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STUDY OF SOLUTE RETENTION IN REVERSED-PHASE HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY ON HYDROCARBONACEOUS AND THREE FLUORINATED BONDED PHASES

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SUMMARY

The retention properties of three fluorinated reversed-phase high-performance liquid chromatography bonded phases were characterized through the use of a wide variety of substituted aromatic test solutes. An identical study was conducted on a hydrocarbonaceous decyl bonded phase enabling the direct comparison of all phases. Functional groups were found to affect retention in a fundamentally different way on each of these bonded phases.

Fluorinated alcohols were used as organic mobile phase modifiers and compared with the non-fluorinated alcohol modifiers through their effects on solute retention.

INTRODUCTION

Straight-chain hydrocarbonaceous bonded phases used in reversed-phase high-performance liquid chromatography (RP-HPLC) have been the subject of intense practical and theoretical study over the past five years. (For reviews see refs. 1–4.) Commercial availability coupled with proven versatility and efficiency in the separations of a wide variety of species have contributed greatly to their extreme popularity. Many groups have documented the effects of varying the nature of the non-polar support surface upon the retention and the selectivity for a given chromatographic separation (*i.e.*, endcapping as well as changing the bonded phase chain length and/or the extent of carbon loading[5–13]). In addition, a much smaller number of studies on the chromatographic characteristics of branched-chain, unsaturated and cyclic (saturated and unsaturated) hydrocarbonaceous bonded phases have been reported[9,14–16]. The use of branched-chain stationary phases typically causes only minor changes in solute retention. However, saturated cyclic and aromatic bonded phases can cause greatly enhanced specificity for solutes which show either steric recognition or undergo π–π interactions, respectively.

A wide range of more polar bonded phases has been produced by incorporating polar functional groups at internal and/or terminal positions on the alkyl chain

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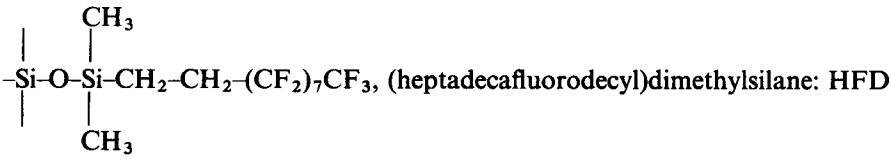
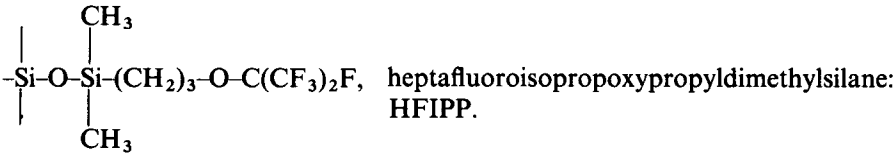
(*e.g.*, -OH[17–19]; -NH_2[20,21]; -CN[20–22]; -NO_2[23,24]). These supports sometimes possess superior chromatographic properties for specific classes of solutes (organic acids, sugars, aromatics, etc.[5,23]). However, short column lifetimes (*e.g.*, cyano phases) or very limited ranges in compatible solvent compositions (*e.g.*, amino phases) can limit their utility.

Recently, highly fluorinated RP-HPLC stationary phases have been introduced[25–30]. These bonded phases probe the extreme non-polar end of the polarity scale. This is reflected by the Hildebrand solubility parameters (δ) of fluorocarbon liquids which are considerably smaller than those of the analogous hydrocarbon liquids[31,32] as well as their very low Kamlet–Taft solvatochromic index[33]. Fluorinated phases are also less polarizable than are hydrocarbonaceous bonded phases as indicated by the refractive indices of the analogous liquids.

The structures of the currently commercially available fluorinated bonded phases are shown below:

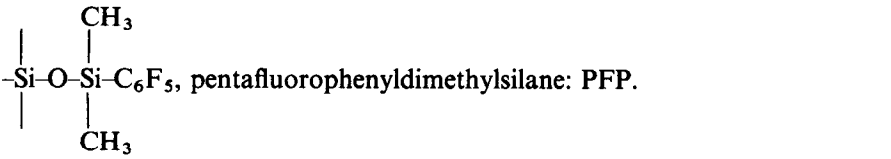


(heptadecafluorodecyl)dimethylsilane: HFD;



heptafluoroisopropoxypropyldimethylsilane: HFIPP.

Additionally, the following perfluorinated bonded phase was studied:



pentafluorophenyldimethylsilane: PFP.

Very few studies have been reported for these fluorinated HPLC bonded phases. Berendsen *et al.*[26], directly compared the HFD support to its hydrocarbonaceous analog, the decyl (C_{10}) bonded phase. Only eleven solutes were used in the study. Billiet *et al.*[25], used a wider variety of test solutes (39) but compared the HFD column with an octadecyl bonded silica. The chromatographic difference between a decyl and an octadecyl bonded phase was assumed to be insignificant. Their study found the HFD bonded phase to have an increased selectivity over the octadecyl bonded phase for esters, ketones, and fluoro-substituted solutes.

The purpose of the present work is to expand the number of solutes tested in order to compare and contrast directly the chromatographic characteristics of the HFD and C_{10} bonded phases. In addition, two other fluorinated phases, HFIPP and PFP, were evaluated under the same mobile phase conditions as the HFD and the C_{10} phases in an attempt to compare qualitatively all four bonded phases.

A series of 42 test solutes comprised of benzene, pyridine, substituted benzenes, 2-picoline, and fused benzenes were examined using a methanol–water (60:40) mobile phase. This wide variety of solutes was chosen to help elicit information as to the role functional groups play in retention when fluorinated bonded phases are employed. As might be expected, the chromatographic characteristics of the various fluorinated bonded phases differ markedly from one another as well as from those of the hydrocarbonaceous phase.

The chromatographic support material can be divided into two regions: the bonded phase itself and the underivatized silica surface (*i.e.*, silanol groups, siloxanes, metals). The physical properties of silanol groups on base silica differ from manufacturer to manufacturer. Engelhardt and Müller[34] have shown that the pK_a values of silanol groups on different base silicas vary from 4 to 10. The values given for the packing materials used in this study are: Hypersil, 9.0; LiChrosorb, 7.8; and Zorbax, 3.9. Chromegabond was not tested in their work. Approximately half of all initially present surface silanol groups remain underivatized, even after an "exhaustive" silanization[35,36]. Not surprisingly, these residual silanol groups have been shown to influence strongly the chromatographic behaviour of polar and hydrogen bonding solutes[37–39].

Not only were the bonded phases compared and contrasted, but the effects of various organic mobile phase constituents were also investigated. A small but constant volume fraction (5%) of methanol was replaced by either ethanol, isopropyl alcohol, trifluoroethanol or hexafluoroisopropyl alcohol and the resulting chromatographic behaviour of a subset of test solutes (sixteen) on the C_{10} and HFD columns was compared. This was done in order to ascertain the relative solvent strengths of the fluorinated *versus* the non-fluorinated alcohols for these packing materials.

Finally, an estimate of the contribution of silanophilic interactions on solute retention was obtained. A series of buffered solvents with and without the silanol group blocking agent, tetramethylethylenediamine (TEMED), was used. Two nonpolar, one weak acid and one weak base were used as test solutes. To guarantee the presence of a very different number of residual silanol groups, two C_{18} packing materials with different carbon loadings were compared under the above conditions using the solute subset.

EXPERIMENTAL

The Chromegabond decyl (C_{10}) (10 μm, 60 Å) and the Chromegabond heptadecafluorodecyl (HFD) (10 μm, 60 Å) packing materials were obtained from ES Industries (Marlton, NJ, U.S.A.). Both packing materials were upward slurry packed into 5 cm × 4.6 mm I.D. column blanks; the C_{10} was packed from 100% methanol and the HFD was packed from 100% tetrachloromethane. The HFIPP and the PFP phases were prepared by the reaction of the appropriate dimethylchlorosilane obtained from Petrarch Systems (Levittown, PA, U.S.A.) with LiChrosorb Si-60 10-μm silica from E. Merck (Darmstadt, F.R.G.) in dry toluene. These were suspended in trichloromethane–isopropyl alcohol (90:10) and packed from 100% methanol. Two prepacked columns were also used: a Hypersil ODS 5 μm, 20 cm × 4.6 mm I.D. column obtained from Hewlett-Packard (Avondale, PA, U.S.A.) and a Zorbax ODS 5–6 μm, 15 cm × 4.6 mm I.D. column obtained from DuPont (Wilmington, DE, U.S.A.).

Methanol, isopropyl alcohol, biphenyl, and uracil were obtained from MCB Manufacturing Chemists (Cincinnati, OH, U.S.A.) N,N,N′,N′-tetramethylethylenediamine from Chemical Dynamics (South Plainfield, NJ, U.S.A.), anisole, iodobenzene, aniline, *p*-hydroxybenzoic acid, 1,4-dihydroxybenzene, *p*-cresol, *o*-cresol, *m*-cresol, *p*-bromotoluene, *p*-nitrobenzylchloride, 2-picoline, *p*-nitrobenzyl bromide, *p*-dichlorobenzene, benzyl alcohol, phenol, *p*-nitrotoluene, 2,2,2-trifluoroethanol, 1,1,1,3,3,3-hexafluoroisopropyl alcohol, benzonitrile, acetophenone, fluorobenzene, chlorobenzene, bromobenzene, pyridine, ethanol, anthracene, ethylbenzene, *n*-propylbenzene, *n*-butylbenzene, *tert.*-butylbenzene, and naphthalene from Aldrich (Milwaukee, WI, U.S.A.), benzylamine, nitrobenzene, *p*-nitrobenzoic acid, *p*-aminobenzoic acid, *p*-nitrophenol, *p*-aminophenol, *o*-nitrotoluene, and *p*-hydroxybenzaldehyde from Eastman-Kodak (Rochester, NY, U.S.A.), benzaldehyde from Mallinckrodt (Paris, NY, U.S.A.), benzene, *p*-ethylphenol from Baker (Phillipsburg, NJ, U.S.A.), benzoic acid No. 428443-p from the National Bureau of Standards (Washington, DC, U.S.A.), and *p*-chlorobenzoic acid from BDH (Poole, U.K.).

Non-fluorinated alcohols and water were filtered through 0.45-μm Zetapor filters obtained from AMF Cuno Division (Meriden, CT, U.S.A.). The fluorinated solvents were used without filtration. Each solvent was degassed prior to use and the appropriate solvents were used to prepare all solute mixtures and completely characterize all the columns tested in each section of the study.

Columns were equilibrated with 30–50 column volumes of new solvent before beginning the solute injections. Triplicate 20-μl injections were made for each solute. For the solutes not capable of strong silanophilic interactions, the retention times were reproducible to better than 1%. Those solutes capable of strong silanophilic interactions (*i.e.*, amines) varied up to 8% in the weakest mobile phase [methanol–water (40:60)]. Each solute was prepared and injected individually. Most solute samples were stable over the entire period of column testing. A few, however, had to be prepared daily (*e.g.*, *p*-aminophenol due to slow air oxidation).

Uracil was used to estimate the void volume of each system. The flow-rate was frequently checked for each solvent system and found to be 0.50 ± 0.01 ml/min. The chromatographic system has been previously described[37]. Column void volumes are listed in Table I.

RESULTS AND DISCUSSION

Stationary phase

As mentioned before, silica-based RP-HPLC supports do not have a homogeneous surface. To minimize the influence of silanol group interactions on the comparisons, solutes capable of hydrogen bonding, strong dipole interactions, or inductive interactions should be avoided. Therefore, the most effective way to compare the hydrophobic characteristics of bonded phases is to fix the mobile phase composition and then choose a solute set whose stationary phase interactions depend exclusively on the non-polar forces. Consequently, the test solutes chosen to study the non-polar character of the columns were benzene and the *n*-alkylsubstituted benzenes (toluene to *n*-butylbenzene). These test solutes also allow us to test an important property of a reversed-phase support: its ability to discriminate between molecules differing by a single methylene group, often referred to as the relative hydrophobicity of the

TABLE I

$\Delta\Gamma$ VALUES FOR METHYLENE GROUPS

Mobile phase, methanol–water (60:40); flow-rate, 0.5 ml/min; test solutes, benzene, toluene, ethylbenzene, *n*-propylbenzene, and *n*-butylbenzene.

Bonded phase	$-RT \ln k'_{benz}$ *(cal/mole)**	V_0 *(ml)***	*y-intercept****	*Slope*§	$\Delta\Gamma$ *(cal/mole)*	ρ§§
PFP	+767	0.78	−1.329 (0.008)§§§	0.327 (0.003)	−190 ± 2	0.9998
HFIPP	+424	0.67	−0.740 (0.011)	0.332 (0.004)	−192 ± 2	0.9997
HFD	+3	0.73	−0.014 (0.015)	0.438 (0.006)	−254 ± 4	0.9998
C_{10}	−445	0.66	+0.763 (0.018)	0.590 (0.007)	−342 ± 4	0.9997

* k'_{benz} is the capacity factor for benzene.
** Void volume, uracil used as "marker".
*** This is the regression calculated value for $\ln k'_{benz}$.
§ These values are used in eqn. 1 to obtain $\Delta\Gamma$ for a methylene group.
§§ Correlation coefficient for all five solutes.
§§§ Parenthetical numbers are standard deviations.

column. Most certainly solute retention will also depend upon the mobile phase and the temperature, but by keeping these constant their effects on retention will be constant.

The retention of the alkylbenzene series was examined on the above columns under identical mobile phase conditions. The results are summarized in Table I. In all cases, excellent linearity for plots of $\ln k'$ *versus* carbon number is observed, the lowest correlation coefficient being 0.9997.

Compared to the hydrocarbonaceous column, all of the fluorinated columns show a decreased sensitivity for a methylene unit. The intercept of these plots, which correspond to the extrapolated retention of a phenyl group, decrease in the same order as the slope. With the exception of the PFP column, a plot of the intercept *versus* the slope is linear. From these results and the solvophobic model of reversed-phase chromatography, the fluorinated columns would be judged as less hydrophobic than the hydrocarbonaceous column. This cannot be true since fluorocarbon liquids are much less soluble in water (and *vice versa*) than are the analogous hydrocarbon liquids. The solutes, therefore, must interact more strongly with the hydrocarbonaceous stationary phase and/or the adsorbed methanol on its surface than with the fluorinated stationary phases. Certainly, this is the case for the HFD column.

To compare the effect of other substituents upon the retention on these columns, a substituent factor was defined as follows:

$$\Delta\Gamma = -RT \ln (k'_x/k'_b) \quad (1)$$

where R is the gas constant, T is the temperature, and k'_x and k'_b are the capacity factors of the substituted benzene and benzene, respectively. The same column was used under the same conditions to obtain $\Delta\Gamma$ values.

Many groups have used eqn. 2 below to estimate the free energy of transfer from the mobile phase to the stationary phase[40–42]:

$$\ln k' = \ln \varphi - \Delta G^\circ/RT \quad (2)$$

Eqn. 2 is not thermodynamically valid unless the retention process is dominated by a single mechanism, *i.e.*, partitioning, adsorption at the mobile phase–stationary phase interface, or silanophilic interaction. The free energy of transfer for a functional group would then be evaluated as:

$$\Delta(\Delta G^\circ)_x = -RT \ln (k'_x/k'_b) \quad (3)$$

In the absence of certain knowledge that one is dealing with a single retention process, $\Delta\Gamma$ was defined in eqn. 1 above. Although $\Delta\Gamma$ has energy units (*e.g.*, cal/mol) and is calculated in a similar manner as the free energy of transfer, it should not be considered a valid extrathermodynamic characteristic as eqn. 2 suggests.

$\Delta\Gamma$ does not contain any contribution from the phase ratio term because the solvent and the stationary phases are unchanged and the experiments are effectively carried out at infinite dilution of the solute. The difference found in the void volume between the C_{10} and the HFD column, since both are bonded to the same base silica, is presumably due to the substantially larger volume occupied by the fluorinated support even though its percentage carbon loading is somewhat lower (8.6% *versus* 10%).

Not surprisingly, Table I indicates that substantial differences in $\Delta\Gamma$ exist for

TABLE II

$\Delta\Gamma$ VALUES FOR FUNCTIONAL GROUPS

Values are in cal/mole at 293°K. Calculated from k' values in methanol–water (60:40).

	Group	C_{10}	*PFP*	*HFD*	*HFIPP*
A	-I	−597 (1), a	−319 (3), b	−153 (7), c	−133 (7), d
B	-phenyl	−549 (2), b	−607 (1), a	−432 (1), c	−386 (1), d
C	fused	−462 (3), a	−424 (2), b	−246 (3), c	−234 (2), d
D	-Br	−443 (4), a	−267 (4), b	−167 (6), c	−142 (5), d
E	$-CH_3$	−349 (5), a	−228 (6), c	−245 (4), b	−186 (4), d
F	-Cl	−348 (6), a	−245 (5), b	−181 (5), c	−136 (6), d
G	$-CH_2-$	−340 (7), a	−192 (7), d	−254 (2), b	−196 (3), c
H	-F	−15 (8), d	−107 (8), b	−118 (8), a	− 66 (8), c
I	$-OCH_3$	+32 (9), c	−89 (9), a	+127 (10), d	−63 (9), b
J	$-NO_2$	+162 (10), d	−28 (10), a	+116 (9), b	+134 (10), c
K	$-COCH_3$	+531 (11), d	+19 (11), a	+241 (11), c	+161 (11), b
L	-CHO	+538 (12), d	+118 (13), a	+388 (13), c	+278 (13), b
M	-CN	+545 (13), d	+78 (12), a	+295 (12), c	+175 (12), b
N	-OH	+801 (14), d	+432 (14), a	+810 (15), c	+687 (15), b
O	$-NH_2$	+947 (15), d	+465 (15), a	+807 (14), c	+505 (14), b
P	-COOH	+1241 (16)	–	+1691 (16)	+1568 (16)
$\Sigma\Delta\Gamma$		+453*	−1394*	+98*	+398*
$\overline{\Delta\Gamma}$		+30.2	−92.9	+65.9	+26.5
S^{2**}		$3.5 \cdot 10^6$	$1.2 \cdot 10^6$	$2.0 \cdot 10^6$	$1.2 \cdot 10^6$

* Carboxylic acid substituent excluded for the last three rows.

** Total variance from the column mean.

$-CH_2-$ between the C_{10} and all of the fluorinated columns. Dispersive interactions dominate between the stationary phase and these non-polar solutes. Contributions to solute retention due to silanophilic interactions should be extremely small. Rather unexpectedly, the HFIPP and PFP bonded phases show remarkably similar $\Delta\Gamma$ val-

TABLE III

SOLUTE CAPACITY FACTORS

Solvent, methanol–water (60:40).

Solute	*Column*			
	PFP	*HFIPP*	*HFD*	C_{10}
Benzene	0.26	0.48	0.99	2.14
Toluene	0.36	0.66	1.53	3.94
Ethylbenzene	0.50	0.91	2.29	6.77
n-Propylbenzene	0.70	1.29	3.67	12.53
n-Butylbenzene	0.98	1.82	5.74	23.06
tert.-Butylbenzene	0.80	1.58	4.84	15.82
p-Xylene	0.55	0.92	2.29	7.11
Pyridine	0.29	0.48	0.75	0.46
2-Picoline	0.35	0.68	1.13	0.73
Fluorobenzene	0.32	0.54	1.22	2.20
Chlorobenzene	0.38	0.61	1.36	3.91
Bromobenzene	0.42	0.61	1.32	4.59
Iodobenzene	0.46	0.60	1.29	5.98
p-Chlorotoluene	0.58	0.84	2.08	7.15
p-Bromotoluene	0.59	0.84	2.04	8.41
p-Dichlorobenzene	0.51	0.76	1.94	6.70
Benzyl bromide	0.35	0.50	0.87	1.60
Phenol	0.12	0.14	0.25	0.53
Benzyl alcohol	0.13	0.18	0.26	0.51
p-Cresol	0.15	0.23	0.39	0.97
m-Cresol	0.18	0.23	0.36	0.94
o-Cresol	0.17	0.21	0.36	1.03
p-Ethylphenol	0.24	0.33	0.61	1.73
Benzonitrile	0.23	0.35	0.59	0.83
Benzyl cyanide	0.20	0.28	0.45	0.78
Nitrobenzene	0.26	0.41	0.77	1.47
p-Nitrotoluene	0.30	0.60	1.23	2.48
o-Nitrotoluene	0.40	0.56	1.13	3.29
p-Nitrophenol	0.13	0.16	0.29	0.76
p-Nitrobenzyl chloride	0.36	0.44	0.93	2.28
p-Nitrobenzyl bromide	0.36	0.42	0.89	2.59
Anisole	0.31	0.43	0.79	2.03
Aniline	0.12	0.20	0.25	0.41
p-Aminophenol	0.14	0.24	0.22	0.03
Benzaldehyde	0.21	0.29	0.51	0.84
p-Hydroxybenzaldehyde	0.10	0.09	0.12	0.30
Acetophenone	0.25	0.36	0.65	0.98
Benzophenone	0.45	0.83	1.47	4.35
Biphenyl	0.76	0.93	2.09	5.53
Naphthalene	0.54	0.71	1.38	6.17
Anthracene	1.15	1.08	2.32	10.56
Benzoic acid	excluded	0.03	0.05	0.25

ues for alkyl substituents (see Table II). Also, variations in the ln k' of benzene, *i.e.*, the intercept values given in Table I, are much greater than would be expected based on the difference in phase ratios. (See Table III for the corresponding experimentally obtained capacity factors from which the $\Delta\Gamma$ values in Tables I and II are derived.)

Although nine different pairs of compounds which differed by only one alkyl group were examined, the $-CH_2-$ and $-CH_3$ groups have a remarkable consistency in their $\Delta\Gamma$ values. In fact, only the PFP column appears to be able to discriminate a $-CH_2-$ group from a $-CH_3$ group based on their values and the total range in values between different compounds. The reproducibility in $\Delta\Gamma$ for a given solute can be determined through the equations of error propogation. With the assumed independence of the variances for the experimental error in the component k' values, $\sigma(\Delta\Gamma)$ varies from *ca.* 130 for the least retained ($\Delta\Gamma = +1000$) to 5 for the most retained ($\Delta\Gamma = -600$) species in Table II. At k' values close to that of benzene, ($\Delta\Gamma = 0$), the uncertainty in $\Delta\Gamma$ is *ca.* 10 units. Thus the range in the $\Delta\Gamma$ values for the $-CH_2-$ and the $-CH_3$ substituted solutes is almost the same as the expected standard deviation for a single material.

$\Delta\Gamma$ values were found to be consistent when there was at most one polar substituent on the ring. For example, the $\Delta\Gamma$ value for the hydroxyl group in *p*-hydroxyphenol was not in accord with the tabulated values. Complex convolution of resonance and inductive effects on the benzene ring electrons when two polar substituents are present prohibit the prediction of solute retention in these cases[43–45].

In comparison, when studying the homologous series phenol, *p*-cresol, *p*-ethylphenol, values for the slopes of carbon number *versus* ln k' plots were similar to the values in Table I. However, the *y*-intercepts were much lower due to the presence of the hydroxyl group (*i.e.*, phenol is better solvated by the mobile phase than is benzene).

These results are in good agreement with those of Billiet *et al.*[25], who found the $-CH_2-$, $-CH_3$, –Br, and –I substituents to be more favorably transferred to the C_{10} support relative to the fluorinated support, but –CHO, $-COCH_3$, and –F prefer the fluorinated support. In this study, the –OH, $-NO_2$, –CN, $-OCH_3$, and –COOH groups were also found to prefer the fluorinated material since their $\Delta\Gamma$ values are less positive for the fluorinated column than for the hydrocarbonaceous column. This cannot, *a priori*, be accredited to the bonded phase itself due to the active role silanol groups play in retention of polar solutes (see below).

The data in Table II are divided into three regions by two horizontal lines. Those solutes above the first horizontal line are better retained than benzene on all columns, whereas those below the second line are more poorly retained than benzene on all columns. Elution of $-OCH_3$ and $-NO_2$ relative to benzene varies from column to column. The C_{10} was chosen as the elution sequence reference column. A numerical index was then assigned in order to reflect the elution sequence of each species from the most retained (1) to the least retained (16). Relative to the elution sequence on the C_{10} column there are a total of 7, 13, and 10 changes in elution sequence on the PFP, HFD, and HFIPP columns, respectively. Such changes in elution order could be very beneficial when optimizing a separation since selectivity (relative retention) has a large effect on the total number of plates required to achieve a desired resolution. Based on the total variance in the $\Delta\Gamma$ values from the column mean, the C_{10} column is in general the most selective column.

To examine the retention of a substituent group with respect to a column, a letter index was assigned: the most retained (a) through the least retained (d). Several patterns become evident. First, the C_{10} column followed by the PFP, HFD, and lastly the HFIPP column generally has the greatest retention (relative to benzene) for non-polar groups (see solutes A, C, D, F, G). However, there is variability in the letter index sequence (solutes B, E, G, H). In contrast, the more polar groups (K–P) invariably exhibit the elution sequence: PFP (most retentive), HFIPP, HFD, and C_{10} (least retentive). This indicates that the PFP column is really rather retentive towards both polar and non-polar functional groups, which is substantiated by the sum of all $\Delta\Gamma$ values. It is most peculiar for a column to show high relative retention for both polar and non-polar moieties. Clearly, the PFP material could be extremely useful, particularly when separating species containing aromatic groups.

The retention of solutes with extensive π systems (*e.g.*, biphenyl, naphthalene, anthracene) may be safely assumed to be free of silanophilic solute interactions. From Table II, the PFP and the C_{10} columns have comparable $\Delta\Gamma$ values and yet the retention mechanism for these solutes must differ. None of these solutes can interact completely with a single bonded phase ligand because the bonded phase moiety itself is smaller than the solute. Formation of "liquid-like" PFP pockets cannot occur to any significant extent thereby precluding partitioning into the bonded phase. The formation of "liquid-like" pockets on the C_{10} column can occur making solute partitioning possible.

Another major factor which can contribute to the retention of non-polar solutes is dispersive interaction. The strength of this interaction is generally taken as being related to the quantity R[46]:

$$R = (n^2 - 1)/(n^2 + 2) \tag{4}$$

where n is the refractive index. The refractive indices for liquids chosen to serve as bonded phase model compounds are: 1.2617, perfluoroheptane; 1.3769, perfluorobenzene; 1.4113, decane[47,48]. From these data and the quantity R given in eqn. 4, the difference in solute retention on the HFD compared to the PFP is not surprising and is reflected by the large difference in $\Delta\Gamma$ values. Only small differences in the $\Delta\Gamma$ values between the PFP and C_{10} columns are observed. If dispersive interactions are the predominant contributor to solute retention, differences may be partially attributed to uncompensated errors in estimating the refractive indices from the model liquids.

Stationary phase modification by adsorbed methanol and solute partitioning will occur to a greater degree on the C_{10} phase than on the PFP phase. In contrast, strong π–π interactions will play a major role only on the PFP phase. As stated previously, these interactions have been shown to play an important role in the retention mechanism of highly conjugated aromatic solutes when phenyl and naphthyl bonded phases were studied[14].

The solute series fluorobenzene, chlorobenzene, bromobenzene, and iodobenzene exhibits a striking difference in retention between the fluorinated bonded phases and the hydrocarbonaceous phase. For the C_{10} phase the $\Delta\Gamma$ value becomes more negative as the refractive index increases as eqn. 4 predicts (see Table IV). This is not the case for the fluorinated bonded phases. Although the $\Delta\Gamma$ value becomes

TABLE IV

PHYSICAL PROPERTIES OF HALOGENATED BENZENES

Substituent	*Refractive index**	*Dipole moment*** *(D)*	*Van der Waals radius**** *(Å)*	*Van der Waals volume**** *(cc/mol)*	*Solubility parameter* $(cal^{1/2}/cm^{3/2})$
-F	1.465	1.60	1.45	5.8	8.5§§
-Cl	1.524	1.70	1.76	12.0	9.3§
-Br	1.558	1.69	1.86	15.1	10.0§
-I	1.620	1.70	2.00	19.6	10.2§§

* Ref. 47.
** Ref. 49.
*** Ref. 50.
§ Ref. 51.
§§ Ref. 52, eqn. 7.

more negative when changing the substituent from fluoro to chloro, the magnitude of the change is almost 2.5 times less than the change on the C_{10} column. Selectivity for the halogenated solutes is unquestionably greater on the C_{10} column as compared to any of the fluorinated columns. Chloro, bromo, and iodo substituents all have very similar $\Delta\Gamma$ values on the fluorinated phases. Clearly, dispersive interactions alone do not predict this behaviour. Dipole moment interactions may play a role in solute retention, but the differences in dipole moments between the halobenzenes (Table IV) are much too small to be able to draw any conclusions as to their relative contribution to retention.

The data indicate that silanophilic interactions play an insignificant role in the retention of weakly hydrogen-bonding halogenated benzenes. The percentage carbon loading for the C_{10} and the HFD columns are 10 and 8.6, respectively, and the base silicas are the same for these columns. The PFP and HFIPP phases are bonded to irregular particle silica and no data on the carbon loading is available. While a wide variation in the number of accessible silanol groups and their pK_a values is quite likely for these packing materials, the spread in $\Delta\Gamma$ is much greater on the C_{10} column

TABLE V

LINEAR REGRESSION RESULTS FOR C_{18}, HFD AND C_{10}

Mobile phase, methanol–water (40:60); flow-rate, 0.5 ml/min.

Column		*y-intercept*	*S.d.**	*C.I.***	*Slope*	*S.d.**	*C.I.***	*Correlation coefficient*	*Solute set size*
Y	*X*								
Hypersil	Zorbax	−0.008	0.113	0.202	0.519	0.003	0.006	0.999	14***
HFD	Zorbax	1.720	0.308	0.544	0.132	0.009	0.017	0.969	15
HFD	Hypersil	1.675	0.326	0.577	0.256	0.019	0.034	0.966	15
C_{10}	Zorbax	0.515	0.211	0.378	0.525	0.006	0.012	0.999	14
C_{10}	Hypersil	0.673	0.299	0.532	1.021	0.017	0.030	0.998	14

* Standard deviation.
** Confidence limits at the 90% level.
*** Test solutes which were outliers at the 99.9% level (see ref. 53) are excluded.

than on any of the fluorinated columns. The halobenzenes probably cannot compete with polar solvents for the silanol group sites. Unfortunately, none of the above can totally explain the difference in the behavior of the halobenzenes on the C_{10} and the fluorinated columns.

The behavior of the four columns can also be compared by examining plots of the capacity factor on one column *versus* a second column. If the retention of all solutes is controlled by only one factor such as hydrophobicity, or if several enter in but do so in strictly the same proportion on both columns, then it is reasonable to anticipate that a plot of the k' values on one column *versus* another will be linear and show a very small intercept.

This hypothesis was tested by determining the retention of fifteen test solutes on Zorbax ODS, Hypersil ODS, C_{10}, and HFD columns. The result of linear regression analyses are given in Table V. The intercept of the plot for the two C_{18} columns is zero and an excellent correlation coefficient is obtained ($\rho = 0.999$) even though the slope is much less than unity. In contrast, significantly non-zero intercepts are obtained in all other plots although the correlation coefficient is still high. In the case of C_{10} *versus* Hypersil ODS and Zorbax ODS, ρ is 0.998 and 0.999, respectively. For HFD *versus* these two octadecyl columns ρ values of 0.966 and 0.969, respectively, are obtained. However, the intercepts in these cases are about three-fold greater than the 90% confidence limit estimates.

Comparisons between fluorinated and C_{10} columns with a variety of solute subsets are reported in Table VI. There are only seven cases where the intercept can be judged to be zero at the 90–95% confidence level: HFD *versus* C_{10} with the non-polar solute subset; PFP *versus* HFIPP with all solutes as well as the three-solute subsets; and PFP *versus* HFD, and HFIPP *versus* HFD with the halobenzene subset. A tentative hypothesis is that the interactions responsible for the retention of the indicated solute subsets are similar on those pairs of columns whose k' *versus* k' plots show a zero intercept but the strength of the interactions are different. None of the slopes are unity even in the case of the C_{10} and HFD columns where the carbon loads are similar.

Whereas the retention due to silanophilic interactions can be neglected relative to hydrophobic interactions for the non-polar, non-hydrogen bonding, and weakly hydrogen-bonding solutes, these interactions cannot be neglected for polar hydrogen bond acceptors or donors.

The importance of silanol groups to solute retention can be qualitatively studied via blocking agents or by use of a silanophilic probe molecule as described previously[38]. Silanophilic interactions for specific solutes, particularly those having Brønsted acid–base properties, can be examined through the use of buffered solvents. Benzoic acid and pyridine were chosen as the solutes sensitive to residual silanol groups. Benzene and toluene were chosen because these solutes undergo strong hydrophobic interactions and silanophilic interactions are insignificant. Four methanol–water (60:40) solvent systems were prepared. One was unbuffered and the others contained 50 m*M* phosphate buffer at pH values of 2.4 and 6.8. A second pH 6.8 solvent contained 5 m*M* TEMED, an active silanol group blocking agent.

Small changes in k' were obtained on both the HFD and C_{10} columns for benzene when the mobile phase was changed from unbuffered to buffered (see Table VII). Therefore, the silanol groups must not play any significant role in the non-zero

TABLE VI

EXTENDED SOLUTE SET LINEAR REGRESSIONS

All data were obtained with methanol–water (60:40) at a flow-rate of 0.5 ml/min.

Column		*Solute set*	*Solutes in set*	*y-intercept*	*S.d.**	*C.l.***	*Slope*	*S.d.**	*C.l.***	*Correlation coefficient*	*Excluded solute*
Y	*X*										
HFD	C_{10}	All	42	0.280	0.055	0.094	0.250	0.009	0.016	0.974	None
		Non-polar	10	0.493	0.243	0.454	0.238	0.022	0.041	0.968	None
		Polar	31	0.251	0.047	0.080	0.239	0.016	0.026	0.944	2-Picoline
		Halogenated	8	0.679	0.187	0.357	0.166	0.033	0.064	0.899	None
PFP	HFIPP	All	39	0.062	0.016	0.027	0.535	0.024	0.041	0.964	2-Picoline
		Non-polar	9	0.128	0.094	0.185	0.464	0.084	0.162	0.915	Anthracene
		Polar	29	0.014	0.011	0.019	0.668	0.024	0.042	0.982	2-Picoline
		Halogenated	8	−0.034	0.063	0.122	0.733	0.092	0.179	0.956	None
HFIPP	C_{10}	All	42	0.219	0.024	0.041	0.081	0.004	0.006	0.955	2-Picoline
		Non-polar	10	0.436	0.058	0.107	0.065	0.005	0.010	0.975	None
		Polar	32	0.180	0.026	0.044	0.091	0.009	0.014	0.888	2-Picoline
		Halogenated	8	0.407	0.048	0.093	0.051	0.009	0.017	0.925	None
PFP	C_{10}	All	39	0.201	0.019	0.032	0.041	0.003	0.005	0.906	Anthracene
		Non-polar	9	0.338	0.070	0.132	0.029	0.006	0.012	0.876	Anthracene
		Polar	30	0.157	0.017	0.028	0.058	0.005	0.009	0.898	None
		Halogenated	8	0.248	0.028	0.054	0.041	0.005	0.010	0.959	None
PFP	HFD	All	39	0.152	0.019	0.032	0.163	0.011	0.018	0.927	Anthracene
		Non-polar	9	0.278	0.080	0.152	0.120	0.025	0.048	0.827	Anthracene
		Polar	29	0.087	0.011	0.018	0.246	0.011	0.019	0.975	*p*-Hydroxyphenol
		Halogenated	8	0.137	0.063	0.122	0.209	0.040	0.078	0.906	None
HFIPP	HFD	All	42	0.149	0.017	0.029	0.320	0.010	0.017	0.980	None
		Non-polar	10	0.318	0.047	0.088	0.267	0.015	0.029	0.987	None
		Polar	32	0.087	0.016	0.028	0.393	0.017	0.029	0.972	None
		Halogenated	8	0.217	0.033	0.064	0.297	0.021	0.041	0.985	None

* Standard deviation.

** Confidence limit at the 90% level.

TABLE VII

BUFFER EFFECTS ON k' VALUES USING 50 m*M* PHOSPHATE

All solvents are methanol–water (40:60).

Solute	*Column*	*Solvent*			
		Unbuffered	*pH 2.4*	*pH 6.8*	*pH 6.8 + TEMED*
Pyridine	HFD	2.86	0.06	2.79	2.15
	C_{10}	1.54	0.02	1.52	1.34
Benzoic acid	HFD	0.48	1.47	0.63	0.03
	C_{10}	0.36	4.45	0.32	0.30
Benzene	HFD	3.89	3.69	3.64	3.40
	C_{10}	9.26	9.20	8.96	8.40
Toluene	HFD	7.59	7.30	7.21	6.67
	C_{10}	24.4	22.0	21.4	21.0

intercepts obtained in the regression analysis with the non-polar test solutes. Although a slight increase in k' due to salting-out effects was anticipated, a small decrease was actually observed. This effect is trivial in comparison to the convoluted effects of buffer and blocking agent on the retention of a polar solute.

At pH 2.4, both benzoic acid (neutral) and pyridine (positively charged) are protonated. At pH 6.8, they are both deprotonated. Silanol groups are fully protonated at pH 2.4, but may be only partially protonated at pH 6.8. Unfortunately, no pK_a value for Chromegabond support material is available, but it may reasonably be assumed to fall between 4 and 8.

The capacity factor for pyridine drops dramatically when the strongly hydrogen-bonding nitrogen lone-pair electrons are no longer available due to protonation. When the nitrogen is deprotonated, the capacity factor increases. Benzoic acid is repelled from the surface when it carries a full negative charge and is better retained when present as a protonated neutral hydrogen-bonding molecule. The results show

TABLE VIII

LINEAR REGRESSION ANALYSIS COMPARING ALCOHOL MODIFIER EFFECTS

Plot of k' in water–methanol–modifier (60:35:5) *versus* water–methanol (60:40).

*Solvent modifier**	*Column*	*y-intercept*	*S.d.***	*C.l.*	*Slope*	*S.d.*	*C.l.*	*Correlation coefficient*
Ethanol	C_{10}	−0.047	0.302	0.535	0.914	0.017	0.030	0.998
	HFD	0.144	0.112	0.195	0.839	0.012	0.020	0.998
Isopropanol	C_{10}	0.123	0.271	0.479	0.733	0.015	0.027	0.997
	HFD	0.272	0.143	0.250	0.740	0.015	0.026	0.997
TFE	C_{10}	0.401	0.210	0.375	0.773	0.012	0.021	0.998
	HFD	0.685	0.162	0.285	0.688	0.020	0.035	0.994
HFIPA	C_{10}	1.584	0.437	0.794	0.459	0.024	0.043	0.982
	HFD	1.220	0.330	0.576	0.432	0.034	0.060	0.953

* Present at the 5% volume level.

** S.d. and c.l. have same meaning as in Table VI.

TABLE IX

PHYSICAL PROPERTIES OF ALCOHOLS (25°C)

Alcohol	*Surface tension (dyne/cm)*	*pK_a (in H_2O)*
Methanol	22.10*	15.5**
Ethanol	21.83*	15.9**
Isopropanol	20.78*	17.1**
TFE	17.4***	12.4§
HFIPA	16.14§§	9.3§§§

* Ref. 54.
** Ref. 56.
*** Ref. 55 at 24.5°C.
§ Ref. 57.
§§ Ref. 48.
§§§ Ref. 58.

the large part played by silanol groups in the retention of hydrogen bonding species. With both solutes the effect of buffer and blocking agent is much greater on the HFD column. Indeed, the retention of benzoic acid at pH 6.8 in the presence of TEMED is essentially zero. Clearly there must be many more unblocked ionized silanol groups on the HFD column than on the C_{10} column.

Mobile phase

Five mobile phases were studied using the C_{10} and HFD columns and the eighteen-solute subset mentioned earlier. The reference solvent was methanol–water (40:60). A small constant volume of methanol (5%) was replaced by one of the following alcohols: ethanol, isopropyl alcohol, 1,1,1-trifluoroethanol (TFE), or 1,1,1,3,3,3-hexafluoroisopropyl alcohol (HFIPA). Table VIII lists the results of the plots of k' in the modified solvent system *versus* the k' in the reference solvent.

Consider the case where the solvent modifier causes no change in solute retention. The resulting plot of k' modified *versus* k' reference would yield a slope of unity and a zero intercept. When the modified solvent is weaker than the reference solvent the slope will be greater than unity. Conversely, a strong modifier will decrease k' values and the resulting slope will be less than unity. The data in Table VIII show that all the alcohols tested are stronger mobile phase modifiers than methanol since the slopes are always less than unity. On both the C_{10} and the HFD columns ethanol always has a slope closest to unity whereas HFIPA causes the smallest slope to result. Isopropyl alcohol and TFE are intermediate in strength, the TFE being slightly stronger for the HFD column and slightly weaker than isopropyl alcohol for the C_{10} column.

In order to explain these results, some important physical properties of these alcohols must be compared. Table IX lists the surface tension and pK_a for each of the alcohols. All the listed alcohols are miscible with water in all proportions. The surface tension difference between the fluorinated and hydrocarbonaceous support (based on analogous liquid values) is large. This results in a higher interfacial surface energy between the fluorinated phase and the methanol–water solvent. All the liquid

TABLE X

LINEAR REGRESSION ANALYSIS FOR ALCOHOL MODIFIER STUDY

Data derived from analysis of a plot of the k' on HFD *versus* the k' on C_{10}.

Solvent modifier	*y-intercept*	*S.d.**	*C.l.*	*Slope*	*S.d.*	*C.l.*	*Correlation coefficient*
Methanol	1.568	0.290	0.514	0.250	0.016	0.029	0.974
Ethanol	1.326	0.283	0.502	0.247	0.017	0.031	0.969
Isopropanol	1.252	0.228	0.404	0.273	0.017	0.031	0.975
TFE	1.603	0.313	0.554	0.234	0.022	0.039	0.946
HFIPA	1.725	0.369	0.654	0.215	0.040	0.070	0.834

* S.d. and c.l. have the same meanings as in Table V.

solutes have intermediate values for surface tension and would therefore be driven into the interfacial region causing a decrease in the interfacial surface tension, *i.e.*, the adsorbed solute molecules essentially become a temporary part of the stationary phase. This also occurs in the hydrocarbonaceous stationary phase, but to a smaller degree since the interfacial surface tension is initially smaller.

A lower stationary phase surface tension would cause an increase in the retention of all liquid solute species because of their intermediate surface tensions. However, solute retentions are seen to decrease markedly for the majority of solutes tested when the modifying alcohol is added. This is due to the alcohol adsorbing to the stationary phase thereby modifying the surface as well as the increased solvating ability of these alcohols.

The acid properties of TFE and especially HFIPA cause noticeable differences in the retention behaviour of acidic and basic solutes such as benzoic acid and pyridine. In these two cases, retention increases dramatically when the methanol fraction is replaced with the fluorinated alcohol. Undoubtedly, the alcohol moieties interact strongly with these solutes. This increased retention may result from solute interaction with surface adsorbent solvent.

Solutes capable of weak hydrogen bonding (*e.g.*, benzonitrile, acetophenone) also show very slight retention increases. However, some very strong hydrogen-bonding solutes (nitrobenzene, phenol) show large retention decreases as do all the nonpolar solutes. This may reflect better solvation by the bulk solvent. The results once again emphasize the overall complexity of the system.

Table X directly compares the bonded phases using the various modified solvents. The slopes are all similar. The mobile phase modification seems to effect solute retention to approximately equal extents on both columns.

CONCLUSION

For these bonded phases it appears that the bulk of the differences in solute retention originates in the differences between the bonded phases. The clear evidence that solute selectivity is quite different on the hydrocarbon and fluorocarbon decyl columns as well as the fluorinated ether and perfluorinated phenyl columns may indicate that basic differences in the retention mechanism exist between hydrocarbonaceous and fluorinated stationary phases.

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