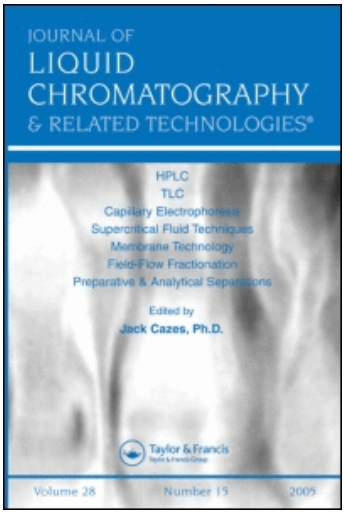

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SYNTHESIS AND CHARACTERIZATION OF A POLYMERIC FLUOROCARBON-DIAMINE REVERSED PHASE WEAK ANION EXCHANGE SILICA HPLC COLUMN PACKING

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ABSTRACT

Polymeric fluorocarbon-diamine silica column packings were synthesized by first reacting a co-polymer of chlorotrifluoroethylene and vinylidene fluoride (Kel-F 800) with piperazine and then reacting this product with aminopropyl silica. This mixed mode reversed phase-weak anion exchange HPLC column and a hydrocarbon (C-8) weak anion exchange silica HPLC column were compared for the separation of aromatic organic acids. Although the fluorocarbon column was generally less retentive than the hydrocarbon one, good resolution and fast analysis of simple mixtures was still possible. Nucleotides were retained longer on the fluorocarbon column. Good alkaline pH stability of the polymeric fluorocarbon-diamine silica column packing was exhibited.

INTRODUCTION

Porous silica modified with polymers for use as HPLC column packings combine the rigidity of a metal oxide with the stability of polymeric materials. A recent review (1) summarizes these chromatographic packings prepared either by physical adsorption of polymers, covalent attachment of polymers, or graft polymerization to silica. Generally amines such as polyN-vinyl pyrrolidone (2), polyethyleneimine (often crosslinked) (3), or poly1-vinyl,2,4 triazole (1) can be effectively adsorbed to porous silica for aqueous weak anion exchange chromatography of proteins. Reaction of polymers such as poly(succinamide)(4) or co-polymers of N-vinyl-pyrrolidone and acryloyl chloride (1) with aminopropyl derivatized silica has been done to prepare hydrophilic weak anion exchange packings with good shielding of the silica surface. Ion exchange of proteins and size exclusion chromatography of biological samples including viruses have been done with these columns. These packings were not considered to have any reversed

phase retention. Graft reactions of vinyl monomers such as styrene or methylmethacrylate with vinyl silyated suspended silica have produced a wide variety of HPLC packings with different polarities (5,6). A review (7) of fluorocarbon bonded phases on silica HPLC packings indicated as a class these reversed phase packings were less retentive than their corresponding hydrocarbon counterparts. Reports of fluorocarbon polymers grafted onto silica HPLC column packing are quite unusual. Sabarov et. al (8) has polymerized tetrafluoroethylene on the surface of silica and characterized these sorbents as having low adsorption activity to most classes of compounds. Effective isolation of t-RNA from proteins and plasmids was demonstrated. Recently, a chlorotrifluoroethylene-vinylidene fluoride co-polymer (Kel-F 800) has been crosslinked to aminopropyl silica to form a fluoropolymeric reversed phase weak anion exchange HPLC column packing. This mixed mode column was particularly useful for the separation of aliphatic anionic surfactants using a naphthalenedisulfonate-acetonitrile mobile phase for indirect photometric detection (9,10). The ion exchange retention was deemed most important for the indirect photometric detection mechanism to be operable.

In this report, we have expanded the versatility of this class of fluorocarbon reversed phase-weak anion exchange packings by modification of the Kel-F 800 first with an organic amine before reaction with the aminopropyl silica. The resultant Kel-F 800 piperazine aminopropyl HPLC column packing was characterized for both ion exchange and reversed phase retention of organic anions. Comparison of this packing to a commercial hydrocarbon based weak anion exchange column was made. Good pH stability of the polymeric fluorocarbon-amine column was found.

EXPERIMENTAL

Instrumentation

The liquid chromatograph was composed of a Model 510 HPLC pump, a Model U6K injector equipped with a 20 μL loop, a Model 490 programmable multiwavelength detector, and a Model 730 data module integrator, all from Waters Chromatography (Milford, MA). A Model LP-121 low pulse dampener from Scientific Systems, Inc (State College, PA) was added to the pump to eliminate baseline noise especially at low detector settings. The HPLC columns used were either the fluorocarbon polymer piperazine (FPP) silica type synthesized in house or the mixed mode C-8 weak anion exchange column purchased from Alltech Associates (Deerfield, IL).

Reagents

Kel-F 800, a copolymer of 78% cholorotrifluoroethylene and 22% vinylidene fluoride, was provided by the 3M Co. (Minneapolis, MN). This polymer (number average M.W. = 29,400; weight

average M.W. = 75,700) (11) is soluble in tetrahydrofuran (THF) but not in acetonitrile or methanol. For the data in Table 1, 10 μm RSil silica from Alltech Associates (Deerfield, IL) was used. For the preparation of the FPP HPLC column packing, 10-14 μm Zorbax diameter silica microspheres provided by the DuPont Co were used and treated as described in reference (12). Basically, the silica was refluxed with 10% HCl and 10% HNO_3 acid overnight, respectively. After the acid treatment, the particles were thoroughly washed with distilled water until pH tested to neutrality, filtered, and then dried at 125 ^{0}C in vacuum overnight. The silica particles were sintered at 850 ^{0}C for one hour to give an optimum surface area of about 40 m^2/g. Then the particles were refluxed in a 75 ppm HF solution overnight. After filtering and a thorough washing with distilled water, the particles were refluxed in distilled water overnight. Finally, the particles were filtered and dried at 125 ^{0}C in a vacuum oven overnight.

The γ-aminopropyltrimethoxysilane, toluene (dry 99%, grade), and piperazine were obtained from Aldrich (Milwaukee, WI). Mobile phases were prepared from HPLC grade acetonitrile (ACN) (EM Science, Morristown, NJ) and distilled water purified with an E-pure water treatment system(Bransted/Thermolyne Corp., Dubuque, IA). The common sample solutes such as the organic acids were obtained from a variety of sources. The biochemical sample components separated in Figures 7 and 8 were received from Sigma Chemical Co (St. Louis, MO).

PROCEDURE

Aminopropyl silica was synthesized in batches by reacting 0.50 g of silica with 120 mL of 2.5% γ-aminopropyltrimethoxysilane in toluene for 18 hours at 80 ^{0}C. The apparatus consisted of a three neck round bottom flask with a thermometer, a N_2 purge line, and a Dean-Stark trap connected to a condenser to remove the ethanol. The resulting particles were filtered and washed 3 times with toluene and then with acetone. Several batches of aminosilica were dried in vacuum at 120^{0}C for 8 hours before use. Reproducibility of the amino silica synthesis for 2 batches was within 0.5% for C and 0.2% for H and N.

The fluorocarbon diamine silica packings were synthesized using a two-step procedure. Kel-F 800 and the crosslinking agent such as piperazine at a 3:1 by weight stoichiometric ratio were reacted with stirring in 150 mL of THF at 57 ^{0}C for 24 hours. Then a known weight of aminosilica was added to the flask and the reaction was allowed to proceed another 46 hours. This two step procedure as opposed to a one step process involving adding the Kel-F 800, crosslinking agent, and aminosilica all together resulted in higher %C, %N and %F from elemental analysis. In the

Table 1 Elemental Analysis Data for Kel-F 800-amine Polymer-Aminosilica (RSil) Reaction Products

Kel-F 800/Amine Polymer	Kel-F 800-amine Polymer/ Amino Silica Reaction Ratio	%C	%H	%N	%F
	0:1	7.96	2.10	2.61	
Kel-F 800/Piperazine	1:1	10.51	2.41	1.85	3.92
Kel-F 800/Piperazine	2.5:1	13.75	3.63	5.52	4.01
Kel-F 800/Piperazine	5:1	7.61	4.73	8.03	4.78
Kel-F 800/N,N' Diethylethylenediamine	5:1	15.63	5.31	7.59	3.93
Kel-F 800/N,N' Dimethyl 1,6-hexanediamine	5:1	29.47	6.73	7.80	4.22

one step process, the %N of the silica product did not increase significantly when a crosslinking agent was added. Initially, small scale reactions were tried; for the 5:1 Kel-F aminosilica packing, 1 g of the Kel-F-piperazine product was reacted with 0.2 g aminosilica. However, this reaction was scaled up to 1 g batch quantities of aminosilica to prepare the column packing. The light orange silica products were washed copiously with THF and light microscopy showed discrete uniform particles with little agglomeration. Elemental analyses were carried out by Microanalysis Inc. (Wilmington, DE). The FPP column (0.40 x 25 cm) was packed from a silica slurry in methanol at 8,000 psi using a Haskel pneumatic pump. The FPP column as well as the Alltech mixed mode column were conditioned with the desired mobile phase for about an hour before use. All separations were carried out at ambient temperature. Retention factors, k', were calculated in the usual way; the solvent front (injection peak) was taken as the retention of an unretained peak.

RESULTS AND DISCUSSION

A characterization of the reaction of Kel-F 800 with diamines to form various silica products was carried out first. Table 1 shows elemental analysis data for a variety of Kel-F 800 aminosilica

reaction products. As the reaction ratio increases from 1:1 to 5:1 for the piperazine product, both the %N and %F increase as expected. Above the reactant ratio of 5:1, agglomeration of particles was observed. Straight chain agents such as N-methyl substituted ethylenediamine and hexanediamine gave similar products as evidenced by the %N and %F data. However agglomeration of the microspheres was evident in these two products.

Based on the fluorescent reaction of aminosilica with o-phthaldehyde and mercaptoethanol, it was estimated about one-third of the aminopropyl groups were unreacted. A schematic of the reaction product in agreement with the proposed mechanism (13) is shown in Figure 1. It is likely that some of the piperazine groups are not crosslinked and secondary amine groups are present. In addition, reaction of both the starting Kel-F polymer and the final amine Kel-F 800 aminosilica product with bromine decolorized both samples indicating some degree of unsaturation is present. Proton NMR also showed a strong band upfield at about 6 ppm for the polymer-silica product indicating double bonds are likely present.

The (FPP) HPLC column packing was based on Zorbax silica with a lower surface area to make this packing more comparable to the C-8 hydrocarbon anion exchange (HAE) column. Elemental analysis data for these two columns are shown in Table 2. The ion exchange capacity from %N for the HAE column can be calculated to be about 0.5 mmole/g (14). For the aminopropyl silica used to make the FPP column, an ion exchange capacity of 0.45 mmole/g was calculated. However, it is likely that many of these aminopropyl groups are not accessible due to steric hindrance by the fluorocarbon polymer. Some of this ion exchange capacity is undoubtedly replaced by the piperazine groups which should be more accessible. However, the increase of %N by 0.35% due to the piperazine is quite modest and it is likely the ion exchange capacity of the FPP column is significantly less than the the HAE column.

A chromatographic comparison of the FPP column was made to the commercial HAE column. The retention factor, k', for a wide variety of analytes was determined as a function of pH on both columns as shown in Figures 2 and 3. However, the mobile phase ionic strength was required to be a factor of 10 greater for the HAE column to get reasonable retention times. For the simple inorganic anion nitrate, retention decreases as the pH increases as expected. Phenol was weakly retained since only reversed phase and no ion exchange interaction was possible. The retention of monoprotic acids increases with mobile phase pH because of their increasing ionization. Many of these organic acids tend to show a maximum retention at pH 5.5 where both the ion exchange and the hydrophobic retention mechanisms should play roles. This maximum retention at pH 5.0 was also observed for short chain organic acids on a C-8 HAE column (15). The retention of benzoic acid and sorbic acid is greater on the HAE column as compared to the FPP column. Both columns showed strong retention for the diprotic aromatic acid, phthalic acid. Note that the k' of phthalic acid (pka_2 = 5.40) is more than double at a pH of 5.5. A further increase in pH decreases

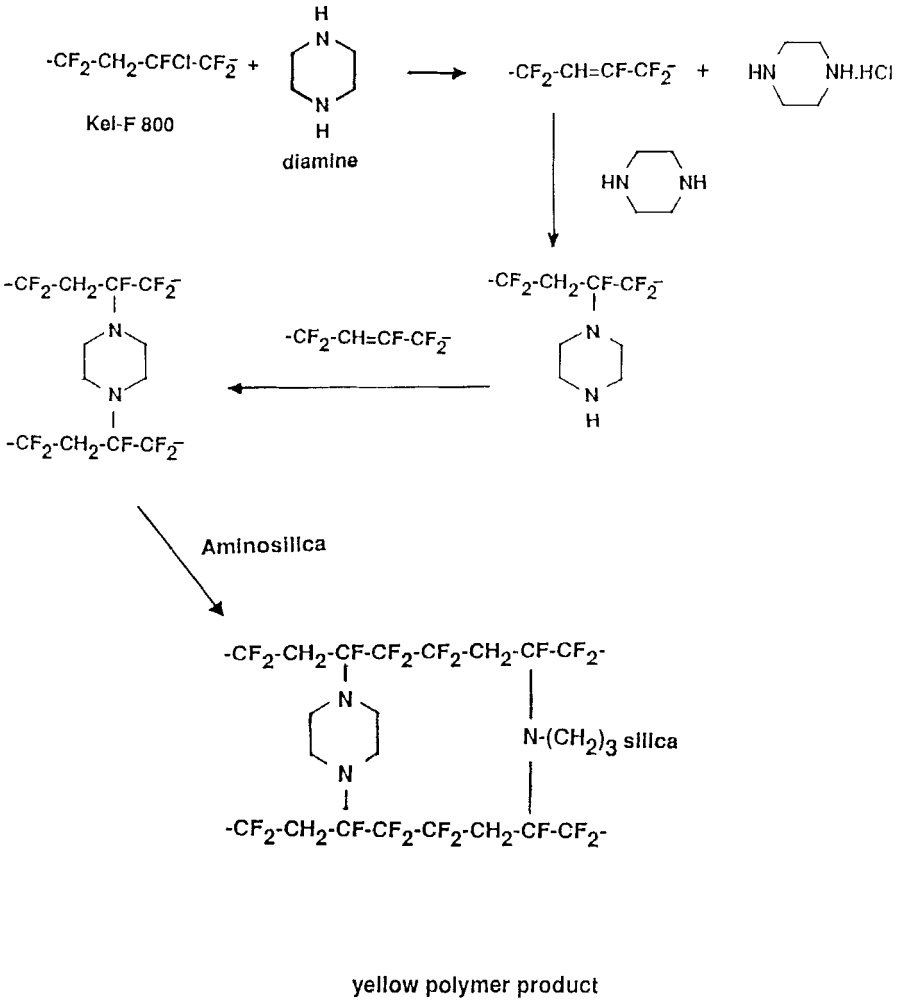
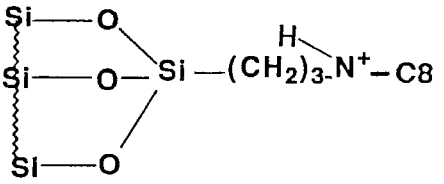


Figure 1: Proposed synthesis of the fluoropolymer-diamine weak anion exchange silica.

Table 2 Elemental Analysis Data for Fluorocarbon Polymer-Piperazine (FPP) / Aminosilica (Zorbax) and the C-8 Hydrocarbon Weak Anion Exchange Packings (Alltech)

Silica Product	%C	%H	%N	%F
Aminopropyl Zorbax	2.55	0.54	0.59	-
FPP(5:1) Zorbax	7.66	1.34	0.94	7.53
C-8 Weak Anion Exchange*	8.20	-	0.60	-

* Proposed structure for C-8 hydrocarbon weak anion exchange packing (Alltech):



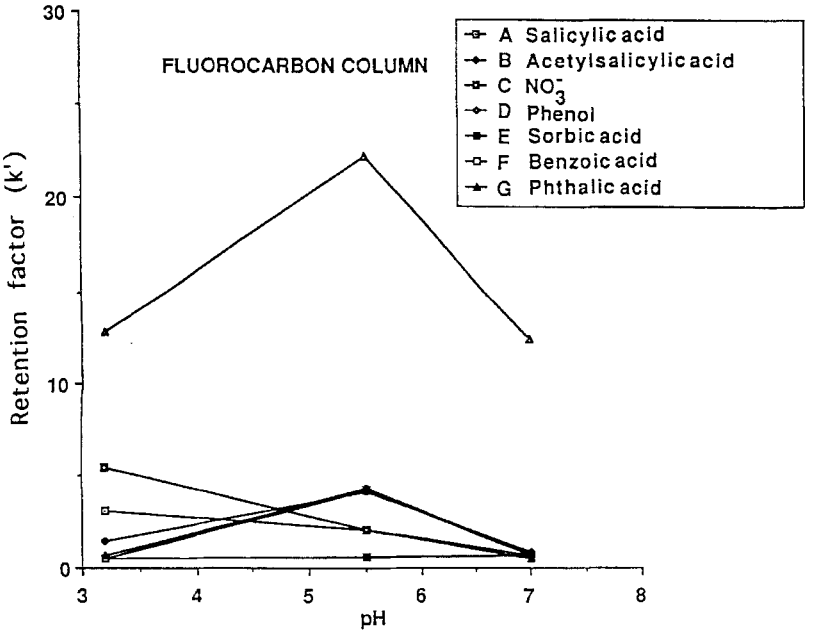


Figure 2: Retention of organic acids as a function of eluent pH on the fluorocarbon column.

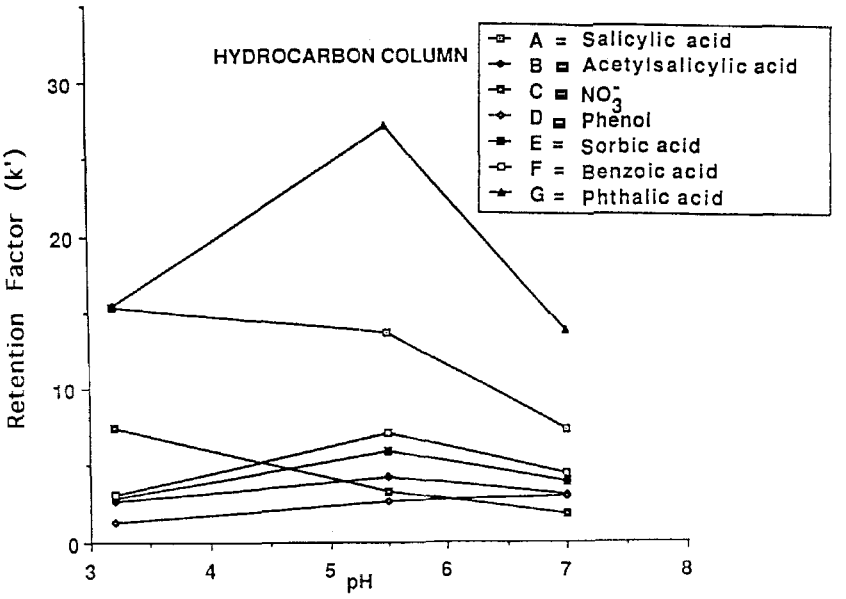


Figure 3: Retention of organic acids as a function of eluent pH on the hydrocarbon column.

the retention as the amine functionalities on both columns become deprotonated. Citric acid being triprotic could not be eluted at this ionic strength on either column. Representative chromatograms for 5 of these solutes are shown in Figure 4. The retention of the first four monoprotic carboxylic acids is about twice as long on the HAE column as that on the FPP column. The diprotic acid, phthalic acid, is eluted at 19 min on the HAE column compared to 14 min on the FPP column. The separation of the isomers maleic acid and fumaric acid isomers with baseline resolution was also possible using 10% methanol-90% acetate buffer (data not shown).

Figure 5 shows the effect of pH on the separation of salicylic acid (pKa= 2.97) and acetylsalicylic acid (pka = 3.49) on three columns: aminosilica, FPP, and HAE. Retention of these salicylates is substantially longer on the aminosilica and HAE columns as compared to the FPP column. Although the concentration of acetate is a factor of 10 higher in the mobile phase for the HAE column, retention of salicylate is still substantial on this column as compared to the aminosilica column. Since the ion exchange capacities of aminosilica and the HAE columns are similar, reversed phase retention must be strong on the HAE column even at 50% ACN. Indeed at pH 3.2, the retention of salicylate is 22 min on the HAE column compared to 12 min on the aminosilica column. The retention of the salicylates on the FPP column is substantially shorter

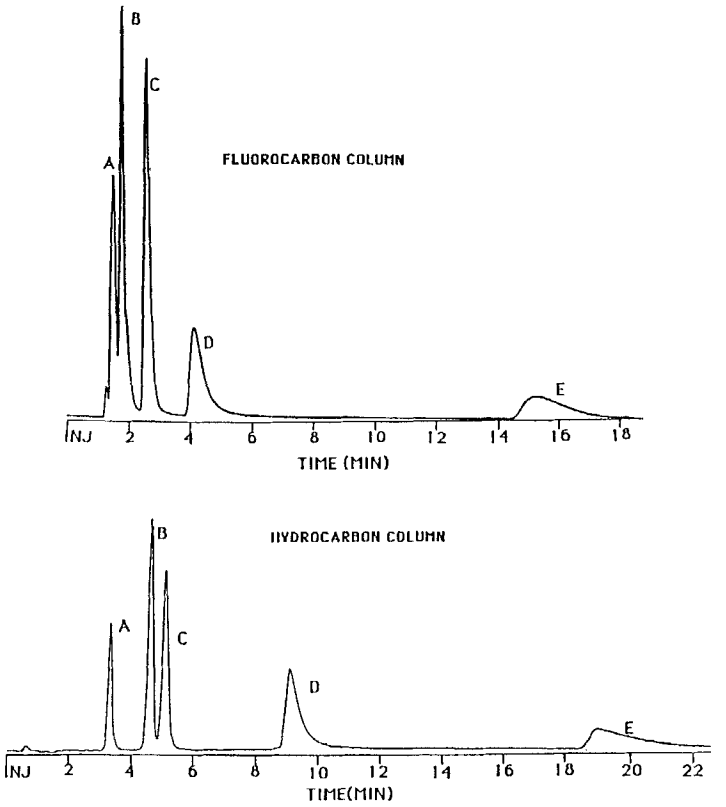


Figure 4: Separation of organic acids. Conditions: flow rate 1.0 mL/min, injection volume 20 μL, UV detection at 254 nm, 0.1 AUFS. Solute concentration: 25 ppm of (A) benzoic acid (B) sorbic acid (E) phthalic acid, and 100 ppm of (C) acetylsalicylic acid and (D) salicylic acid. Mobile phase is 50% 0.010 M sodium acetate/ 50% ACN pH = 3.2 for the fluorocarbon (FPP) column and 50% 0.100 M sodium acetate /50% ACN pH = 7.0 for the hydrocarbon (HAE) column.

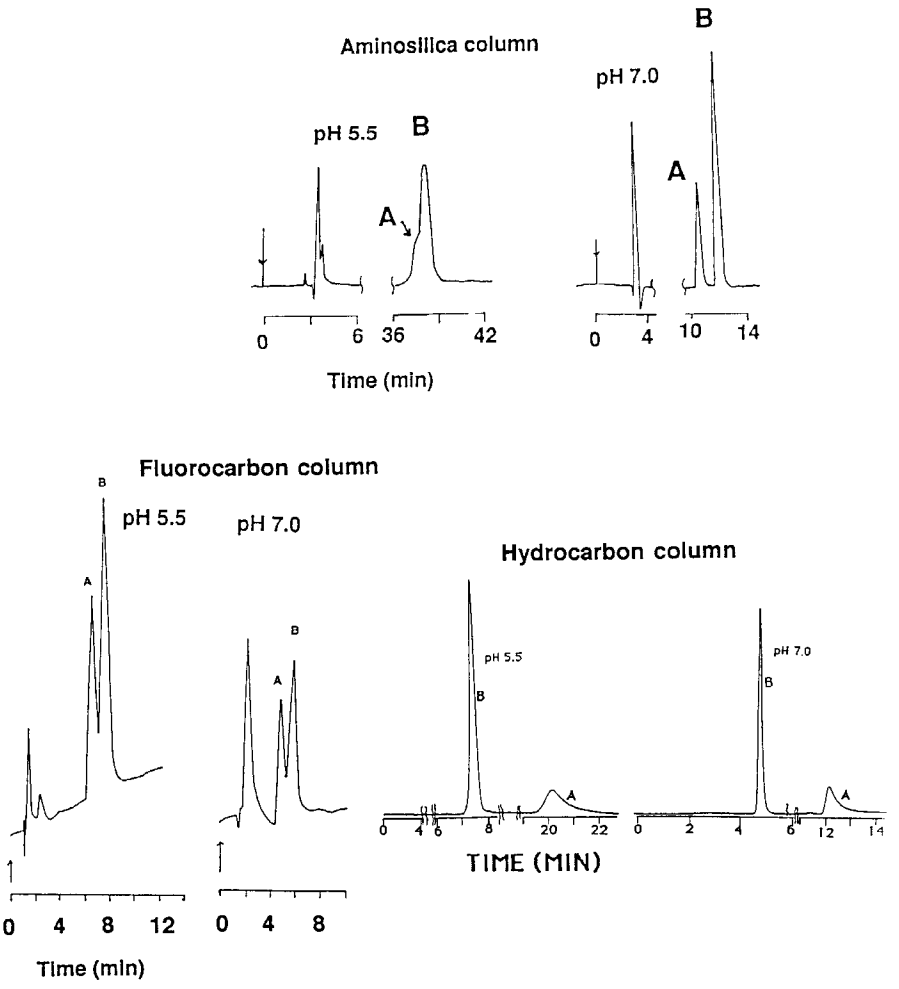


Figure 5: Effect of eluent pH on the retention of salicylate (A) and acetylsalicylate (B) on the aminosilica, fluorocarbon (FPP), and hydrocarbon (HAE) columns. Conditions are similar to those in Figure 4. Mobile phases: 50% 0.010 M sodium acetate, pH = 5.5 or 7.0/ 50% ACN for the aminosilica and the FPP columns; 50% 0.10 M sodium acetate, pH = 5.5 or 7.0/ 50% ACN for the HAE column.

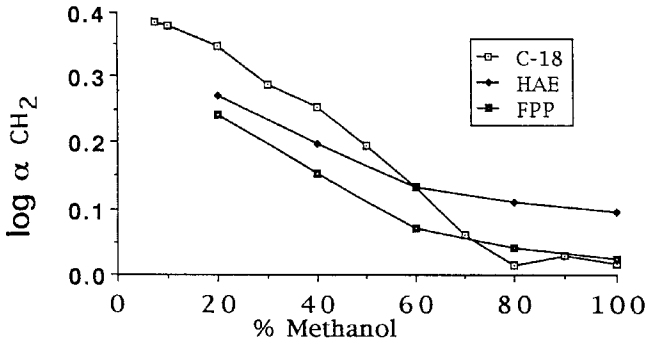


Figure 6: Plot of log α for phenylethyl alcohol and benzyl alcohol as a function of methanol content in the mobile phase for a C-18 silica, the HAE, and FPP columns.

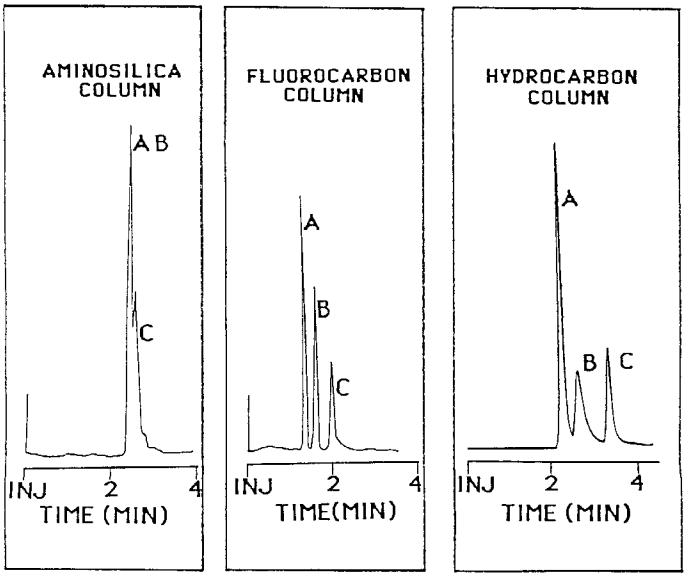


Figure 7: Separation of 10 ppm each of (A) pyridoxamine (B) pyridoxal and (C) pyridoxine. Conditions are the same as in Figure 4 except mobile phase is 20% ACN/80% H_2O on the aminosilica column and the fluorocarbon (FPP) column but 5% ACN/ 95% H_2O, 0.01% CH_3COOH on the hydrocarbon (HAE) column.

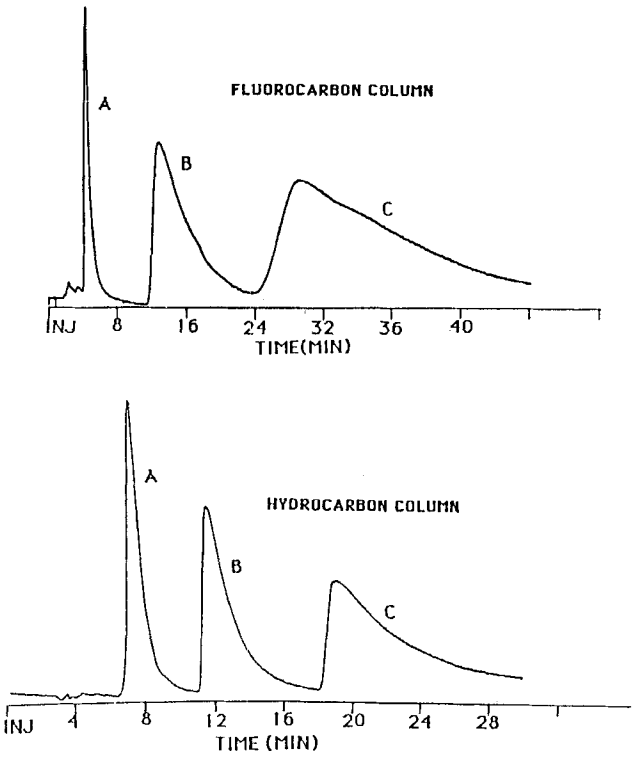


Figure 8: Separation of nucleotides on the fluorocarbon (FPP) and hydrocarbon (HAE) columns. Conditions: flow rate 1.2 mL/min, injection volume 20 μL, UV detection at 254 nm, 0.1 AUFS, mobile phase. 0.200M sodium acetate - 10% ACN. Solute concentration: 25 ppm each of (A) adenosine monophosphate (AMP), (B) adenosine diphosphate (ADP), (C) adenosine triphosphate (ATP).

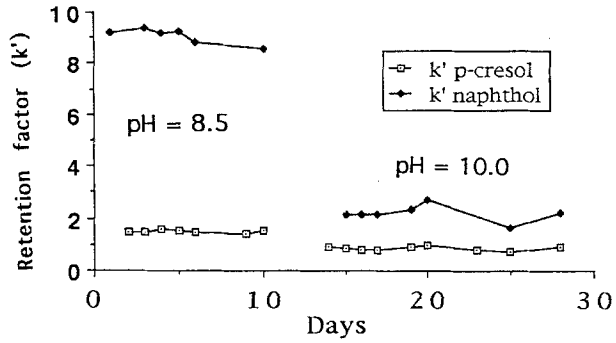


Figure 9: Retention factors for p-cresol and naphthol as a function of time the FPP column was in contact with a basic mobile phase (65% methanol-35% borate buffer, pH 8.5 or 10).

indicating both less retention by ion exchange as compared to aminosilica and less retention by reversed phase as compared to the HAE column. Interestingly, the retention order of the salicylates at pH 3.2 for both the aminosilica and FPP columns switches to that found for the HAE column at any of the three pH values. The retention order difference between the HAE column and the FPP column is unclear but may be due to differences in the type of base silica or in the hydrophobic retention mechanism.

The selectivity for the methylene group ($^{\alpha}CH_2$) when plotted semilogarithamically versus % organic solvent in the mobile phase has been found to be linear to about 75% methanol for reversed phase silica HPLC columns (16). Similar plots for both the reversed phase-ion exchange FPP and HAE columns were compared to that found for a C-18 column (Figure 6). The log α data is consistently ordered C-18 > HAE > FPP to about 70% methanol which is expected considering the relative hydrophobicities of C-18, C-8, and fluorocarbon substituents. Above 70% methanol, the retention of the methylene group for the mixed mode columns tends to plateau out. This may signal a change in the reversed phase retention mechanism. In high organic content mobile phases, retention is thought to be based on attractive dispersive interactions between the solute and the stationary phase not the repulsion of the solute out of the mobile phase to the stationary phase which is more likely in high water content mobile phases (17).

Figure 7 shows the separation of compounds that constitute vitamin B_6 (pyridoxamine, pyridoxine and pyridoxal) on both mixed mode columns. The retention is the reverse of that found for cation exchange chromatography (18). Retention is dominated by hydrophobic effects since little resolution is seen on the hydrophilic aminosilica column. This is consistent with the separation of this same vitamin B_6 mixture on a picolyl Kel-F polymeric HPLC column which is also a mixed mode reversed phase weak anion exchange column (19). Addition of acetic acid to the mobile phase used for the HAE column was necessary to reduce silanol interaction and ensure a narrow peak shape for pyridoxine. Even with 20% ACN- 80% water (no acetic acid), the retention of pyridoxine was about 10 min with a 3 min peak width at half height.

The separation of the highly charged nucleotides, adenosine monophosphate (AMP), adenosine diphosphate (ADP), and adenosine triphosphate (ATP), was carried out on both mixed mode columns (Figure 8). Retention of these solutes was stronger on the FPP column even though the same mobile phase was used for both separations. Fluorocarbon polymers have been previously reported effective for the separation of nucleotides (20).

Finally, stability of the FPP column was found to be good for at least 4 weeks even when operated at pH 8.5 or 10 (Figure 9). At pH = 8.5, the k' for naphthol was about 3 times longer than that at pH = 10. The retention factors for the mixture of p-nitrophenol, cresol, and naphthol remained constant within 10% during each pH trial period.

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REFERENCES

1. A. E. Ivanov, V. V. Saburov, V. P. Zubov, Advances in Polymer Science., 104: 1-41 (1992).
2. L. Letot, J. Lesec, C. Quivoron, J. Liq. Chromatogr., 4: 1311 (1981).
3. A. Alpert, F. E. Regnier, J. Chromatogr., 185: 375 (1979).
4. A. Alpert, J. Chromatogr., 266: 23 (1983).
5. B. B. Wheals, J. Chromatogr., 107: 402 (1975).
6. M. Hanson, K. K. Unger, Trends. Anal. Chem., 11: 368 (1992).
7. N. D. Danielson, L. G. Beaver, J. Wangsa, J. Chromatogr., 544: 187 (1991).
8. V. V. Saburov, M. R. Muidinov, S. A. Guryanov, A. D. Kataer, S. I. Turkin, V. P. Zubov, Zh. Fiz Khim., 65: 2692 (1991). Chem. Abstr. 115: 274916e.
9. S. A. Maki, J. Wangsa, N. D. Danielson, Anal. Chem., 64: 583 (1992).
10. N. D. Danielson, S. A. Shamsi, S. A. Maki, J. High Resol. Chromatogr., 15: 343 (1992).
11. L. G. Beaver, N. D. Danielson, Polymer Bulletin., 30: 47 (1993).
12. J. Kohler, J. J. Kirkland, J. Chromatogr., 385: 125 (1987).
13. K. L. Paciorek, L. C. Mitchell, C. T. Lenk., J. Polymer Sci., 45: 405 (1960).
14. S. A. Shamsi, N. D. Danielson, J. Chromatogr Sci., in Press.
15. R. Saari-Nordhaus, J. M. Anderson., Anal. Chem., 64: 2283 (1992).
16. B. L. Karger, J. R. Gant, A. Hartkopf, P. H. Weiner, J. Chromatogr., 128: 65 (1976).
17. J. G. Dorsey, W. T. Cooper, Anal. Chem., 66: 857A (1994).
18. R. C. Williams, D. R. Baker, J. A. Schmit, J. Chromatogr Sci., 11: 618 (1973).
19. M. Kruempelman, N. D. Danielson., Anal. Chem., 57: 340 (1985).
20. D. A. Usher, Nucleic Acids Res., 6: 2289 (1979).

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