34	0.76	0.38	23	[17]
$\log k_w^{OS}$	6.21 ± 0.61	-1.01 ± 0.50	0.85 ± 0.24	-3.10 ± 0.26	-0.87 ± 0.39	43	0.96	0.15	-	[8]
$\log k_w^{ODP}$	5.37 ± 2.06	-1.11 ± 1.33	-0.40 ± 1.12	-3.49 ± 1.10	0.19 ± 0.88	32	0.87	0.49	34	[17]
$\log k_w^{ODP}$	5.16 ± 0.86	-0.17 ± 0.45	-0.27 ± 0.40	-3.20 ± 0.49	-0.26 ± 0.40	40	0.98	0.22	-	[8]
$\log k_w^{SB}$	6.38 ± 1.05	-1.07 ± 0.66	0.72 ± 0.29	-3.12 ± 0.37	-0.86 ± 0.54	30	0.93	0.16	23	[17]
$\log k_w^{IN}$	6.24 ± 0.88	-0.43 ± 0.52	0.02 ± 0.26	-2.67 ± 0.40	-0.68 ± 0.58	42	0.88	0.20	66	[17]

a) stationary phase: DB = DeltaBond^{TM} C_8; ODS = octadecylsilane; OS = octylsilane; ODP = octadecyl polyvinyl-alcohol copolymer; SB = RP select B; IN = Inertsil C_8.

b) See Table 1.

The resulting statistical artifacts may in turn mask or exaggerate the structural information. Thus, a set of structurally diverse, well-balanced compounds is imperatively needed in order to draw sound conclusions from a solvatochromic analysis of lipophilicity indices.

In the present study, our first goal was to select by cluster analysis (CA) an optimal set of compounds covering a wide and regular range in the structural parameters needed to perform LSER analyses. The octanol/water partition coefficients (log P_{oct}) were also included in the parameter hyperspace used in the cluster analysis. Then, the retention of this optimal set of compounds was measured in a novel ODS stationary phase (Supelcosil LC-ABZ column) using methanol/water mixtures as mobile phases. We were particularly interested in this novel stationary phase because of its electrostatic coating avoiding silanophilic interactions and diminishing specific polar interactions. The LSER approach was used to compare the RP-HPLC indices at different mobile phase compositions with the partition coefficients (log P_{oct}). In order to test the predictive power of the LSER equations, we used a test set of neutral compounds not included in the optimal set. To determine the practical limitations of the new Supelcosil LC-ABZ phase, the retention of other polar solutes, namely cyclodipeptides and zwitterionic dipeptides, was also examined.

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MATERIALS and METHODS

Solutes

All compounds were obtained from commercial sources (Merck, Darmstadt, Germany; Fluka, Buchs, Switzerland; Janssen, Beerse,

Belgium; Aldrich, Steinheim, Germany) and in the highest available purity. Analytical grade methanol was purchased from Romil Chemical (GB), 3-morpholinopropane sulfonic acid (MPS) from Merck and HPLC grade methanol from Machler (Basel, Switzerland). Deionized water was used throughout.

Multivariate Statistical Analysis

The LSER models were generated by multivariate regression using both the QSAR module in the Sybyl software (Tripos Associates, St-Louis, MO, USA) and the TSAR program (Oxford Molecular, Oxford, GB). To express molecular volumes, we used van der Waals volumes (V_W) calculated with the standard software MOLSV (QCPE N° 509) and the atomic radii of Gavezzotti [18], instead of tabulated V/100 volume parameters. The geometries used to generate van der Waals volumes were optimized with the Tripos force field including an electrostatic term calculated with a dielectric constant $\varepsilon = 1$ until the gradient norm was less than 0.001 kcal • $Å^{-1}$. For the 253 compounds in the starting set, the correlation between the two volume parameters is excellent ($r^2 = 0.988$), and the molecular modeling approach to generate molecular volumes is adequate even for complex compounds.

Selection of an Optimal Set of Compounds Using Cluster Analysis

253 compounds with known solvatochromic parameters (V_W, π^*, α and β) and known octanol/water partition coefficients (log P_{oct}) [12] were selected as a starting set and analyzed by cluster analysis in order to obtain maximal structural and property diversity with a

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minimum number of compounds. The TSAR software was used to perform cluster analysis with a single-link hierarchical clustering algorithm based on weighted mean distances generated from data transformed by standardization using the mean and the standard deviation.

The visual inspection of the dendogram produced by cluster analysis of the initial set of 253 compounds allows the identification of 78 pairs of most similar compounds. For each pair, one compound was eliminated based on the random criterion of a higher ranking number. Three similar runs were then performed allowing the elimination of, respectively, 57, 12 and 26 compounds. The optimal set (in terms of cluster analysis) was thus composed of 80 compounds (Tables 3 and 4).

However, due to the non-availability of some compounds (**66-70**), the poor solubility of others (**71-80**) in mobile phases and to experimental problems associated with basic compounds (**62-64**) and with DMSO (**61**), the set was reduced to 60 compounds (Table 3).

In order to determine the performance of the selection process, an independent series of 18 compounds (**81-98**) was used as a test set (Table 6).

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Measurement of Capacity Factors (log k')

The chromatograph (Kontron MT1) was equipped with a MSI T-660 Auto-sampler, an HPLC pump model 420, a column oven 480, an oven controller 480 and an UV/Vis detector model 430 with variable wavelength (all from Kontron, Switzerland). For the UV inactive compounds, a Refractive Index (RI) detector (Erma refractometer,

TABLE 3. Investigated compounds in the optimal set.

N°	Solutes	V_W	π^*	α	$\beta^{a)}$	log P_{oct}	log k_w	log k'_{40}
1	n-C_5H_{12}	98.8	-0.08	0.00	0.00	3.39	3.22	-
2	CH_2Cl_2	58.4	0.82	0.10	0.13	1.15	0.94	-0.30
3	$CHCl_3$	72.6	0.58	0.10	0.20	1.94	1.80	0.71
4	CCl_4	87.4	0.28	0.10	0.00	2.63	2.43	1.16
5	CH_2ClCH_2Cl	75.4	0.81	0.10	0.00	1.48	1.16	0.40
6	$CHCl_2CHCl_2$	105.2	0.95	0.10	0.00	2.39	2.16	0.99
7	1-C_4H_9Cl	94.9	0.39	0.10	0.00	2.64	2.17	0.99
8	$(C_2H_5)_2O$	88.0	0.27	0.47	0.00	0.89	0.71	-0.08
9	(n-$C_3H_7)_2O$	124.4	0.27	0.46	0.00	2.03	1.72	0.72
10	CH_3COOCH_3	71.4	0.60	0.42	0.00	0.18	0.09	-0.47
11	$CH_3COOC_2H_5$	88.9	0.55	0.45	0.00	0.73	0.67	-
12	$CH_3COOC_4H_9$-n	124.0	0.51	0.45	0.00	1.82	1.91	0.68
13	CH_3CN	46.7	0.75	0.31	0.09	-0.34	-0.45	-0.74
14	CH_3CH_2CN	64.4	0.70	0.31	0.00	0.10	0.00	-0.53
15	CH_3-CO-N$(CH_3)_2$	92.3	0.88	0.76	0.00	-0.77	-0.44	-0.98
16	CH_3-CO-N$(C_2H_5)_2$	127.9	0.84	0.78	0.00	0.34	0.59	-0.28
17	C_2H_5OH	52.9	0.40	0.45	0.33	-0.25	-0.44	-0.71
18	n-C_3H_7OH	70.1	0.40	0.45	0.33	0.28	0.02	-0.37
19	$(CH_3)_3COH$	86.7	0.40	0.57	0.32	0.36	0.46	-0.20
20	n-$C_5H_{11}OH$	104.9	0.40	0.45	0.33	1.40	1.39	0.40
21	$CH_3CH_2C(CH_3)_2OH$	105.0	0.40	0.57	0.32	0.93	0.98	0.15
22	1-$C_6H_{13}OH$	122.0	0.40	0.45	0.33	2.03	1.73	0.68
23	HCOOH	36.8	0.65	0.38	0.65	-0.54	-0.39	-0.68
24	CH_3COOH	53.6	0.60	0.45	0.56	-0.24	-0.39	-0.67
25	n-C_3H_7COOH	88.6	0.56	0.45	0.56	0.79	0.63	-0.06
26	n-C_4H_9COOH	106.2	0.54	0.45	0.56	1.39	1.30	0.33
27	n-$C_4H_9NO_2$	99.7	0.76	0.25	0.00	1.47	1.28	0.37
28	Tetrahydrofuran	77.5	0.58	0.55	0.00	0.46	0.06	-0.57
29	$C_6H_5CH_3$	104.4	0.55	0.11	0.00	2.69	2.43	1.11
30	C_6H_5-CO-CH_3	122.5	0.90	0.49	0.04	1.58	1.57	0.46

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TABLE 3 continued

N°	Solutes	V_w	π^*	α	$\beta^{a)}$	log P_{oct}	log k_w	log k'_{40}
31	$C_6H_5NO_2$	105.4	1.01	0.30	0.00	1.85	1.74	0.65
32	$C_6H_5OCH_3$	112.5	0.73	0.32	0.00	2.11	1.77	0.75
33	$C_6H_5COOC_2H_5$	147.6	0.74	0.41	0.00	2.64	2.30	1.07
34	C_6H_5-CO-C_2H_5	139.7	0.88	0.49	0.00	2.20	1.98	0.78
35	$C_6H_5COOCH_2C_6H_5$	205.8	1.32	0.50	0.00	3.97	3.38	-
36	2-$ClC_6H_4NO_2$	120.5	1.11	0.26	0.00	2.24	2.18	0.98
37	$C_6H_5CH_2CN$	123.2	1.34	0.41	0.00	1.56	1.49	0.4
38	$C_6H_5CH_2$-CO-CH_3	139.2	1.30	0.58	0.00	1.44	1.39	0.37
39	$C_6H_5CH_2CH_2$-O-CO-CH_3	165.8	1.14	0.55	0.00	2.30	2.20	0.89
40	Pyridine	82.5	0.87	0.44	0.00	0.65	0.48	-0.26
41	Acridine	175.1	1.02	0.44	0.00	3.40	2.74	1.32
42	1-Naphthalenecarboxylic acid	158.4	0.84	0.40	0.59	3.10	3.27	1.57
43	2-Naphthylamine	146.2	0.83	0.50	0.35	2.28	2.09	0.87
44	$C_6H_5NH_2$	99.4	0.73	0.50	0.26	0.90	1.04	0.06
45	$C_6H_5NHC_2H_5$	133.6	0.82	0.47	0.17	2.16	1.79	0.76
46	2-Cl-$C_6H_4NH_2$	113.9	0.83	0.40	0.25	1.91	1.85	0.70
47	2-NH_2-C_6H_4-C_6H_5	175.0	1.32	0.60	0.26	2.84	2.60	1.22
48	4,4'-$(NH_2)_2$-Biphenyl	187.7	1.46	1.00	0.62	1.34	1.44	0.29
49	4-NO_2-C_6H_4-NH_2	118.8	1.25	0.48	0.42	1.39	1.67	0.52
50	C_6H_5OH	93.9	0.72	0.33	0.61	1.49	1.26	0.37
51	3-Cl-C_6H_4OH	109.6	0.77	0.23	0.69	2.49	2.24	0.98
52	3-CH_3-C_6H_4COOH	129.6	0.70	0.41	0.59	2.37	2.10	0.80
53	$C_6H_5CH_2COOH$	128.6	1.19	0.55	0.60	1.46	1.49	0.59
54	3-Cl-$C_6H_4CH_2COOH$	144.1	1.31	0.45	0.62	2.09	2.25	1.15
55	$C_6H_5CH_2CH_2CH_2COOH$	163.0	1.15	0.55	0.55	2.42	2.41	1.07
56	$C_6H_5CH_2OH$	111.1	0.99	0.52	0.39	1.08	1.04	0.19
57	4-Cl-$C_6H_4CH_2OH$	126.5	1.11	0.42	0.40	1.96	1.78	0.71
58	4-NO_2-C_6H_4-OH	113.0	1.15	0.32	0.82	1.92	2.12	0.87
59	1,3-$C_6H_4Cl_2$	116.5	0.75	0.03	0.00	3.48	3.06	1.59
60	Biphenyl	163.5	1.18	0.20	0.00	3.90	3.47	-

a) Taken in ref [12]

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Table 4. Unavailable or non-usable compounds in the optimal set.

N°	Solutes	V_W	π^*	α	$\beta^{b)}$	log P_{oct}
61	CH_3SOCH_3	70.8	1.00	0.00	0.76	-1.35
62	$(CH_3)_3N$	76.5	0.16	0.00	0.65	0.22
63	$(C_2H_5)_3N$	127.8	0.14	0.00	0.71	1.36
64	$(n\text{-}C_3H_7)_3N$	178.8	0.14	0.00	0.69	2.79
65	$C_6H_5CH_2N(CH_3)_2$	150.3	0.75	0.00	0.67	1.91
66	$3\text{-}Cl\text{-}C_6H_5OCOCH_3$	144.1	1.19	0.00	0.42	2.32
67	$C_6H_5CH_2COOC_2H_5$	171.7	1.21	0.00	0.68	1.41
68	$C_6H_5OCH_2CON(CH_3)_2$	176.5	1.60	0.00	0.99	0.77
69	$C_6H_5OCH_2CH_2CH_2OCH_3$	164.1	0.86	0.00	0.56	2.70
70	$C_6H_5OC_3H_7\text{-}n$	147.4	0.67	0.00	0.30	3.18
71	Naphthalene	133.7	0.70	0.00	0.15	3.35
72	$1,3,5\text{-}C_6H_3(CH_3)_3$	137.6	0.47	0.00	0.13	3.84
73	$n\text{-}C_9H_{19}COOH$	194.1	0.42	0.55	0.45	4.09
74	$2\text{-}C_{12}H_9Cl$	178.1	1.30	0.00	0.17	4.30
75	$1,2,4,5\text{-}C_6H_2Cl_4$	145.9	0.70	0.00	0.00	4.51
76	$n\text{-}C_7H_{16}$	132.4	-0.02	0.00	0.00	4.66
77	$C_6H_5CH_2CH_2C_6H_5$	196.9	1.10	0.00	0.22	4.80
78	$C_6H(CH_3)_5$	196.9	0.39	0.00	0.17	4.56
79	$2,5\text{-}C_{12}H_8Cl_2$	193.0	1.30	0.00	0.13	5.10
80	$1\text{-}C_{12}H_{25}OH$	225.8	0.42	0.33	0.45	5.13

a) Taken in ref [12]

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Tokyo, Japan) was used. Both RI- and UV-detection modes were employed for a number of UV-active compounds to verify the results calculated from RI detection method.

The column was a Supelcosil LC-ABZ (150x4.6 mm ID, Supelco, Bellefonte, Pennsylvania, USA) of 5 μm packing and 100 Å pore size. This ODS stationary phase is pretreated with an electrostatic coating to suppress free silanophilic groups.

For all analyses, the flow rate was set at 1 ml/min, the column oven temperature to 25 ± 1 °C and the detection wavelength was varied according to λ_{max} of analytes. All solutes were dissolved in the mobile phase and the injection volume was 10 µl. The columm dead time (t_0) was defined as the retention time of a non-retained compound (MeOH or uracil) [19]. The isocratic capacity factor log k' was calculated from solute retention time (t_R) using equation 2:

$$\log k' = \log \frac{t_R - t_0}{t_0} \qquad 2$$

Four to five log k' values were measured using methanol/buffer mixtures containing 10-50 % (v/v) methanol for very hydrophilic compounds, 30-70 % (v/v) methanol for compounds of moderate polarity and 50-80 % (v/v) methanol for very lipophilic compounds. The buffer was an MPS (0.02 M) solution of pH = 7.4, except for acidic compounds (pH = 3.0). The log K_W values were thus calculated by extrapolation using linear regression to 100 % water.

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RESULTS AND DISCUSSION

Structural Diversity of the Optimal Set of Compounds

The optimal set of 80 compounds derived as described above by cluster analysis of 253 candidates is given in Tables 3 and 4. The dendogram in Fig. 1 shows the similarities in the parameter hyperspace among the selected compounds which are well distributed and without overlap. The population distribution in the optimal set of 80 compounds, in the set of 60 compounds finally used, and in the

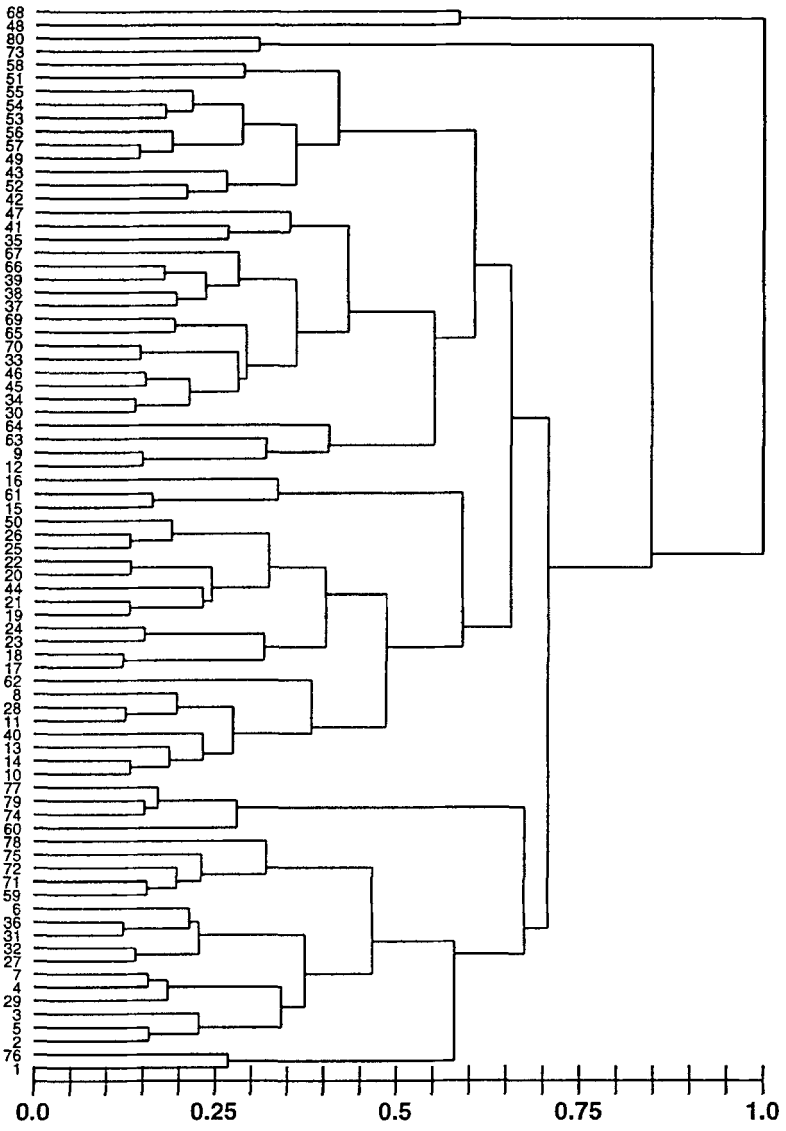

FIGURE 1:
Dendogram illustrating the even distribution in the five-parameter hyperspace of the 80 compounds in the optimal set.

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original data were examined for each parameter (Fig. 2). These three distributions are comparable confirming that the selection process by cluster analysis eliminates only compounds with similar properties in the five-parameter space. It should also be noted that no correlation between the four structural parameters (V_W, π^*, α, β) was found in any of the three sets of compounds.

Some interesting features emerge from Figure 2. An approximate Gaussian distribution is observed in the van der Waals volume, ranging from 35 to 205 $Å^3$. In contrast, the population distribution in the set with respect to dipolarity/polarizability is an asymmetric Gaussian curve due to the limited number of alkanes, which have a negligible π^* value and are difficult to measure because of their very long retention and low solubility in the mobile phase. Since all aprotic compounds have a negligible α values, a high population at $\alpha = 0$ is observed. The hydrogen bond acceptor capacity is largely populated for β values between 0.4 and 0.5. This is due to the fact that most alcohols, carboxylic acids and esters have a β value in this range. The log P_{oct} values in the three sets cover the range between -1.3 and 4.0 following a Gaussian distribution centered on log P_{oct} values around 2 (not shown).

Hence an optimal set of compounds has been selected which retains the distribution of properties of the original set and spans as regularly as possible the full property space explored. To the best of our knowledge, such an approach is used here for the first time in order to define an optimal set of compounds for characterizing RP-HPLC phases.

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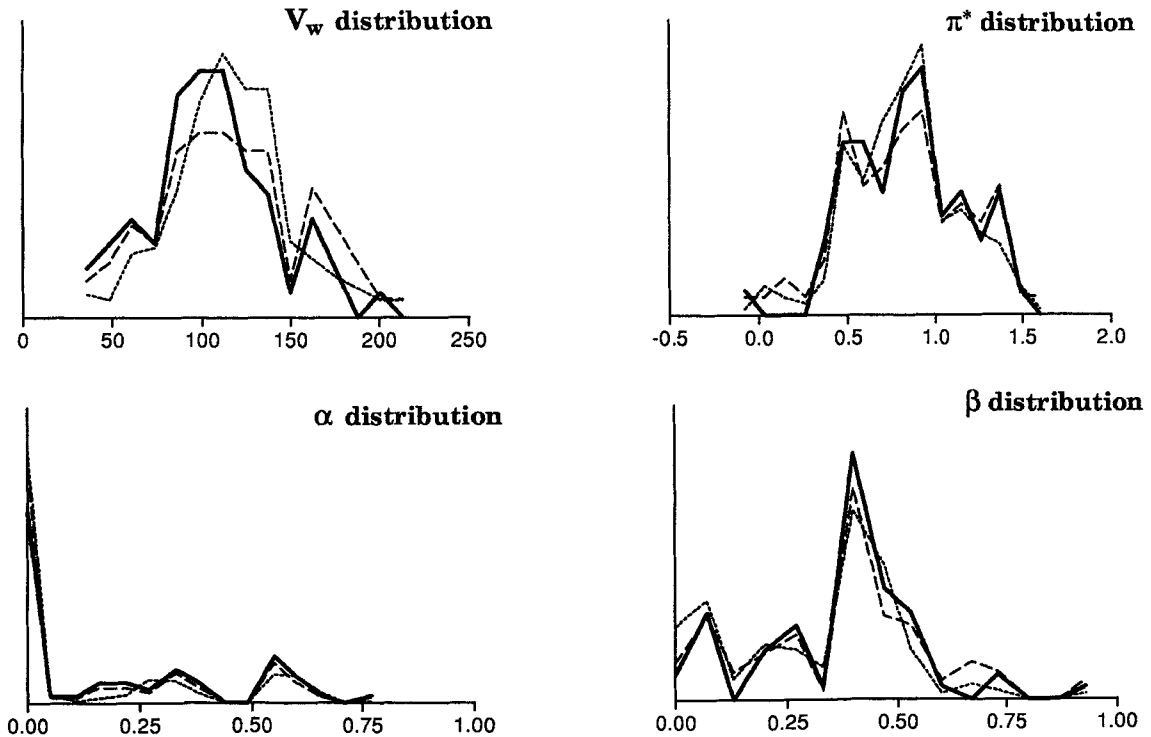

FIGURE 2:
Distribution of compounds in parameter spaces for the starting set (····· 253 compounds), the optimal set (– – – 80 compounds) and the final set (— 60 compounds).

Chromatographic Behavior of Solutes on the LC-ABZ Stationary Phase

With the LC-ABZ stationary phase, the chromatograms of relatively nonpolar solutes such as biphenyl and acridine show very symmetrical peaks compared to other C-18 stationary phases, which is indicative of predominating partitioning mechanisms with little or no adsorption phenomena. For strong hydrogen bond donors such as 1-naphthol or phenol, no "tailing" was observed in the chromatograms even for retentions as long as one hour. Taken together, these results suggest that, for neutral compounds, only partitioning is involved in the retention on the LC-ABZ stationary phase.

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The isocratic capacity factors of the 60 selected compounds were determined. Representative results for a 40 % methanol eluent are listed in Table 3. The log k_W values were obtained using linear regression of four or more points (average $r^2 > 0.99$). No parabolic curve (as been with some C-18 stationary phases [20]) was observed for any compound examined.

In contrast to other C-18 stationary phases, the log k_W values of neutral acidic compounds (measured with a mobile phase at pH = 3.0) are different from the log k_W calculated by correcting for ionization the log k_W values of the anions (measured with a mobile phase at pH = 7.4). This observation suggests that, for anionic compounds, the partioning mechanism in the LC-ABZ column is perturbed by specific interactions between the stationary phase and charged species. It follows that for this phase, the neutral form of acidic compounds must be measured directly.

It should be also noted that the ABZ column is not suitable for strong basic compounds. Indeed, measuring protonated species and correcting for ionization appears to give yield erratic results, while the unstability of the stationary phase at pH value above 7.4 does not allow the measurement of neutral species.

Retention Mechanisms on the LC-ABZ Stationary Phase

Application of linear solvation free-energy relationships (LSERs) to our optimal set of compounds gives a statistically significant equation 3 which describes the structural properties governing the retention mechanisms on the LC-ABZ stationary phase:

$$\log k_w = 3.05 \bullet 10^{-2}(\pm 0.20 \bullet 10^{-2}) \bullet V_w - 0.48(\pm 0.22) \bullet \pi^* + 0.42(\pm 0.19) \bullet \alpha - 3.73(\pm 0.28) \bullet \beta - 0.12(\pm 0.21) \quad (3)$$

$$n = 60; \quad q^2 = 0.96; \quad r^2 = 0.97; \quad s = 0.19; \quad F = 405$$

In this and the following equations, 95 % confidence limits are in parentheses; q^2 is the cross-validated correlation coefficient [21]. Mager's standardization [22] of equation 3 gives the relative contibutions of each variable to the LSER model, namely 53.2 % for V_w, 7.7 % for π^*, 33.9 % for β and 5.2 % for α. Eqn (3) indicates that V_w and β are the principal structural descriptors contributing to log k_w, while the importance of π^* and α are only marginal.

To compare the RP-HPLC lipophilicity index to the partition coefficient measured in the octanol/water system, the latter was similarly analyzed using the same series of compounds (Eq. 4). This equation and the relative contribution of each variable, namely 52.9 %

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for V_w, 9.2 % for π^*, 35.3 % for β and 2.6 % for α, confirm the close analogy between log P_{oct} and log k_w.

$$\log P_{oct} = 3.30 \bullet 10^{-2}(\pm 0.14 \bullet 10^{-2}) \bullet V_w - 0.62(\pm 0.12) \bullet \pi^* + 0.23(\pm 0.16) \bullet \alpha - 4.23(\pm 0.26) \bullet \beta + 0.09(\pm 0.16) \quad 4$$

$$n = 60; \quad q^2 = 0.98; \quad r^2 = 0.98; \quad s = 0.15; \quad F = 729$$

This similarity is also seen in the relationship between the two lipophilicity descriptors (Eq. 5).

$$\log P_{oct} = 1.07(\pm 0.06) \bullet \log k_w + 0.03(\pm 0.10) \quad 5$$

$$n = 60; \quad q^2 = 0.97; \quad r^2 = 0.97; \quad s = 0.20; \quad F = 1798$$

Thus, the log k_w derived from the LC-ABZ stationary phase appears as a promising surrogate of log P_{oct} as shown by their similar structural information content.

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Information content of isocratic capacity factors

To obtain more rapidly RP-HPLC derived lipophilicity indices and to eliminate the problem of linear versus parabolic extrapolation to log k_w, it is of interest to examine the retention mechanism on the LC-ABZ phase with different mobile phase compositions and the ability of isocratic capacity factors to predict octanol/water partition coefficients. Multilinear equations (not shown) were derived for the six compositions of the mobile phase used to calculare log k_w, but only the relative contribution of each solvatochromic variable is given in Table 5.

At different ratios of methanol/water in the mobile phase, the nature of both mobile and stationnary phases is modified. The decreasing

Table 5. Solvatochromic analysis of isocratic capacity factors

Mobile Phase[a)]	N	$\%V_w$	$\%\pi^*$	$\%\alpha$	$\%\beta$[b)]
30/70	44	53.3	5.7	7.1	33.7
40/60	56	50.6	6.2	6.3	36.9
50/50	51	46.6	8.1	7.1	38.1
60/40	54	44.8	8.0	7.7	39.6
70/30	44	41.8	9.5	8.9	39.8
80/20	27	42.8	11.7	8.8	36.7

a) Composition of mobile phase: % methanol/water (v/v)
b) Relative contribution of each independent variable, based on normalized equations

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contribution of V_W with increasing methanol concentration may be due to the more hydrophobic character and lower hydrogen-bond capacity of methanol compared to water. These results further suggest that at high concentrations of methanol in the mobile phase, other polar interactions with the stationary phase not taken into account by the structural descriptors used here become significant. They also suggest a poorer correlation of isocratic capacity factors (log k') with log P_{oct} at high concentrations of methanol in the mobile phase, as indeed seen: 30% methanol, $r^2 = 0.96$; 40% methanol, $r^2 = 0.97$; 50% methanol, $r^2 = 0.96$; 60% methanol, $r^2 = 0.93$; 70% methanol, $r^2 = 0.86$; 80% methanol, $r^2 = 0.72$. Interestingly, a single determination with 40 % methanol in the mobile phase (log k'_{40}) seems sufficient to obtain a good lipophilicity index based on the similary of the regression coefficients in the solvatochromic equations. The interest of log k'_{40} is also seen in equation 6:

$$\log P_{oct} = 1.57(\pm 0.09) \bullet \log k'_{40} + 0.85(\pm 0.06) \quad (6)$$
$$n = 56;\ q^2 = 0.97;\ r^2 = 0.97;\ s = 0.18;\ F = 1707$$

The slope of this linear equation is higher than 1, suggesting that log k'_{40} is also useful to extend the experimental range of measurable lipophilicity.

RP-HPLC of a test set of compounds

To determine the ability of both log k_w and log k'_{40} to be used as a predictor of log P_{oct}, the predictive power of equations 5 and 6 was established on a test set of 18 model molecules (**81-98**) closely related to the compounds used in the optimal set. The experimental results are given in Table 6.

In the lipophilicity range (-1.0 to 4.0) explored by the test set, the two indices log k_w and log k'_{40} give a satisfactory estimation of log P_{oct} as shown by equations 7 and 8. It should be noted that the quality of prediction is better when using log k'_{40} than log k_w presumably due to the uncertainty in the linear extrapolation to 100 % water.

$$\log P_{oct} = 0.99(\pm 0.11) \bullet \log P(\text{est. from } \log k_w) + 0.10(\pm 0.23) \quad (7)$$
$$n = 18;\ q^2 = 0.97;\ r^2 = 0.98;\ s = 0.20;\ F = 615$$

$$\log P_{oct} = 0.99(\pm 0.05) \bullet \log P(\text{est. from } \log k'_{40}) + 0.04(\pm 0.08) \quad (8)$$
$$n = 17;\ q^2 = 0.99;\ r^2 = 0.99;\ s = 0.09;\ F = 2445$$

RP-HPLC of a test set of peptides

The results described above have demonstrated the performance of the Supelcosil LC-ABZ column to estimate the log P of neutral model

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Table 6. Test sets

N°	Solute	log P_{oct}	log k_w	log P[a)]	log k'_{40}	log P[b)]
81	1-C_3H_7Cl	2.04	1.53	1.67	0.69	1.93
82	CH_3COCH_3	-0.24	-0.14	-0.11	-0.69	-0.23
83	$HCON(CH_3)_2$	-1.01	-0.88	-0.91	-1.18	-1.0
84	2-C_3H_7OH	0.13	-0.12	-0.1	-0.47	0.11
85	C_6H_6	2.13	1.68	1.83	0.76	2.04
86	C_6H_5CHO	1.48	1.23	1.35	0.32	1.35
87	C_6H_5F	2.27	1.71	1.86	0.83	2.15
88	C_6H_5Cl	2.84	2.36	2.56	1.18	2.70
89	C_6H_5Br	2.99	2.94	3.18	1.29	2.88
90	1-Naphthol	2.91	2.88	3.11	1.41	3.07
91	2-Naphthol	2.81	2.82	3.05	1.33	2.94
92	1-Naphthylamine	2.24	2.08	2.26	0.92	2.30
93	1,3,5-$C_6H_3Cl_3$	4.02	3.54	3.82	-	-
94	2-Cl-C_6H_4OH	2.15	1.92	2.08	0.80	2.11
95	2-F-C_6H_4OH	1.71	1.41	1.54	0.46	1.57
96	2-Br-C_6H_4OH	2.35	2.11	2.29	0.94	2.33
97	4-F-C_6H_4OH	1.77	1.58	1.72	0.57	1.75
98	4-I-C_6H_4OH	2.91	2.61	2.82	1.32	2.93
99	c-Trp-Tyr[c)]	1.05	-	-	0.23	1.21
100	c-Gly-Tyr	-0.69	-	-	-0.86	-0.51
101	c-Gly-Phe	0.05	-	-	-0.38	0.25
102	c-Phe-Phe	1.59	-	-	0.78	2.08
103	c-Phe-Ser	-0.45	-	-	-0.49	0.08
104	c-Ser-Tyr	-1.09	-	-	-1.03	-0.77
105	Leu-Phe	-1.13	-	-	0.00	0.85
106	Trp-Tyr	-1.38	-	-	-0.11	0.68
107	Phe-Phe	-0.99	-	-	0.22	1.20
108	Phe-Leu	-1.24	-	-	0.03	0.89
109	Trp-Phe	-0.52	-	-	0.36	1.42
110	Phe-Tyr	-1.83	-	-	-0.31	0.36
111	Tyr-Leu	-1.81	-	-	-0.45	0.14
112	Met-Leu	-1.92	-	-	-0.51	0.05
113	Leu-Tyr	-1.95	-	-	-0.70	-0.25

a) Partition coefficient predicted by equation (5)

b) Partition coefficient predicted by equation (6)

c) For dipeptides and cyclodipeptides, a pH of 6 was used.

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compounds and the necessity to perform measurements on the neutral form of ionized compounds. Due to the limited stability of the stationary phase, the second condition restricts the range of ionizable compounds to those neutral in the pH range between 2 and 7.5. To define the application domain of the LC-ABZ phase, two other classes of compounds were studied, namely six neutral cyclodipeptides (**99 - 104**) and nine zwitterionic dipeptides (**105 - 113**) (Table 6).

Although higher than for the first test set, the mean deviation between the measured log P_{oct} and the log P_{oct} predicted by equation 6 (0.32 ± 0.16) is still acceptable for the neutral cyclodipeptides. However, for the zwitterionic dipeptides, this deviation is much larger (2.01 ± 0.16) indicating either a change in the equilibrium between neutral and zwitterionic species in the 40 % methanol eluent, or specific interactions between charged amino or carboxylate groups and the stationary phase. In the light of results on ionized acids and bases, the latter phenomenon is the most likely. Work is on progress in our laboratory to clarify the retention mechanisms of ionised species on the Supelcosil LC-ABZ column.

Interestingly, in an homogenous series like the dipeptides, the deviation between log P_{oct} and log P predicted is largely constant (2.01 ± 0.16) suggesting that isocratic capacity factors could be used to characterize lipophilicity variations in the series thus giving direct access to hydrophobic substituent constants similar to those obtained from log P_{oct} values.

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CONCLUSION

In conclusion, the LC-ABZ stationary phase is shown to retain neutral solutes by a balance of intermolecular forces closely resembling that underlying octanol/water partition coefficient. The isocratic capacity factor at 40% methanol appears even better than log k_w in resembling log P_{oct}. For anions and zwitterions, specific interactions occur with the stationary phase whose nature remains to be established but which affect lipophilicity prediction.

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