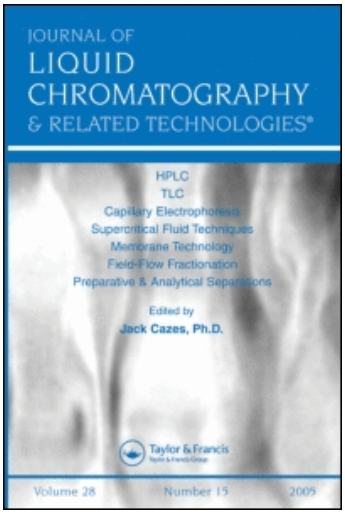

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PERFLUOROOCTYL AND PERFLUOROBUTYL BONDED ALUMINA STATIONARY PHASES FOR HIGH PERFORMANCE LIQUID CHROMATOGRAPHY

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ABSTRACT

The preparation and properties of perfluorooctylalumina (PFOA) and perfluorobutylalumina (PFBA) high performance liquid chromatographic stationary phases have been investigated. The PFOA phase was produced by chemisorption of perfluorooctanoic acid onto the surface of alumina. The PFBA phase was produced by a similar adsorption of perfluorobutylphosphonic acid onto alumina. Both phases exhibit reverse phase liquid chromatographic properties. Elemental analyses of these materials indicated that alkyl group surface coverage of the PFBA phase is higher than that of the PFOA phase. In contrast, retention of solutes on the PFBA phase is lower than that of PFOA. Isocratic capacity factors of over 20 compounds on the PFOA and PFBA phases were determined and compared with those obtained on octadecylalumina (ODA) and octadecylsilica (ODS) phases. In contrast to the greater

retention of phenols than other compounds that was evident on the unfluorinated ODA phase, the retention of phenols on the PFOA and PFBA phases was not found to be significantly different from that of other compounds. These results are attributed to a reduced degree of hydrogen bonding interactions between phenolic solutes and the PFOA and PFBA phases compared to those which occur between phenols and the ODA phase. Preliminary investigations of the utilization of the PFOA phase for the separation of peptides and the employment of the PFBA phase for the rapid separation of phenols are also described.

INTRODUCTION

Aluminum oxide has long been used as a stationary phase in liquid chromatography. Its unique molecular structure, amphoteric nature and high mechanical stability imparts to it a unique selectivity, which has enabled alumina to be employed for separations of a variety of organic compounds under normal phase and ion exchange conditions.[1] Only recently, however, chemical processes have been developed which allow the modification of the surface of alumina with nonpolar alkyl functionalities, including octyl and octadecyl moieties.[2-7] Such surface modified aluminas have been employed as stationary phases in high performance liquid chromatography (HPLC) under reverse phase conditions. Owing to their stability in mobile phases over a wide range of mobile phase pH's, the unique shape of their particles and the absence of interfering acidic sites on their surfaces, these new reverse phase aluminas have been demonstrated to be equal or superior to commonly-used silica based phases for a variety of applications, including the separations of alkaloids,[4,5] peptides and proteins,[6,7] and the determination of octanol-water partition coefficients of organic compounds.[5]

Surface modified alumina HPLC stationary phases may be prepared by adsorbing organic phosphonic or carboxylic acids onto chromatographic-grade alumina.[3,8] The adsorbed acids create a hydrophobic surface on the alumina, enabling it to be employed in reverse phase HPLC. This method of preparation allows for the modification of the surface of alumina by any organic moiety which can be attached to a carboxylic acid (- COOH) or phosphonic acid ($-PO_3H_2$) functional group.

In the past, we have reported on the chromatographic properties of octyl-

bonded alumina (OCA) and octadecyl-bonded alumina (ODA) stationary phases prepared by the process described above.[4-7] We have recently utilized similar processes to prepare perfluorooctyl bonded alumina (PFOA) and perfluorobutyl bonded alumina (PFBA) stationary phases, which have selectivity and stability which are somewhat different from those of the previously prepared unfluorinated OCA and ODA phases. In this report, we describe the preparation of the PFOA and PFBA phases, and discuss some of their chromatographic properties and applications.

EXPERIMENTAL

Materials

The octapeptide samples listed in Table 1 were obtained from the Protein Chemistry Core Facility of the University of Florida (Gainesville, FL, USA). Glass distilled acetonitrile and trifluoroacetic acid (TFA) was obtained from Fisher Scientific (Fair Lawn, NJ, USA). The reverse phase test mixture D consisted of approximately 0.1 mg/mL each of uracil, phenol, benzaldehyde, N-N,diethyltoluamide, toluene, and ethyl benzene in acetonitrile. It was obtained from Alltech Associates (Deerfield, IL, USA). Perfluorooctanoic acid was obtained from Aldrich Chemical Company (Milwaukee, WI, USA) and Fluowet PL 80™ (perfluorobutylphosphonic acid, 80% aqueous solution) was obtained as a gift from Hoechst Celanese Corp. (Charlotte, NC, USA). Other organic compounds used in this study were obtained from various sources, mostly from Aldrich Chemical Company (Milwaukee, WI, USA). Spherisorb™ spherical alumina, (A5Y: 5 micron particle diameter; A10Y: 10 micron particle diameter) was purchased from Phase Separations Inc.

Table 1

Octapeptide Standards

Peptide	Amino Acid Sequence	Molecular Weight
Lys-Ser-R	Lys-Ser-Ala-Lys-Phe-Nph-Arg-Leu	1166
Lys-Ala-R	Lys-Ala--Ala-Lys-Phe-Nph-Arg-Leu	1150
Leu-Pro-R	Leu-Pro--Ala-Lys-Phe-Nph-Arg-Leu	1151

Nph = nitrophenylalanine

(Norwalk, CT, USA) and the Unisphere™ fused microplatelet alumina (particle diameter: 5.6 microns) was purchased from Biotage (Charlottesville, VA, USA). Potassium bromide was purchased from Aldrich Chemical Company (Milwaukee, WI, USA).

Instrumentation

Infrared spectra of stationary phases and other materials were obtained as KBr pellets on a Mattson Instruments Model 4020 Fourier Transform Infrared Spectrometer. All columns were packed using an Alltech Slurry Column Packer.

The HPLC system consisted of a Perkin-Elmer Series 410 quaternary solvent delivery system, a Rheodyne Model 7125 injector (10 microliter loop) and a Perkin-Elmer Model LC-135 diode array UV-VIS detector. All chromatographic data were recorded and processed on a Perkin-Elmer Omega Data System.

Elemental analyses of PFOA and PFBA phases were performed by Atlantic Microlabs, Inc. (Norcross, GA, USA).

Sample Preparation and Analysis

The octapeptide samples (Table 1) were prepared as 1 mg/mL solutions in 0.1% aqueous TFA. All other compounds and mixtures to be analyzed were prepared as 1 mg/mL solutions in acetonitrile. All samples were analyzed by injecting 10 uL of the solutions into the HPLC system. The wavelength monitored for the octapeptide analyses was 280 nm. A wavelength of 255 nm was employed during the analyses of all other compounds. Except where noted, the mobile phase flow rate was set at 1 mL/min.

Columns

Four different columns were used in these evaluations. Each column consisted of stainless steel and had an internal diameter of 4.6 mm and a length of 150 mm. The slurry method, described by Snyder and Kirkland,[1] was used to pack all of the columns.

The Adsorbosphere™ octadecylsilica (ODS) column was obtained from Alltech Associates (Deerfield, IL, USA). The particle diameter of this material was 5 microns. An experimental octadecylalumina (ODA) column (not available for purchase), packed with surface modified Spherisorb A5Y spherical alumina, was obtained from the Aluminum Company of America (Alcoa Center, PA).

The method which was used to modify the alumina with monomeric octadecyl groups is described by Wieserman et al.[3] and Haky et al.[4] The PFOA and PFBA columns were packed with the stationary phases described below.

Synthesis of the PFOA and PFBA Phases

The perfluorooctylalumina (PFOA) phase consisted of Spherisorb A10Y spherical alumina which had been chemically modified with a perfluorooctanoic acid using the method described by Wieserman et al.[8] Approximately 10 g of the hydrated alumina was combined with 40 mL of a 0.06 M aqueous solution of perfluorooctanoic acid. The reaction mixture was heated for 2 hours at 50°C with constant stirring. The product was isolated by vacuum filtration and then washed with 100 mL of 0.1 M aqueous sodium bicarbonate in order to remove excess acid which was weakly adsorbed. The product was then washed with water and dried for 1 hour at 60°C.

The perfluorobutylalumina (PFBA) phase was prepared from Unisphere fused microplatelet alumina particles which were surface modified with perfluorobutylphosphonic acid by the process described by Wieserman et al.[3,8] Approximately 10 g of the hydrated alumina were mixed with 40 mL of a 0.06 M aqueous solution of perfluorobutylphosphonic acid and stirred for 2 hours at 25°C. The product was then washed and dried by the same method used for the PFOA phase, as described above.

Characteristic infrared spectral bands and assignments for the PFOA and PFBA phases are shown in Table 2.

Stability Study of PFOA

As part of the stability study of the PFOA phase, TFA was adsorbed to the surface of alumina by reacting it with solutions of TFA as described below. Approximately 100 mg of Spherisorb A10Y spherical alumina was mixed with

Table 2

Characteristic Infrared Spectral Band Assignments for PFOA, PFBA and TFA-Treated Alumina

PFOA	PFBA	TFA-Treated Alumina	Mode
3400	3400	3400	O-H stretch
1248, 1213, 1153	1244, 1215, 1115	1221, 1159	C-F stretch
825, 543	825, 543	825, 543	Al-O stretch

All spectral bands are expressed in cm^{-1}

20 mL of 0.1% TFA in ACN and stirred for one day . The solid material was isolated by filtration, washed with water and dried in an oven at 120°C for 2 hours. Characteristic infrared spectral bands and assignments for this material are shown in Table 2.

The PFOA phase itself was then also reacted with 0.1% TFA in ACN, using the same procedure described above. The infrared spectra of the resulting material was identical to that of the alumina which had been reacted with the TFA, as described in Table 2.

Calculations

Capacity factor (k') values were calculated by using the equation $k' = (t_r - t_o) / t_r$ where t_r is the retention time of the compound and t_o is the retention time of an unretained solute. The value of t_o was determined by injecting a 1 mg/mL aqueous solution of sodium nitrate which is not retained on any column under any of experimental conditions.

Reduced capacity factor (k'_r) values were calculated by the equation: $k'_r = k'/ A$, where A is the surface area (in m^2 / g) of the stationary phase support, as supplied by the manufacturers.

The surface coverage, SC, for each phase, expressed in terms of micromoles of monomeric sites per unit area, was calculated by equation $SC = (10^6) (\%C) / [(100)(12.01)(M)(A)]$, where %C is the percentage of

carbon in the stationary phase obtained from elemental analysis and M is the number of carbon atoms in the monomeric unit (i.e., M = 8 for PFOA; M= 4 for PFBA).

Peak asymmetry factors (AF's) were calculated from the equation AF = b/a, where b is the distance between the end of the peak and a vertical line which bisects the top of the peak, measured at 10% of the peak height and a is the distance between the beginning of the peak and the vertical line which bisects the top of the peak, measured at 10% of the peak height.

RESULTS AND DISCUSSION

We chose to investigate the chromatographic properties of fluorinated alkyl-bonded alumina stationary phases because of their potentially unique and interesting properties. On the basis of previous work with perfluoroalkyl-bonded silica phases,[9-18] the perfluoroalkyl alumina phases could be expected to have selectivities which are different from the unfluorinated alumina-based phases that had been previously investigated. Moreover, the high polarity of the C-F bond in such phases would be expected to minimize van der Waals interactions between such phases and solutes interacting with them, thus minimizing problematical irreversible adsorption caused by such interactions during HPLC separations of hydrophobic compounds on unfluorinated phases.[19-22]

The perfluoroalkyl alumina-based stationary phases examined in this study were perfluorooctylalumina (PFOA) and perfluorobutylalumina (PFBA). These phases can be easily prepared by using simple and efficient reactions which result in alumina containing strongly bonded fluoro-alkyl groups on the surface. The PFOA phase was prepared by reacting hydrated alumina with perfluorooctanoic acid. Synthesis of the PFBA phase was accomplished by reacting hydrated alumina with perfluorobutylphosphonic acid. These two particular acids were chosen for this study because they were commercially available and enable comparisons between fluorinated alkyl groups of different lengths bonded to the surface of the alumina.

Surface Alkyl Group Coverage

The conditions for the preparation of the PFOA and PFBA phases shown in the experimental section are optimized to produce the highest surface alkyl group coverage possible. Adsorption of carboxylic acids onto alumina is not as

strong or as fast as the corresponding adsorption of phosphonic acids.[23-25] For this reason, the preparation of the PFOA phase, produced by carboxylic acid adsorption onto alumina, required higher reaction temperatures than the preparation of the PFBA phase, which is produced by a phosphonic acid adsorption.[3,8] For the same reason, the PFOA phase was also found to be less stable than PFBA in mobile phases containing organic acids, as will be discussed later.

As shown in previous studies, alkyl group surface coverage can affect the reverse phase properties of an HPLC column.[1] Table 3 lists the alkyl group surface coverages and related data for the PFOA and PFBA phases , along with similar data for an unfluorinated octadecylalumina (ODA) phase and a typical octadecylsilica (ODS) phase. Expressed in terms of monomeric sites per unit surface area, the surface coverages of all 3 alumina-based phases are higher than that of the silica-based phase. As might be expected, surface coverage is highest among the alumina-based phases for PFBA, whose alkyl groups are the smallest and thus interfere the least with each other during the bonding process.

While the alkyl groups of the PFOA phase are also smaller than those of the ODA phase and thus might also be expected to have higher surface coverage than the octadecyl-bonded alumina phase, this was not observed. As shown in Table 3, surface coverage is nearly equivalent for the ODA and PFOA phases. Apparently the weaker bond which is formed between the carboxyl group of perfluorooctanoic acid and alumina in the PFOA phase reduces the effect of the diminished steric crowding on the alumina surface. This results in the PFOA phase having a similar surface alkyl coverage to the ODA phase, which has a bulkier alkyl group but is prepared by forming a stronger bond between alumina and the phosphoryl group of octadecylphosphonic acid.

Table 3

Surface Coverage Data

Phase	Surface Area (m^2/g)	Weight % Carbon	Surface Coverage ($mmoles/m^2$)
ODA	93	7.2	3.6
ODS	200	12	2.8
PFOA	90	3.0	3.5
PFBA	105	4.5	8.9

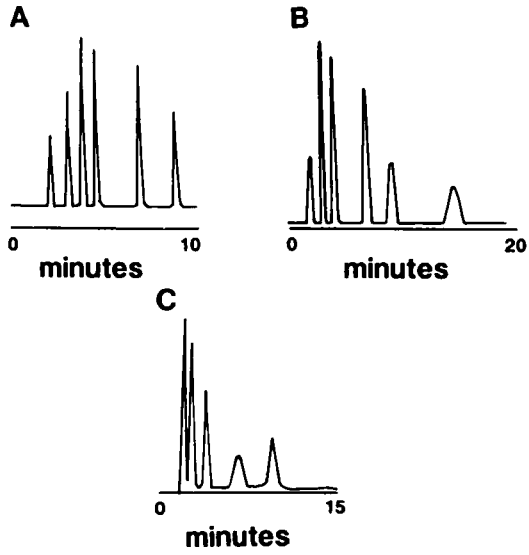

Figure 1. Chromatograms of reverse phase test mixture D on three columns. A: ODS column; B: PFOA column; C: PFBA column. Mobile phases: ODS: 65% acetonitrile, 35% water; PFOA: 30% acetonitrile, 70% water; PFBA: 10% acetonitrile, 90% water. Elution order for ODS and PFOA columns is uracil, phenol, benzaldehyde, N,N-diethyltoluamide, toluene and ethyl benzene. Elution order for PFBA column is the same as that described above, except that uracil and phenol coelute.

Comparison of Solute Retention

Since their surfaces are both less polar than that of their alumina backbones, the PFOA and PFBA phases can generally be used in the reverse phase mode, employing aqueous mobile phases similar to those employed with conventional ODS phases. Figure 1 shows chromatograms for a common reversed test mixture obtained on the PFOA and PFBA columns, along with a chromatogram obtained of the same test mixture on a commercial ODS column. Similar separations of the components of these mixtures were obtained on all three columns, indicating a general similarity of the retention mechanisms in all three phases. More significant, however, is the need to employ mobile phases containing lower percentages of organic modifier to achieve these separations on the PFOA and PFBA columns than on the conventional ODS column. Indeed, even with a mobile phase containing only

Table 4

Capacity Factor Data for Comparison of Relative Solute Retention

Column	ODS		ODA		PFOA		PFBA	
Parameter	k'	k'_r	k'	k'_r	k'	k'_r	k'	k'_r
Phenol	1.78	.0089	1.44	.0155	.351	.0039	.189	.0018
Benzaldehyde	3.76	.0188	7.28	.0783	.702	.0078	.386	.0035
Toluene	4.34	.0217	14.46	.1555	2.69	.0299	1.28	.0122
Mean	3.29	.0165	7.73	.0831	1.25	.0139	.612	.0058

Mobile phase: 30% acetonitrile, 70% water

10% acetonitrile, the two least hydrophobic compounds in the mixture, phenol and uracil, were not separated on the PFBA column at all. These results suggest that the perfluoroalkylalumina phases PFOA and PFBA are significantly less retentive than the conventional ODS phase.

A more realistic method of comparing the relative retention of stationary phases is to compare isocratic capacity factors (k's) of test solutes on each column employing aqueous mobile phases of identical compositions.[26] Table 4 shows such capacity factor data for three test compounds on the PFOA and PFBA phases, along with similar data obtained for the unfluorinated octadecylalumina (ODA) and ODS columns.

Average k' s of the three test solutes for all four columns are displayed in the bar graph in Figure 2A. On the basis of the relative magnitudes of these average k's, these data indicate that the ODA phase is the most retentive phase of those investigated, followed by ODS, PFOA and PFBA, respectively. However, retention of solutes can be influenced by a stationary phase's surface area, which differs among each of the phases investigated here. A more useful measure of relative retention of these phases, therefore, are the reduced capacity factors (k'_r), which can be obtained by dividing the capacity factors by the respective surface areas of stationary phase particles.[12] These k'_r data are displayed in Table 4, and the average k'_rs are displayed in bar graph form in Figure 2B. The capacity factors corrected in this manner still indicate ODA phase is the most retentive of the phases tested in this study. Average k'_rs for the alumina-based PFOA and silica-based ODS are very similar, indicating

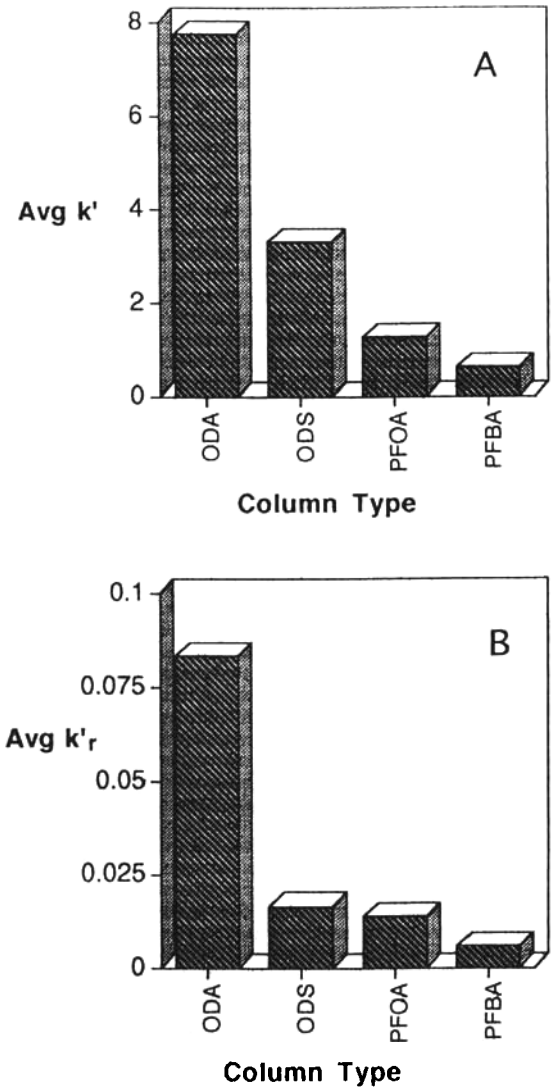

Figure 2. Bar graphs comparing the average capacity factors (A) and average reduced capacity factors (B) for three compounds on four columns using the mobile phase described in Table 4.

similar solute retention for these two phases in spite of PFOA's lower alkyl chain length. On the other hand, the PFBA column has the lowest verage k'_r,

Table 5

Asymmetry Factors for Test Compounds

Compound	AF (ODS)	AF (PFOA)	AF (PFBA)
Uracil	2.2	1.5	1.0
Phenol	1.4	1.0	1.0
Benzaldehyde	1.9	1.5	1.02
N,N-Diethyltoluamide	2.7	1.1	0.80
Toluene	1.2	1.3	0.60
Ethylbenzene	1.1	1.2	1.0
Mean value	1.7	1.3	0.90

Conditions: Same as in Figure 1.

consistent with low solute retention on other reverse phase columns with short alkyl chain lengths.[1]

Peak Symmetry and Interfering Mechanisms

One of the demonstrated advantages of alumina-based HPLC stationary phases is the absence of interferences of unbonded acidic sites on their surfaces. The presence of such sites in reverse phase silica-based columns often causes substantial peak tailing, especially during the analysis of basic solutes.[1,4,6] Similar to alkyl-bonded silica, reverse phase alumina surfaces might contain unbonded hydroxyl groups. However, if they are present, these groups are not nearly as acidic as those in silica. Earlier studies have demonstrated this by comparing symmetry factors for chromatographic peaks for a wide variety of compounds on ODA and ODS phases. Substantially higher degrees of peak symmetry were found on the alumina-based ODA column, especially for basic solutes.[4,6]

Table 5 lists asymmetry factors (AF's) for six compounds on PFOA and PFBA phases used in the present study, along with corresponding values determined for a conventional ODS phase. Consistent with earlier results, peak symmetries for all compounds are equal or superior for the alumina-based PFOA column than for the silica-based ODS column. Especially significant is the excellent symmetry of the peak corresponding basic solute N,N-

diethyltoluamide (AF = 1.1), in contrast to its poor symmetry on the ODS column (AF = 2.7). This again confirms the absence of acidic unbonded sites on alkyl-bonded alumina phases.

While average peak symmetry on the PFBA phase is also superior to that found on the ODS column (Table 5), asymmetry factors for some of the test compounds are less than unity, indicating peak fronting, an unusual occurrence in HPLC. This phenomenon results when solute molecules in the beginning of a band passing through an HPLC column interact with the stationary phase and enhance retention of solute molecules passing through later in the same band.[27] Such peak fronting has been observed during the normal phase liquid chromatographic analysis of some polar compounds on unbonded alumina.[28] Analogous to what was proposed for that phenomenon, a possible explanation for peak fronting occurring on the PFBA column is that retention is enhanced by solute molecules in the leading edge of their bands as they pass through the HPLC column, resulting in the increased retention of solute molecules in the middle and end of the solute bands. This explanation is especially appealing, considering the inherently low solute retention on the PFBA phase, as discussed earlier.

Selectivity Comparison

While comparison of reduced capacity factors of a few compounds provided a measure of the relative retention of solutes on the PFOA and PFBA phases, a detailed evaluation of selectivities of these phases requires additional studies involving a larger number of compounds. The method used to compare the selectivities of the phases investigated in the present work is based on one used in an earlier investigation of the chromatographic properties of the octadecyl-bonded alumina (ODA) phase.[4] In that study, isocratic capacity factors of over 20 compounds of different structure and functionality were determined on an ODA column and compared with those obtained on a standard ODS column. A logarithmic plot of the capacity factors of the compounds on the ODA column vs the capacity factors on the ODS column gave a straight line with excellent correlation for all compounds with the exception of the phenols. These compounds appeared to form a separate correlation line slightly above the line for the other compounds.

In the present study, a similar comparison of capacity factors of compounds on the PFOA and PFBA phases with those obtained on an ODS column was performed. Capacity factor data were also obtained on an ODA column to confirm the results found in the earlier study. The compounds used

Table 6

Capacity Factor Data for Correlation Study

Compound	Log k' (ODS)	Log k' (ODA)	Log k' (PFOA)	Log k' (PFBA)
m-Aminophenol	-0.334	-0.383	-0.795	--
Benzamide	-0.081	-0.019	-0.324	-0.719
Resorcinol	-0.082	0.021	-0.943	-1.128
Aniline	0.262	0.172	0.036	-0.738
Benzyl Alcohol	0.151	0.228	-0.228	-0.711
Phenol	0.254	0.366	-0.316	-0.699
Ethyl Propionate	0.485	0.254	0.165	-0.395
Benzonitrile	0.568	0.579	0.342	-0.210
Acetophenone	0.668	0.406	0.706	0.428
m-Cresol	0.427	0.713	0.033	-0.375
Benzene	0.797	0.786	0.265	-0.365
Quinoline	0.677	0.635	0.600	-0.910
Methyl Benzoate	0.751	0.921	0.571	0.101
Trichloroethylene	0.951	1.089	0.440	-0.113
Methyl Salicylate	0.862	1.294	0.038	0.313
Chlorobenzene	1.061	1.173	0.544	0.303
Ethyl Benzoate	0.963	1.113	0.631	0.412
1-Naphthol	0.710	1.447	0.425	0.032
Toluene	1.049	1.236	0.645	0.024
2-Naphthol	0.641	1.389	0.442	0.067
Bromobenzene	1.123	1.360	0.577	0.076
Thymol	1.029	1.146	0.979	0.468
Naphthalene	1.250	1.672	0.877	0.328
Anisole	0.773	0.870	0.344	-0.202

Mobile phases: ODS: 40% acetonitrile, 60% water; ODA: 20% acetonitrile, 80% water; PFOA: 10% acetonitrile, 90% water; PFBA: 10% acetonitrile, 90% water. Flow rate: 2 mL/min

in this study and k' data on all columns are shown in Table 6.

Logarithmic plots of k's of the test compounds of the ODA, PFOA and PFBA columns vs their k's on the ODS column are shown in Figures 3, 4 and 5

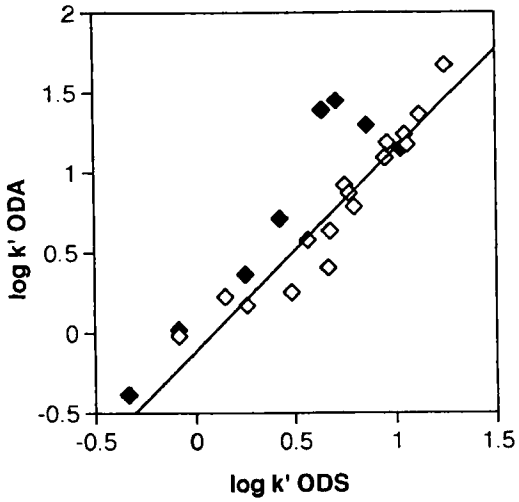

Figure 3. Logarithmic plot of the capacity factors of 24 compounds on the ODA column vs their capacity factors on the ODS column. Darkened squares correspond to phenols.

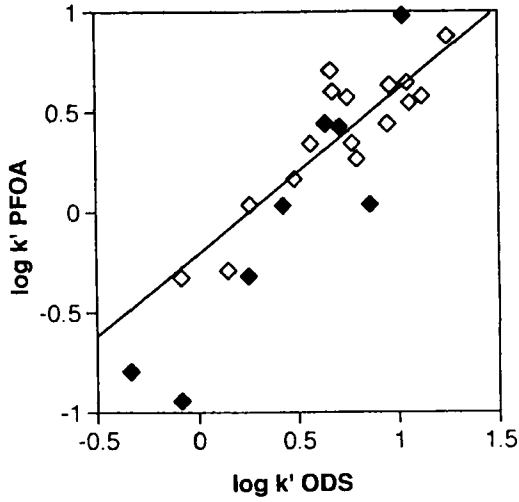

Figure 4. Logarithmic plot of the capacity factors of 24 compounds on the PFOA column vs their capacity factors on the ODS column. Darkened squares correspond to phenols.

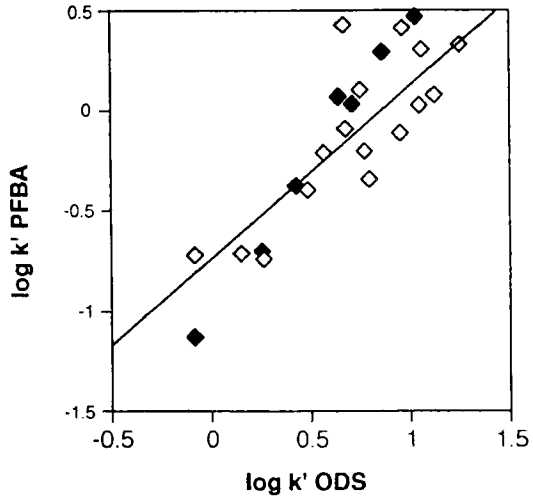

Figure 5. Logarithmic plot of the capacity factors of 23 compounds on the PFBA column vs their capacity factors on the ODS column. Darkened squares correspond to phenols.

respectively, and linear regression parameters for all graphs are shown in Table 7. In accord with the previous study, phenolic compounds are retained longer than other compounds on the ODA phase when compared with ODS, as indicated by their points generally being above the general correlation line for other compounds (see Figure 3) . Moreover, as shown in Table 7, removal of phenols from the ODA-ODS correlation substantially improves the degree of linear fit, as indicating by an increase in the correlation coefficient. Additionally, when the phenols are grouped together, they form an line with an excellent degree of correlation and a higher y-intercept than the line formed with the other compounds.

In contrast to the unique correlation of the phenolic compounds on the ODA-ODS curve, similar graphs produced for PFOA and PFBA columns exhibit no discernible unique retention behavior for phenols or any other class of compounds when compared to ODS. As shown in Figures 4 and 5, phenolic compounds do not appear to be consistently above or below the general correlation lines found for other compounds. Removal of the phenols does not substantially improve the correlation coefficients for these graphs (Table 7). These data indicate that neither phenols nor any class of compounds in consistently retained to a different degree or manner on the PFOA or PFBA phase than on a standard ODS phase.

Table 7

Linear Regression Parameters for Selectivity Comparison Study

Data set	Parameter	ODA/ODS	PFOA/ODS	PFBA/ODS	PFBA/PFOA
All Compounds	Slope	1.185	1.059	1.026	0.872
	Intercept	0.034	-0.411	-0.821	-0.396
	R^2	0.816	0.807	0.749	0.788
All except Phenols	Slope	1.261	0.847	0.866	1.049
	Intercept	-0.118	-0.285	-0.735	-0.518
	R^2	0.912	0.719	0.667	0.844
Phenols only	Slope	1.365	1.208	1.496	0.844
	Intercept	0.151	-0.567	-1.013	-0.271
	R^2	0.870	0.849	0.988	0.826

A comparison of selectivity of the PFOA with the PFBA phase is supplied by Figure 6, which is a plot of k's of the test compounds on the the PFOA column vs their k's on the PFBA column. This indicates a slightly enhanced retention of phenols on the PFOA phase than on the PFBA phase. Similar to the curve obtained using the ODA-ODS capacity factor data, the general correlation of the data improves when phenols are removed from the data set, and again the phenols appear to form their own unique correlation line (Table 7).

Hydrogen Bonding Retention Mechanism

The following conclusions can be reached from the analysis of the graphs and data discussed in the previous section:

1. Phenols are retained to a greater relative degree on the unfluorinated ODA stationary phase than on a standard ODS phase.

2. When compared to a standard ODS phase, the relative retention of phenols on the fluorinated PFOA and PFBA phases is not discernibly different from any other class of compounds.

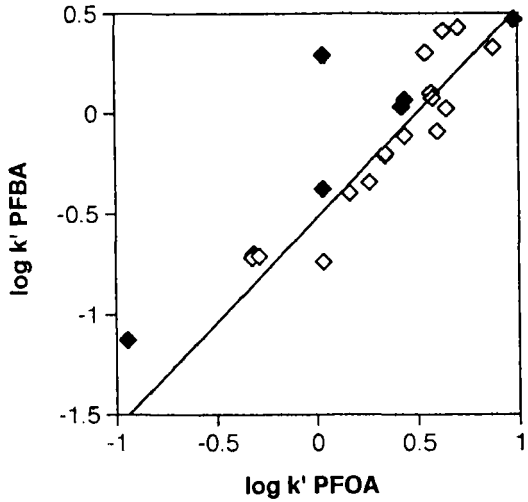

Figure 6. Logarithmic plot of the capacity factors of 23 compounds on the PFBA column vs their capacity factors on the PFOA column. Darkened squares correspond to phenols.

3. When compared to each other, the relative retention of phenols on the PFBA phase is slightly higher than on the PFOA phases.

In previous studies, differences in hydrogen bonding capabilities of the alumina backbone of the ODA phase and the silica backbone of the ODS phase were used to explain the different degrees of retention of phenols on the two phases.[4,29] The new data obtained in the present study indicate similar differences between the hydrogen-bonding capabilities of the ODA, PFOA and PFBA phases. Such differences can be justified through a consideration of the expected effects of the structural differences in the ODS, ODA , PFOA and PFBA phases on such hydrogen bonding interactions.

The most likely sites for hydrogen-bonding interactions between solutes and silica-based or alumina-based stationary phases are at the oxygen atoms directly bonded to the silicon or aluminum atoms of these materials. These oxygen atoms have unbonded electron pairs, which can serve as hydrogen-bond accepting sites for hydrogen-bond donating solutes, such as phenols. Such hydrogen-bonding would involve an interaction between a partial negative

charge on the oxygen atom bonded to alumina (or silica) and a partial positive charge on the hydrogen atom bonded to the oxygen atom of the phenol. These interactions would lead to increased retention of solutes such as phenols when compared to solutes which cannot be involved in hydrogen-bonding interactions.

Since phenols are retained to a greater relative degree on the ODA column than other compounds when compared to their retention on a silica-based ODS column, it appears that the potential for such hydrogen-bonding interactions is greater on the alumina-based ODA stationary phase than on ODS. This implies that a greater electron density (and a resulting more intense partial negative charge) exists on the oxygen atoms of the ODA phase than on the oxygen atoms of the ODS phase.

Since the structures and bonding chemistries of the ODA and ODS phases are so different, it is difficult to quantitatively assess the differences between electron densities and hydrogen-bonding capabilities of the oxygen atoms of the two phases on a theoretical basis. However, if such differences are primarily governed by the presence of either silicon or aluminum in the materials, a consideration of effects of these phases' silicon and aluminum atoms on the electron densities of adjacent oxygen atoms involved in hydrogen bonding may qualitatively indicate the relative hydrogen-bonding accepting abilities of the oxygen atoms of the ODA and ODS phases. Silicon is more electronegative than aluminum (Pauling electronegativities: 1.9 and 1.6 respectively[30]), and thus it should have a greater effect on reducing electron density around the oxygen atoms bonded to it. The intensity of any hydrogen bonding interactions to such oxygen atoms should be lower than interactions involving oxygen atoms bonded to aluminum. Thus, all else being equal, a silica-based phase such as ODS would be expected to have a lower degree of hydrogen-bonding accepting interactions with solutes than an alumina-based phase such as ODA. On this basis, hydrogen-bond donors such as phenols should be expected to have greater relative retention than other compounds on the ODA column when they are compared to their retention on an ODS column. As shown in Figure 3 and in a previous study,[4] this is in accord with the experimental results.

In contrast to the results obtained the ODA phase, retention of phenols on the fluorinated PFOA and PFBA phases is not discernibly different from that of non-hydrogen bonding compounds, relative to their retention on the silica-based ODS column (Figures 4 and 5). On the basis of the discussion in the previous paragraph, this would indicate that the potential for hydrogen-bonding interactions between solutes and the oxygen atoms bonded to aluminum in

these fluorinated phases is lower than that in the unfluorinated ODA phase. It also implies that the electron density and resultant partial negative charges on these oxygen atoms of the PFOA and PFBA phases is lower than that on corresponding atoms of the ODA phase.

Since the ODA, PFOA and PFBA phases are all based upon an aluminum-containing backbone, the differences in the hydrogen bonding capabilities of these phases must be related to the differences in their alkyl-group containing substituents. In fact, on a theoretical basis, these substituents would be expected to have different effects on electron density around the oxygen atoms in these phases, and the resulting hydrogen bonding interactions of these phases with solutes. In contrast to ODA, the alkyl groups of the PFOA and PFBA phases contain highly electronegative fluorine atoms, which, by pulling electrons toward them, reduce electron density about the oxygen atoms in these phases. This in turn reduces the intensity of any hydrogen bonding interactions with solutes, compared to similar interactions which occur in the ODA phase. Thus retention of phenols on the PFOA and PFBA columns would not be expected to be increased by such hydrogen-bonding interactions as much as on the ODA column, which does not contain highly electronegative atoms in its alkyl groups. Relative retention of phenols on PFOA and PFBA should therefore be more similar to their retention on ODS, which has low hydrogen-bonding capabilities, than that on ODA, whose hydrogen bonding capabilities are inherently stronger. This prediction is, of course, in accord with the experimental results. Moreover, since the alkyl groups of the PFOA phase contain a larger number of electronegative fluorine atoms than the smaller alkyl groups of PFBA, reduction of electron density and hydrogen-bonding interactions around the oxygen atoms bonded to aluminum would be expected to be greater for the PFOA phase than for the PFBA phase. This should result in lower hydrogen-bonding interactions between phenols and the PFBA phase than between phenols and the PFOA phase. In accord with this prediction, slightly higher retention of phenols over other compounds on the PFOA column compared to their retention on the PFBA column is observed, as suggested by the graph in Figure 6.

Applications of the PFOA Phase

As discussed earlier, the high polarity of the C-F bond in the perfluoroalkylalumina phases reduces intermolecular interactions caused by induced dipoles, and thus should minimize such van der Waals interactions between these phases and solutes interacting with them. This in turn should reduce the degree of irreversible adsorption of solutes and increase rates of

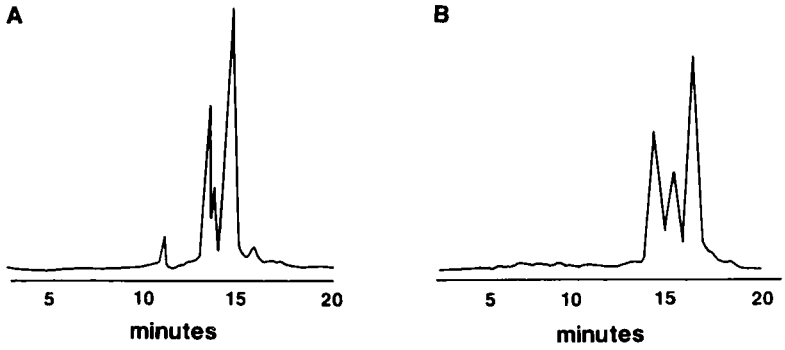

Figure 7. Chromatograms of mixture of octapeptide standards on PFOA column. Mobile phase gradients: A: 5 % - 95% aqueous acetonitrile containing 0.1% TFA; B: 5 % - 95% aqueous acetonitrile containing 0.1% perfluorooctanoic acid. Gradient time was 20 minutes for both chromatograms. Elution order corresponds to the order in which the octapeptides appear in Table 1. Minor peaks correspond to unknown impurities.

mass transfer between the stationary phase and the solutes. Irreversible adsorption and poor mass transfer is often a problem during the HPLC separation of high molecular weight proteins and peptides on conventional ODS and other nonpolar phases.[19-22] A potential application of the perfluoroalkylalumina phases, therefore, is the enhanced separation and preparative isolation of proteins and peptides by HPLC. For the same reasons, similar applications have been investigated for perfluoroalkyl-bonded silica-based phases.[12]

In a preliminary study, the HPLC separation of a mixture of three octapeptides shown in Table 1 was achieved using the PFOA column and a standard water-acetonitrile mobile phase gradient containing 0.1% trifluoroacetic acid (TFA). The TFA serves to protonate the peptides and act as an ion-pairing agent, which generally enhances the separations.[31,32] The chromatogram in Figure 7A shows that these conditions resulted in a reasonably good separation of the three octapeptides on the PFOA column. Unfortunately, the PFOA phase was not stable in this mobile phase. After about 5 hours of use with the TFA-containing mobile phase the PFOA column could no longer achieve separations of any kind.

As discussed in the experimental section , the infrared spectrum of the PFOA phase obtained after treatment with TFA-containing solvents was

virtually identical to that of unmodified alumina treated with the same solvents. This indicates that TFA had displaced the perfluorooctanoic acid from the surface of the PFOA phase, resulting in an unusable material in which trifluoroacetic acid is bonded to the alumina surface.

An easy solution to the problem described above was achieved by replacing the TFA in the mobile phase gradient with perfluorooctanoic acid. This acid has previously been demonstrated to serve the same role as TFA for protein and peptide separations by reverse phase HPLC.[32] Additionally, since perfluorooctanoic acid is one of the compounds from which the PFOA phase was produced, the phase could not degrade as a result of its presence in the mobile phase. Figure 7B shows a chromatogram of the octapeptide mixture on the PFOA column using an acetonitrile-water gradient containing 0.1% perfluorooctanoic acid. Good separation of the three peptides is evident. Additionally, the PFOA column remained stable for over 500 hours of use with the perfluorooctanoic acid-containing mobile phase.

Applications of the PFBA Phase

While the low solute retention of the PFBA phase rendered it unusable for separations of peptides and proteins under standard conditions, this same property enabled it to be employed for fast separations of smaller molecules using mobile phases little or no organic modifier. The advantages of reducing the percentage of organic modifier in the mobile phase are twofold: it reduces costs, and it minimizes waste disposal problems.

An example of such an application of the PFBA phase is shown in in Figure 8. It consists of a chromatogram of three phenols (m-cresol, 2-naphthol and thymol) obtained with the PFBA column using a mobile phase consisting of 99.8% water and 0.2% TFA. Good separation of these compounds was obtained in less than 6 minutes.

Unlike PFOA, which, as discussed earlier, would have been destroyed with a TFA-containing mobile phase such as that described above, the PFBA phase suffered no apparent short-term or long-term degradation when such mobile phases were employed. Apparently, the perfluorobutylphosphonic acid of PFBA is more strongly bound to the alumina surface than the octanoic acid of PFOA, and thus is more resistant to displacement by TFA. This is consistent with other studies which have demonstrated that phosphonic acids are more strongly adsorbed to alumina than carboxylic acids.[23-25]

Figure 8. Chromatograms of phenol mixture on PFBA column. Mobile phase: 0.2% aqueous TFA. The flow rate was 2 mL/min for both chromatograms. Elution order: m-cresol, 2-naphthol and thymol.

CONCLUSIONS

In this study, perfluorooctylalumina and perfluorobutylalumina stationary phases were prepared and demonstrated to be useful for HPLC separations of compounds in the reverse phase mode. The mechanisms involving the retention of compounds on these and other alumina-based phases were demonstrated to be similar to those which are involved with separations on standard octadecylsilica phases. However, subtle differences can be detected for the retention of phenolic compounds on different alumina-based phases . These differences are apparently the result of hydrogen-bonding interactions of these phenolic solutes with oxygen atoms on the alumina surface. The extent of such interactions is at least in part controlled by the net electron-withdrawing power of the alkyl groups bonded to the alumina.

In the past, fluoroalkyl-bonded stationary phases have been used primarily for very specialized applications, such as the separation of fluorinated organic compounds. While they are only preliminary, the separation studies described in the present work have demonstrated potential applications of perfluoroalkylalumina stationary phases of much broader scope, such as the separation of peptides and the rapid separation of lower molecular weight compounds using low cost, nontoxic mobile phases. Further research is needed

to systematically evaluate the new perfluoroalkylalumina phases for these and other applications, and to compare their performance with more conventional stationary phases such as ODS. These studies are continuing in our laboratories.

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