Journal of Chromatography, 544 (1991) 187–199
Elsevier Science Publishers B.V., Amsterdam

CHROM. 22 943

Review

Fluoropolymers and fluorocarbon bonded phases as column packings for liquid chromatography

NEIL D. DANIELSON*, LARRY G. BEAVER and JULIE WANGSA
Department of Chemistry, Miami University, Oxford, OH 45056 (U.S.A.)

ABSTRACT

The use of both fluoropolymers and their derivatives as well as fluorinated bonded phases on silica as high-performance liquid chromatography column packings is reviewed. In general, retention of small molecules is less on fluorocarbon phases as compared to hydrocarbon materials. However, specific effects such as π electron interactions can be magnified using fluorinated phenyl phases. The potential of fluorocarbon phases for the separation of biomacromolecules appears to be good.

CONTENTS

1. INTRODUCTION

Although the use of fluorinated column packing materials for high-performance liquid chromatography (HPLC) is not widespread, a number of interesting columns have been developed for both broad and specialized applications. In this review article, the types of column packings will be classified into two main groups: (1) fluoropolymers and their derivatives and (2) fluorinated bonded phases on silica. Although the second topic has been reviewed in part previously [1], the first topic has not. Therefore slightly more attention will be placed on the fluoropolymer topic. In addition, both categories will be subdivided into sections describing columns that

0021-9673/91/$03.50

separate either small molecules or large biomolecules. Examples of small molecules are pharmaceuticals, polyaromatic hydrocarbons, and a variety of substituted aromatics. Examples of large biomolecules are DNA fragments and proteins.

2. FLUOROCARBON POLYMERS AND THEIR DERIVATIVES

2.1. Small-molecule separations

The use of fluorocarbon polymers to separate small organic molecules has been focused primarily on two methodologies. Both involve largely the use of the fluoropolymer Kel-F or polychlorotrifluoroethylene (PCTFE).

First, carbon adsorbents have been prepared from the reduction of Teflon or polytetrafluoroethylene (PTFE) using alkali-metal amalgams. The PTFE sheet is immersed in an lithium amalgam under argon while being heated for 8 h at 100°C [2]. The dark product is crumbled in water and washed to remove residual Li and Hg. It was postulated that LiF covering the reduced carbon chains of polyne $(C\text{–}C\equiv C\text{–})_n$ or polycumulene $(=C=C=)_n$ is washed away upon extraction with water. With a particle distribution of about 4–12 μm, high surface area values on the order of 2000 m^2/g were found. After packing at 20 MPa, columns 300 × 3 mm I.D. were less efficient than corresponding silica types. However, the separations of five aliphatic alcohols using acetonitrile or two carborane isomers using heptane as the respective mobile phases were possible [3]. Using gas chromatography, these carbon particles were characterized to retain aliphatic hydrocarbons through penetration of the carbon skeleton while more polar compounds such as alcohol and water interacted physically with the cavities of the particles [4]. Carbon adsorbents prepared in a similar way but with a low oxygen content were suitable for the separations of aliphatic hydrocarbons using pentane or 1-alkanols using methanol as the eluents. The use of binary solvent mixtures was not explored.

A similar carbon packing with oxygen functional groups was prepared by reduction of PCTFE particles using a lithium amalgam [6]. The carbon surface was modified either through silylation or Grignard chemistry to prepare a reversed-phase C_8 packing. Although the bonding was similar to that found for C_8 silica, a much stronger (× 40) interaction of polyaromatic molecules such as 2-naphthol over that of C_8 silica was still generated by the carbon substrate.

The second approach in the use of fluoropolymers as column packings for the separation of small molecules is to functionalize the PCTFE particles. The PCTFE polymer itself has a number of desirable properties such as high chemical resistance, total hydrophobicity, and good rigidity. Because of the presence of halogen atoms on the carbon–carbon chain, the polymer exhibits a helical rather than a zig-zag conformation. This arrangement forms a cylindrical polymer chain having an outer sheath of halogen atoms resulting in a polymer with high chemical resistance [7]. Because of the presence of a chlorine instead of a fluorine atom, PCTFE chains are not as closed packed as those in PTFE resulting in a lower melting point for PCTFE. However, this structural difference of halogen atoms does cause greater interchain attraction for PCTFE making this polymer more rigid than PTFE.

To increase the versatility of PCTFE particles as an HPLC column packing, Danielson and co-workers discovered that organolithium [8] and organomagnesium [9] reagents will derivatize the polymer with a variety of functional groups. The

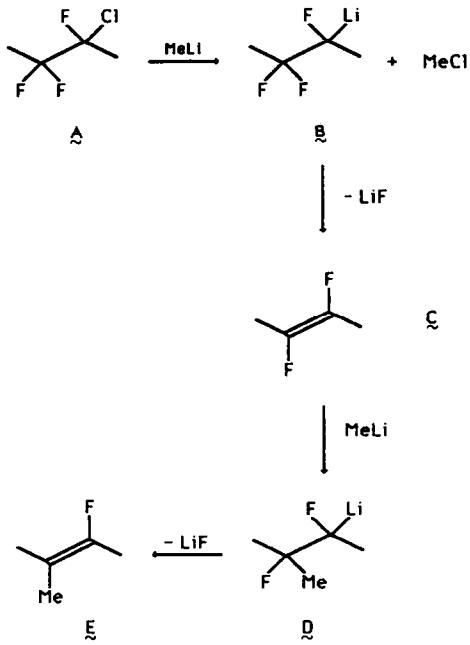

Fig. 1. Mechanism of reaction between methyllithium and polychlorotrifluoroethylene. Me = Methyl. From ref. 10.

reaction of several alkyllithium reagents such as *n*-butyllithium and alkylmagnesium reagents with PCTFE results in loss of chlorine and the introduction of the alkyl group. Aryllithium such as phenyllithium and arylmagnesium reagents also react in an analogous fashion with PCTFE. Dias and McCarthy [10] proposed a two-electron, elimination–addition mechanism to explain the observed reaction between methyllithium and PCTFE oil. In this mechanism (Fig. 1), a halogen–metal exchange affords the lithiated intermediate and subsequent elimination of lithium fluoride is rapid, even at low temperatures (−78°C). The loss in fluorine and the gold-brown color of the reacted polymer have been attributed to the reducing capabilities of organometallics resulting in the formation of carbon-carbon double bonds [11]. We have trapped the lithiated PCTFE oil intermediate formed from *n*-butyllithium at −125°C by quenching with dry ice to form the carboxylated *n*-butyl PCTFE product [12]. A similar reaction mechanism is believed to occur for organomagnesium reagents and PCTFE [12].

Organolithium and organomagnesium reagents (3:1 excess) were most effective in derivatizing PCTFE particles when reacted at 25°C for 2 h using tetrahydrofuran (THF) as the solvent. Quenching the reaction with dilute mineral acid instead of methanol was important because the later solvent would introduce methoxy functional groups. However, introduction of unwanted oxygen containing functional groups into the derivatized polymer was still possible using THF and ambient or reflux conditions. The order of reactivity of organolithium reagents in producing a substituted PCTFE derivative was *n*-buthyllithium ≈ methyllithium > phenyllithium > *tert.*-butyl-

lithium, indicating possible steric considerations [12]. The extent of functionalization of PCTFE decreased with increasing chainlength (C_1, C_8, C_{14}) of the organomagnesium reagent as well. Carbon ESCA (electron spectroscopy for chemical analysis) data indicated the surface coverage of the derivatized PCTFE was somewhat spotty as evidenced by the presence of fully halogenated carbons [13]. The extent of functionalization using *n*-butyllithium could be increased 2–3 times by using a co-polymer of PCTFE such as Kel-F 6300 (chlorotrifluoroethylene–vinylidene fluoride, 97:3) [8]. Because PCTFE is not a co-polymer, it has a higher degree of crystallinity and greater hardness. This fluoropolymer is therefore less prone to swelling by the THF solvent causing its lower reactivity.

The chromatographic potential of *n*-butyl PCTFE particles packed in 200 × 4.6 mm I.D. columns was demonstrated with the resolution of solutes such as *p*-nitroaniline, toluene and phenanthrene using methanol–water (75:25) mobile phase. A similar column packed with underivatized PCTFE provided no resolution under the same mobile phase conditions and only poor resolution when the methanol content was reduced to 40%. Electron microscopy of *n*-butyl PCTFE showed the surface had been etched and pitted. A surface area increase from about 1.6 to 3.5 m^2/g was measured. The extent of C_4 functionalization was estimated to be about 2 mmol/g from elemental analysis data. Other small aromatics such as aniline, nitrobenzene, toluene, and xylene could be separated.

A detailed chromatographic study of an organolithium functionalized PCTFE, phenyl PCTFE, has been made [14]. Using 15–35-μm particles packed at 9000 p.s.i. into 150 × 4.1 mm I.D. columns, parameters such as reduced plate height ($h = 5.7$), sample capacity (100 μg/g packing), column porosity, and permeability indicated phenyl PCTFE is pellicular in nature. Plots of the methylene group selectivity (α) *vs.* the surface tension of the acetonitrile or methanol mobile phase were linear indicating a reversed-phase mechanism explained by the solvophobic theory was valid. The retention order of a variety of substituted benzene molecules was identical to that found for C_{18} silica. Some of the compounds such as benzaldehyde, cyanobenzene, nitrobenzene, methoxybenzene, and benzene itself were retained longer on the phenyl Kel-F column. The separation of aniline and several N-alkylanilines is shown in Fig. 2.

Phenyl Kel-F has been modified to form both the strong cation and anion exchange packings [15]. The capacity of the ion exchange materials was about 0.2 mequiv./g. Permeability of both columns was good, as evidenced by an operating pressure of about 700 p.s.i. at 1.0 ml/min for a water–methanol (50:50) mixture. The effects of pH, ionic strength and mobile phase counter-ion on solute retention showed conventional ion-exchange behavior. A combination of reversed-phase and ion-exchange properties was particularly useful for separating mixtures containing neutral and ionic molecules. Baseline resolution of four sulfa drugs, an important class of antibacterial compounds, was possible at pH 7.0. Sulfanilamide and sulfamerazine are neutral molecules at pH 7 and were retained least due only by a reversed-phase interaction. Sulfanilic acid, ionized at this pH, was retained primarily by ion exchange. Sulfathiazole, both ionic and hydrophobic in nature, was retained the longest since both retention mechanisms could be involved.

A weak anion exchange packing, picolyl PCTFE, prepared from 4-picoline using organolithium chemistry, was also found to have reversed-phase characteristics. This is probably due to the fact that the ion-exchange ligand density is very low (22 μg/g) at

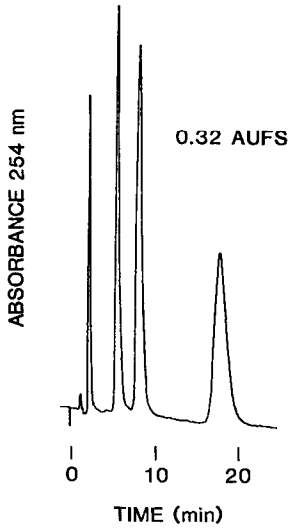

Fig. 2. Separation of aniline, N-ethylaniline, N,N-dimethylaniline and N,N-diethylaniline. Column: Phenyl Kel-F 6061 (20 μm), 150 × 4.6 mm I.D. Mobile phase: acetonitrile–0.01 *M* borate buffer pH 10 (55:45, v/v). Flow-rate: 0.7 ml/min. Each solute concentration was 88 ppm. From ref. 14.

the surface of the support [16]. Definite weak anion-exchange behavior for nitrate and *m*-chlorobenzoic acid was observed. When using this column in the reversed-phase mode at neutral pH, a preference for retention of organic cations such as *o*-nitroaniline ($pK_a = 10$) and neutral species such as *p*-chloroaniline ($pK_a = 4$) or phenol was found. Drugs of closely related structure such as pyridoxamine, pyridoxal, and pyridoxine could be separated even though column efficiency was modest because of the wide particle distribution.

Although it was easier to prepare organomagnesium reagents with a wide variety of functional groups for PCTFE derivatization, no significant advantage of using these PCTFE derivatives (such as phenyl PCTFE) instead of the organolithium-derivatized PCTFE as column packings was noted [13]. In fact, C_8 and C_{18} organomagnesium-derivatized PCTFE particles when packed into an HPLC column were not pressure stable due to the long alkyl chains on the PCTFE surface enhancing compaction of the particles. In general, organometallic functionalized PCTFE particles may be useful as adsorbents for the preconcentration of trace organics from water but are no longer competitive polymer-based HPLC column packings. Present work by us directed toward the cross-linking of PCTFE polymers on silica will be described in a later section.

2.2. Biomacromolecule separations

Unreacted PCTFE particles have been utilized as a column packing for the liquid chromatography of certain classes of biomolecules. Pearson *et al.* [17] coated PCTFE with Andogen 464, a trialkyl (C_8–C_{10}) methylammonium reagent to permit the preparative ion-exchange separation of transfer RNA molecules. Glass columns (240 cm × 1 cm I.D.) packed with 140–230 mesh PCTFE particles previously coated

with the quaternary ammonium compound (RPC-5) provided better separations than analogous columns packed with silylated diatomaceous earth. Since then, similar RPC-5 columns have been used for the separation of nucleosides [18], nucleotides [19] and DNA fragments [20]. Column lifetimes of the RPC-5 packing were limited by the "bleeding" of the surfactant. To avoid this problem, Usher [21] used uncoated PCTFE as a support for the analytical separation of DNA fragments and riboligonucleotides. Using $< 32\ \mu m$ particles packed in 30 × 0.4 cm columns, separations at least as good as the RPC-5 columns were possible (Fig. 3). A separation of 2′- and 3′-phosphate monoester isomers of heptauridylate was possible on the PCTFE column but not with a conventional C_{18} column. The DNA loading capacity of the PCTFE columns was restricted to μg quantities.

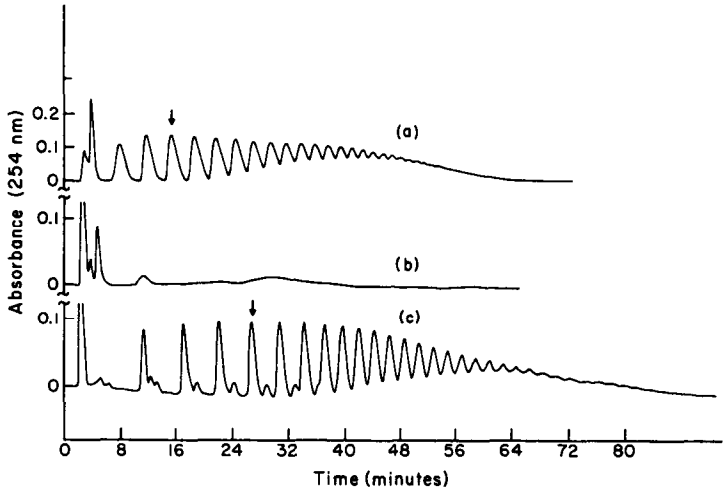

Fig. 3. Separation of oligouridylate 2′,3′-cyclic phosphates. (a) 30 × 0.4 cm column of Kel-F particles coated with Adogen 484. Mobile phase: 0.01 *M* Tris–HCl, pH 8.1 with a linear gradient of 0.005 to 0.05 *M* $KClO_4$ in 1 h. (b) as (a) but using a 30 × 0.4 cm column of uncoated Kel-F 82. (c) 30 × 0.4 cm column of uncoated Kel-F. Mobile phase: 0.1 *M* triethylammonium acetate (TEAA) (pH 6.8) with a 0.1 *M* TEAA in acetonitrile–water (11:89) gradient from 0–11%. The sample contained about 10% of the acyclic (2′ + 3′) phosphates U_nU_p. The arrow indicates the hexamer $U_5U > p$. From ref. 21.

The hydrophobic attraction of proteins to fluoropolymers has been documented through immobilization studies as well as chromatography. Both the enzymes urease immobilized on PTFE film [22] and lactate dehydrogenase immobilized on PTFE particles in a column [23] showed enzymatic activity and resistance to leaching by aqueous solutions. The capacity of 5–10-μm PTFE particles was approximately 0.7 mg of enzyme/g with about 4% retention of activity. A more extensive analytical study of a variety of enzymes immobilized on PCTFE particles packed in short 5–10-cm columns has also been reported [24]. The extent of immobilization of enzymes on PCTFE such as urease, glutamate dehydrogenase, and alcohol dehydrogenase was 70–85% similar to that capacity and retention of activity found for PTFE. Using a flow-rate of 0.2 ml/min and a urease–glutamate dehydrogenase column, urea in serum after removal of the protein could be assayed. Lifetimes of the columns were 1–2

months or about 100 injections. Alkaline pH solutions required for optimum activity of dehydrogenase enzymes were not a problem. Flow-rates on the order of 1–1.5 ml/min did not displace any enzyme and Hjertén [25] has also noted increasing the pH or ionic strength did not displace plasma protein adsorbed to PTFE during hydrophobic chromatography.

Some success has been shown using either PCTFE or functionalized PCTFE polymer columns for the separation of proteins [12]. A successful separation of insulin, ribonuclease A, lysozyme, myoglobin and ovalbumin was accomplished in 12 min under reversed-phase conditions on a 10-cm column packed with 6–7-μm PCTFE particles. Slightly better resolution of some peak pairs was possible using a butyl-PCTFE derivative. A weak cation-exchange column, PCTFE-S-CH_2COO^-, retained the proteins longer, especially ovalbumin. Williams *et al.* [26] evaluated a proprietary fluorocarbon PTFE-like material as an HPLC packing for the separation of peptides and proteins. These 25-μm polymer particles were porous with an average pore diameter of 330 Å and a surface area of 5 m^2/g. Good gradient separations of various peptide and protein mixtures under acidic or basic conditions were shown (Fig. 4). Lifetime of the column was fine with a maximum pressure stability of 2200 p.s.i. and a normal working back pressure of only 150 p.s.i.

3. FLUORINATED BONDED PHASES ON SILICA

The following section describes fluorinated liquid phases generally bonded to silica particles via silane reactions. Both aliphatic and aryl phases have been developed primarily for the separation of aromatics. Only a few applications of fluorinated bonded phases for large biomolecules have been made.

3.1. Small-molecule separations

Fluorinated silica was prepared by reacting (diethylamino) sulfur trifluoride with silica for eventual investigation as a column packing for normal-phase LC [27]. Approximately 30–50% of the OH groups were replaced by fluorine atoms corresponding to a coverage of 400–600 μmol/g. Retention of organic bases such as pyridine was longer on fluorinated silica as compared to silica itself. Hydrolysis of the Si–F bond by water diminishes the lifetime of these columns, however, performance could be maintained for about a month.

The first report of a fluorinated bonded phase described the preparation of (heptadecafluorodecyl) ($CF_3(CF_2)_7CH_2CH_2$–)-silica from the corresponding dimethylchlorosilane [28]. This column packing (HFD) was compared to the propyl (RP-3) and the *n*-decyl (RP-10) hydrocarbon analogues. Selectivity (α) of the HFD phase for various pairs of fluorinated and hydrocarbon compounds was most similar to the RP-3 materials. Retention of fluorine containing compounds such as perfluorooctanol, was greater on the HFD column than the hydrocarbon columns. Benzene and fluorobenzene could be separated on the HFD but again not on the alkyl phases. Improved separation of herbicides containing only one fluorine atom was also seen on the HFD column. In general, retention increased with more fluorine atoms in the molecule. A follow-up report gave a more detailed comparison of the HFD phase with C_{18} [29]. Retention data of nineteen solutes including fluorinated aromatics showed consistently lower values for the HFD column as compared to an RP-18

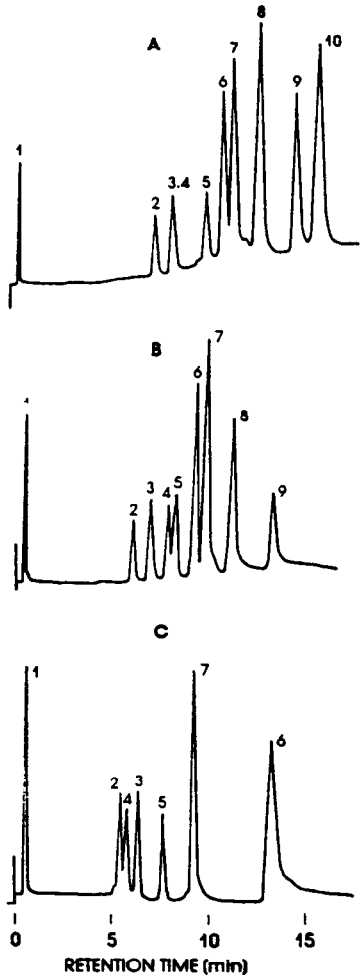

Fig. 4. Effect of mobile phase pH on separation of proteins. Column: 35-μm fluorocarbon resin, 8 cm × 6.2 mm I.D. Mobile phase: Buffer to acetonitrile–buffer solution (70:30, v/v) in 20 min at 2 ml/min. Detection: UV at 220 nm. (A) 0.1% Trifluoroacetic acid, pH 2; (B) 0.1 *M* sulfuric acid, pH 1. (c) 0.1% ammonium hydroxide, pH 11. Peaks: 2 = enkephalin (leucine); 3 = enkephalin (methionine); 4 = insulin chain A; 5 = angiotensin I; 6 = ribonuclease; 7 = insulin; 8 = lysozyme; 9 = carbonic anhydrase; 10 = ovalbumin. From ref. 26.

column. This trend was not due to the shorter chain length of the fluorinated phase. Using multi-fluorine substituted aromatics, only hexafluorobenzene was retained longer on the HFD phase compared to the RP-18 phase. The use of trifluoroethanol in the mobile phase could mask any fluorine–fluorine interactions substantially reducing retention on the HFD column. Some non-fluorine containing solutes such as dimethylphthalate and acetophenone gave a longer retention on the HFD column. The separation of steroids using a similar aliphatic fluorinated column packing has been reported [30].

Normal-phase chromatography of polycyclic aromatic hydrocarbons (PAHs) using a perfluoroethoxyethanol bonded phase has been compared to that with a silica column [31]. Although retention was roughly a factor of 4 less on the fluorinated silica, fraction collection of a crude oil distillate indicated alkyl aromatics eluted in groups of different ring types. This weak retention was explained as being due to induction forces acting between the C–F dipoles and π electrons of the solutes.

Reversed-phase gradient elution of stereoisomers of polystyrene oligomers (average molecular weight 800) on fluorodecyl (HFD) bonded silica columns was investigated by Lewis *et al.* [32,33]. Although the oligomers could be separated, stereoisomers could not be effectively resolved. Aromatic phases such as ethylphenyl silica behaved in the same way. In contrast, alkyl phases ranging from C_1 to C_{18} when eluted with an acetonitrile–water to acetonitrile–methylene chloride gradient could separate the stereoisomers. Some improvement in resolution was achieved as the chain length increased.

A variety of pentafluoroaromatic phases have been synthesized and briefly characterized. Phenyl- and pentafluorophenyl (PFP) silica phases were compared, although both the coverage of silyl groups and plate count were somewhat less for the fluorophase [34]. Whereas a similar separation of benzene and naphthalene was observed on both columns, fluorobenzene, fluorotoluene, *p*-fluorophenol, and *p*-fluoroaniline were retained longer and with greater selectivity on the PFP column than the corresponding hydrocarbon analogues. In a similar study [35], retention of other halogen containing aromatics such as mono-, di- and trichlorobenzenes was enhanced on the PFP column. A pentafluorophenethyl-silica phase used in the normal-phase mode with hexane as the mobile phase separated PAHs according to the number of condensed rings, apparently through weak donor–acceptor interactions [36]. Nitrogen-containing aromatics were retained longer than the corresponding PAHs while sulfur aromatics were retained less. Studies describing the normal-phase separation of PAHs on pentafluoroanilinopropyl-silica found a similar retention order but broader peaks [37]. When the hexane mobile phase was saturated with water, a notable decrease in retention of the PAHs was observed. This could either be due to solvophobic effects or unreacted, exposed silanols. Both normal- and reversed-phase HPLC using a pentafluorobenzamidopropyl-silica column indicated preferred retention of PAHs in the latter mode [38].

A comparison of chiral *R*-N-(pentafluorobenzoyl)phenylglycine and the 3,5-dinitrobenzoylphenyl glycine phases was made [39]. It was speculated that the presence of the five fluorine atoms instead of the two nitro groups might make the benzoyl ring electron poor and influence the resolution of certain enantiomers based on π–π interactions. Larger α values of racemates containing nitrogen such as succinimides and hydantoins were found using the fluorinated phase. In contrast, smaller values were noted for alcohol racemates. A column packed with a mixture of the two phases gave acceptable α values for both nitrogen and hydroxy containing racemates.

A comparison of the PFP and HFD phases with the corresponding hydrocarbon analogues has been made [40]. The PFP phase gave larger capacity factors (k') for aromatics, halogenated aromatics, and PAHs than the phenyl phase using ethanol–water (70:30). This is due to donor–acceptor complex formation similar to that found for a tetranitrofluorenone phase. Although ligand density was greater with the fluorinated aliphatic phase than the aliphatic hydrocarbon phase, the former phase

gave no advantage in the separation of small molecules or PAHs. Lower solute retention on the fluorinated phase was attributed to the rigidity of the C–F chains producing a "brush-like" network which limited solute contact sites.

The retention properties of three fluorinated phases, HFD, heptafluoroisopropoxypropyl (HFIPP) and PFP were compared with a C_{10} alkyl phase using 42 test solutes consisting primarily of substituted benzenes [41]. Based on plots of ln k' *vs.* carbon number for a homologous series of substituted aromatics, the three fluorinated phases showed less affinity for the methylene group than the C_{10} phase. The substituent factor, $\Delta\Gamma$, proportional to ln k'_x/k'_b where k'_x = substituted benzene solute and k'_b = benzene, was calculated to compare the retention ability of each of the four columns. Substituted aromatics with OH, NO_2, CN, F, CHO, OCH_3, or COOH groups were retained preferentially on the fluorinated phases as compared to the C_{10} phase. Surprisingly, selectivity for the separation of halogenated aromatics was greater with the C_{10} column. About 10 changes in elution sequence of the solutes occurred on the fluorinated phases compared to the sequence on the C_{10} column. The unblocked silanols on the HFD phase were more prevalent than the C_{10} phase and could play a role in the retention of hydrogen bonding species.

The comparison of these three fluorinated bonded phases with the C_{10} phase was extended to consider the effect of different methanol–water mobile phases [42]. The retention order of the methylene increment as a function of methanol content in the mobile phase is C_{10} > HFD > HFIPP ≫ PFP. The $\Delta\Gamma$ values were quite constant with mobile phase composition for some polar substituents such as –OH and –CN. These trends were consistent with all the columns. For non-polar substituents, the C_{10} column provided the best retention followed by the PFP, HFD and HFIPP columns. The PFP column also retained polar substituted aromatics well but the C_{10} column was judged most selective. The HFD column was more susceptible to attack by strongly basic mobile phases than the C_{10} column. Again the possibility exists that the rigid "brush-like" character of this stationary phase does not allow sufficient interaction between chains to form a "protective blanket" on the surface.

The HFD and the F-decyl $C_{10}F_{21}C_2H_4$ columns have been compared with the C_{18} column for the separation of a series of perfluoro-alkyliodides, -iodoethanes and -ethenes [43]. Retention times of these compounds were consistently longer on the fluorinated phases compared to the hydrocarbon phase. Plots of log k' *versus* carbon number indicated excellent linearity allowing calculation of the transfer energy per CF_2 group, ΔG^0. The ΔG^0 values were about 1 kJ/mol for the fluorinated phases compared to only 0.35 kJ/mol for the C_{18} column. Better retention and separation of fluorinated surfactants such as fluoroalkylmaltosides and fluoroalkyl propionates were found using the HFD and fluorodecyl phases.

Several fluorinated phase columns such as the PFP, HFD, fluoroether and fluoropropyl types are commercially available [44]. Using the Chromegabond PFP column, nine vanillin analogues could be separated in 20 min but incomplete resolution of the same mixture using a phenyl column was found (Fig. 5). Components of a blood root extract were also effectively resolved on the HFD column.

3.2. Biomacromolecule separations

Because fluorinated aliphatic phases generally retain non-polar solutes less than the corresponding hydrocarbon phases, it was proposed that proteins could be eluted

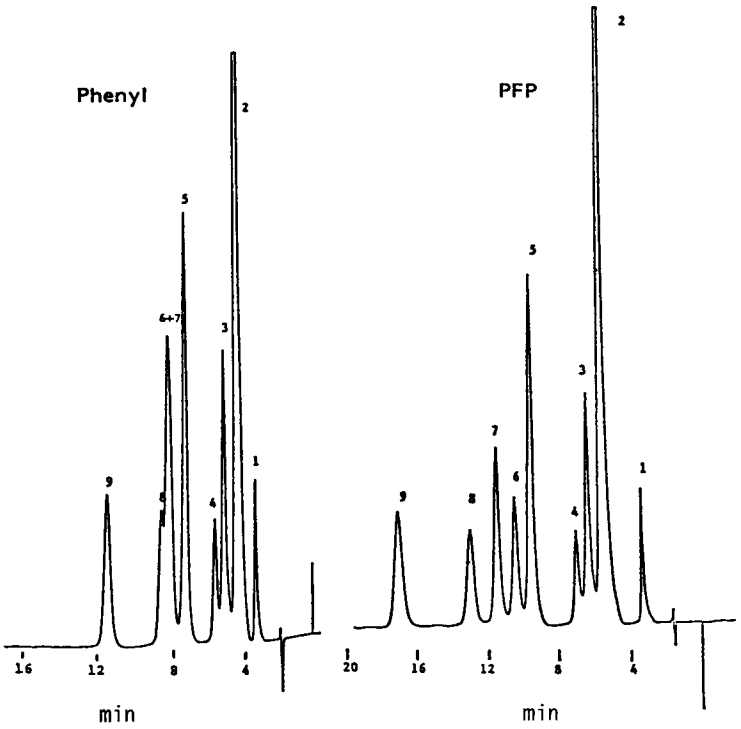

Fig. 5. Comparison of phenyl and PFP silica columns for the separation of vanillin analogues. Column: 15 × 1.6 mm, 5-μm particles. Mobile phase: methanol–0.1% orthophosphoric acid (40:60). Flow-rate: 1 ml/min. Detection: UV at 254 nm. Peaks: 1 = vanillyl alcohol; 2 = vanillic acid; 3 = *p*-hydroxybenzoic acid; 4 = syringic acid; 5 = vanillin; 6 = syringaldehyde; 7 = acetovanillone; 8 = acetosyringone; 9 = ethylvanillin. From ref. 44.

with less organic solvent. This would be an advantage in the case of proteins where high concentrations of organic solvent diminish recovery of biologically active species. Xindu and Carr [45] first reported such a study comparing the reversed-phase separation of proteins on the HFD column and the C_{10} hydrocarbon column. Retention of the proteins ribonuclease, insulin and cytochrome *c* under the same mobile phase conditions was about half that for the same separation on the C_{10} column (Fig. 6). Selectivity of the C_{10} column for these proteins plus lyoszyme, bovine serum albumin and thyroglobulin as a function of increasing isopropanol was generally superior. The more hydrophobic the protein, the greater observed k' difference for the two types of columns. Interestingly, thyroglobulin could only be eluted with a k' of 60 or greater on the hydrocarbon column compared to a k' of 5.37 for the HFD phase using 32% isopropanol. Plots of log k' *versus* % isopropanol from 20–32% for ribonuclease, insulin and lysozyme were curved with steeper slopes for the C_{10} column. Recoveries of the protein were generally near 100% for the HFP column but only 80% for the C_{10} column.

Recently a trifluoroacetamido silyl phase, $-(CH_2)_3NHC=OCF_2CF_3$, has been shown to be useful for the characterization of papain [46]. During reversed-phase

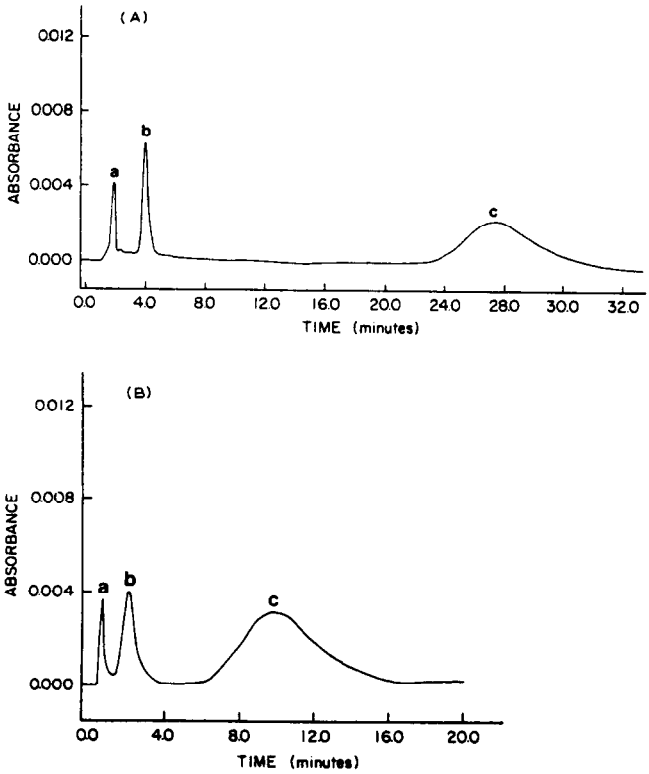

Fig. 6. Chromatograms of protein mixture using (A) C_{10} and (B) HFD columns (50 × 4.6 mm). Mobile phase: isopropanol–0.05 *M* KH_2PO_4 (19:81), pH 3.0. Temperature: 47°C. Proteins: a = ribonuclease; b = insulin; c = cyctochrome *c*. From ref. 45.

separation, a significant percentage of the protein remains in the active form. We have prepared a weak anion exchange phase by cross-linking a fluorocarbon co-polymer with piperazine and then reacting it with amino silica. The resulting stable packing should have some reversed-phase character as well permitting mixed retention mode separations of peptide and proteins with good recovery [47].

Finally, a dual zone material (DZM) that permits direct injection of serum for drug analysis has been developed using a mixed fluorocarbon–hydrocarbon phase [48]. Perfluorobutylethylenedimethylsilyl (PFB) groups were bonded on the external particle surface to minimize adsorption of lipophilic solutes such as blood serum protein while octadecyl chains were bonded inside the pores to permit retention of the small drug molecules. Using phenobarbital and carbamezipine as test solutes, lifetime of the DZM column was about four times longer than a control column tolerating 4000 injections.

In the future, it is expected that the use of fluorocarbon polymers or silica bonded fluorinated phase columns will be directed toward the separation of biological samples. With the emphasis on analysis of smaller and valuable samples, complete recovery of proteins or other biomacromolecules is critical. In addition, mixed fluorocarbon and hydrocarbon phases for certain applications should continue to be developed.

REFERENCES

1 P. Varughese, M. E. Gangoda and R. K. Gilpin, *J. Chromatogr. Sci.*, 26 (1988) 401.
2 F. P. Dousek and J. Jansta, *C.A.*, 89 (1978) 181874; 90 (1979) 7777a; and 90 (1979) 188217m.
3 Z. Plzak, F. P. Dousek and J. Jansta, *J. Chromatogr.*, 147 (1978) 137.
4 V. Patzelova, J. Jansta and F. P. Dousek, *J. Chromatogr.*, 148 (1978) 53.
5 E. Smolkova, J. Zima, F. P. Dousek, J. Jansta and Z. Plzak, *J. Chromatogr.*, 191 (1980) 61.
6 T. A. Zwier and M. F. Burke, *Anal. Chem.*, 53 (1981) 812.
7 K. J. Saunders, *Organic Polymer Chemistry*, Chapman & Hall, London, 1973, p. 143.
8 J. A. Huth and N. D. Danielson, *Anal. Chem.*, 54 (1982) 930.
9 N. D. Danielson, R. T. Taylor, J. A. Huth, R. W. Siergiej, J. G. Galloway and J. B. Paperman, *I&EC Prod. Res. Dev.*, 22 (1983) 303.
10 A. J. Dias and T. J. McCarthy, *Macromolecules*, 18 (1985) 1826.
11 S. Wu, *Polymer Interface and Adhesion*, Marcel Dekker, New York, 1982, p. 280.
12 L. B. Beaver, *Ph.D. Dissertation*, Miami University, Oxford, OH, 1987.
13 N. D. Danielson, S. Ahmed, J. A. Huth and M. A. Targove, *J. Liq. Chromatogr.*, 9 (1986) 727.
14 R. W. Siergiej and N. D. Danielson, *Anal. Chem.*, 55 (1983) 17.
15 R. W. Siergiej and N. D. Danielson, *J. Chromatogr. Sci.*, 21 (1983) 362.
16 M. Kruempelman and N. D. Danielson, *Anal. Chem.*, 57 (1985) 340.
17 R. L. Pearson, J. F. Weiss and A. D. Kelmers, *Biochim. Biophys. Acta*, 228 (1971) 770.
18 R. P. Singhal, *Biochim. Biophys. Acta*, 319 (1973) 11.
19 B. Z. Egan, *Biochim. Biophys. Acta*, 299 (1973) 245.
20 M. Bina, M. F. Radonovich and B. A. Roe, *Anal. Chem.*, 114 (1981) 105.
21 D. A. Usher, *Nucleic Acids Res.*, 6 (1979) 2289.
22 J. H. Fishman, *U.S. Pat.*, 3 843 443 (1974).
23 N. D. Danielson and R. W. Siergiej, *Biotech. Bioeng.*, 23 (1981) 1913.
24 N. D. Danielson, T. M. Bossu and M. Kruempelman, *Anal. Lett.*, 15 (1982) 1289.
25 S. Hjertén, *J. Chromatogr.*, 159 (1978) 47.
26 R. C. Williams, J. F. Vasta-Russell, J. L. Glajch and K. Golebiowski, *J. Chromatogr.*, 371 (1986) 63.
27 M. K. L. Bicking, M. L. Bicking and W. E. Longman, *Anal. Chem.*, 58 (1986) 501.
28 G. E. Berendsen, K. A. Pikaart, L. de Galan and C. Olieman, *Anal. Chem.*, 52 (1980) 1990.
29 H. A. H. Billiet, P. J. Schoenmakers and L. de Galan, *J. Chromatogr.*, 218 (1981) 455.
30 W. Li and J. Wang, *Kexue Tongbao*, 26 (1981) 1214; *C.A.*, 96 (1982) 52376.
31 W. Ecknig, B. Trung, R. Radeglia and U. Gross, *Chromatographia*, 16 (1982) 178.
32 J. J. Lewis, L. B. Rogers and R. E. Pauls, *J. Chromatogr.*, 264 (1983) 339.
33 J. J. Lewis, L. B. Rogers and R. E. Pauls, *J. Chromatogr.*, 299 (1984) 331.
34 F. M. Rabel and E. T. Butts, *LC·GC*, 1 (1983) 42.
35 E. Csato, N. Fulop and G. Szabo, *J. Chromatogr.*, 511 (1990) 79.
36 G. Felix and C. Bartrand, *J. High Resolut. Chromatogr. Chromatogr. Commun.*, 9 (1987) 411.
37 G. Felix, A. Thienpont and C. Bartrand, *Chromatographia*, 23 (1987) 684.
38 G. Felix and C. Bartrand *J. High Resolut. Chromatogr. Chromatogr. Commun.*, 8 (1985) 362.
39 J. Kip, P. van Haperen and J. C. Kraak, *J. Chromatogr.*, 356 (1986) 423.
40 A. Haas, J. Kohler and H. Hemetsberger, *Chromatographia*, 14 (1981) 341.
41 P. C. Sadek and P. W. Carr, *J. Chromatogr.*, 288 (1984) 25.
42 P. C. Sadek, P. W. Carr and M. J. Ruggio, *Anal. Chem.*, 59 (1987) 1032.
43 M.-P. Krafft, F. Jeannaux, M. LeBlanc, J. G. Reiss and A. Berthod, *Anal. Chem.*, 60 (1988) 1969.
44 E. S. Industries, *Application Notes*, Marlton, NJ.
45 G. Xindu and P. W. Carr, *J. Chromatogr.*, 269 (1983) 96.
46 B. E. Boyes and J. J. Kirkland, presented at the *14th International Symposium on Column Liquid Chromatography, Boston, MA, May 20–25, 1990.*
47 J. Wangsa and N. D. Danielson, presented at the *200th American Chemical Society Meeting, Washington, DC, August 29, 1990.*
48 D. W. William and P. M. Kabra, *Anal. Chem.*, 62 (1990) 807.