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Review

Fluorocarbon stationary phases for liquid chromatography applications

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Dedicated to Prof. Dennis P. Curran on the occasion of receiving the 2008 American Chemical Society Award for Creative Work in Fluorine Chemistry.

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ABSTRACT

This article presents an overview on fluorocarbon stationary phases for liquid chromatography (LC) applications. Fluorocarbons developed as alternative reverse phases have revealed previously unknown separation mechanisms and special utilities. Solvophobicity and fluorophilicity of the fluorinated phases provide enhanced selectivity for organofluorine compounds. The dual normal- and reverse-phase characteristics make fluorinated phases suitable for analysis of polar pharmaceutical and biological samples such as proteins, peptides, nucleotides, steroids, and alkaloids. Fluorinated phases for other applications including supercritical fluid chromatography (SFC), micellar electrokinetic liquid chromatography (MEKC), ion chromatography (IC), open tubular electrochromatography (OTEC), and liquid chromatography–mass spectrometry (LC–MS) are also highlighted.

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1. Introduction

Organofluorines have a number of unique properties such as their small size, high electronegativity, low polarizability, strong

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lipo- and hydrophobicity, and good thermal and chemical stability [1]. Some of these properties have been exploited in the design of fluorinated alkyl and phenyl stationary phases. The fluorinated phases have offered many utilities that could not be accomplished by conventional C_8, C_{18}, and phenyl reverse phases [2].

Over the years, following major advancements have been made in the development of fluorinated phases for liquid chromatograph (LC) applications. (1) The first paper that described the preparation of fluorinate phase was reported by the de Galan group in 1980. The strength of the fluorinated phase for separation of fluorinated compounds from the corresponding non-fluorinated analogs was discovered [3,4]. (2) In 1983, Xindu and Carr reported the application of C_8F_{17} phase for separation of proteins using high concentrations of organic modifier in the mobile phase [5]. The so-called "U-shape" retention of fluorinated phases was recognized and later applied to the separations of other polar analytes such as peptides, nucleotides, steroids, and alkaloids [2b]. It has also been used for liquid chromatography–mass spectrometry (LC–MS) analysis of basic pharmaceutical and bioorganic samples [6]. (3) In 2001, the Curran group introduced the fluorous mixture synthesis (FMS) technique and used the fluorinated phases for separation of synthetic mixtures containing different lengths of fluorous tags [7]. (4) In the last decade, a number of research groups extended the application of fluorinated phases for supercritical fluid chromatography (SFC) [8], micellar electrokinetic liquid chromatography (MEKC) [9], ion chromatography (IC) [10], open tubular electrochromatography (OTEC) [11], and liquid chromatography–mass spectrometry [12]. Better understanding of separation mechanisms and discovery of new applications for fluorinated phases continuously attract much attention of scientists from analytical, synthetic, and biochemical areas.

This article discusses some selected papers on fluorinated phases for analysis and prep-scale HPLC separations. Extended applications of fluorinated phases for other chromatography and related techniques are also highlighted.

2. Special characteristics of fluorinated stationary phases

Starting from the early 1980s, the research work on fluorinated phases was directed towards the development of modified reverse phases for alternative retention and selectivity. A series of fluoroalkyl and fluorophenyl phases have been prepared and systematically compared with the traditional C_8, C_{18}, and phenyl phases [2]. Some major differences between fluorinated and non-fluorinated phases have been observed and highlighted as follows [13]:

(1) for common hydrocarbon molecules, fluorinated phases exhibit much weaker retention than corresponding non-fluorinated phases;
(2) fluorinated compounds usually have much stronger retention on the fluorinated phases than on the non-fluorinated phases;
(3) a mixture of fluorinated compounds can be resolved on the fluorinated phase according to its fluorine content;
(4) mixtures containing both fluorinated and non-fluorinated compounds might be separated on the fluorinated phases;
(5) high levels of organic solvent in the mobile phase can increase the retention of many polar and basic compounds;
(6) fluorinated phases have improved performance for many chromatography methods including supercritical fluid LC, micellar LC, ion chromatography, electrochromatography, and LC–MS;
(7) fluorinated phases usually have better reproducibilities and longer lifetimes than the non-fluorinated phases.

The significant differences between fluorinated and non-fluorinated stationary phases have provided strong indications that fluorinated phases are not simply the modified reverse phases. They could have different separation mechanisms and a great potential for a series of new applications.

2.1. Solvophobicity and fluorophilicity of fluorinated phases

Traditional reverse phases are designed to operate primarily by hydrophobic interaction and exhibit minimal polar interaction. The less polar molecule has a longer retention time and the more polar molecule has a short retention time. Fluorinated phases could have different separation mechanisms depending on what kind of analytes they are dealing with. Fluorinated phases are solvophobic towards organic and aqueous solvents. Common lipophilic and hydrophilic analytes have low partition coefficiency in the fluorinated stationary phases, so the non-fluorinated compounds have short retention times. If the analyte is a fluorine-containing molecule, the solvophobicity has a tendency to repulse the fluorinated compound away from the mobile phase (such as MeOH–H_2O or MeCN–H_2O) and allows it to participate into the fluorinated stationary phase. The specific fluorine–fluorine interaction between the analyte and the stationary phase results in a strong retention. The analyte containing more fluorine atoms is more fluorophilic and has a longer retention [14]. However, since silica gel-based fluorinated phases have a significant amount of silanol residues, high concentrations of organic modifiers in the mobile phase can make a fluorinated phase exhibit normal-phase-type character to provide strong retention to non-fluorinated polar molecules, especially for the basic molecules, since silanol residues are acidic.

2.2. "U-shape" retention profile of polar analytes on fluorinated silica gels

The commercially available fluorous silica gels, including the end-capped versions, usually have greater than 50% free silanol residues [15], just like the C_{18} reverse-phase silica gels. These silanol groups could act as a normal phase to retain polar compounds. The fluorinated phase has a so-called "U-shape" retention profile (Fig. 1) [12]. At the left part of the "U shape" the retention of a polar solute is decreased with the increase of organic content (MeOH or MeCN) in the mobile phase, which is a typical reverse-phase behavior. However, after the bottom point of the "U shape", the polar compound gets longer retention when the organic content in the mobile phase is further increased. At the right part of the "U shape", the stationary phase shows normal-phase-type property and provides strong retention for the polar solutes. The mechanism for the "U-shape" retention is still under active investigation. One

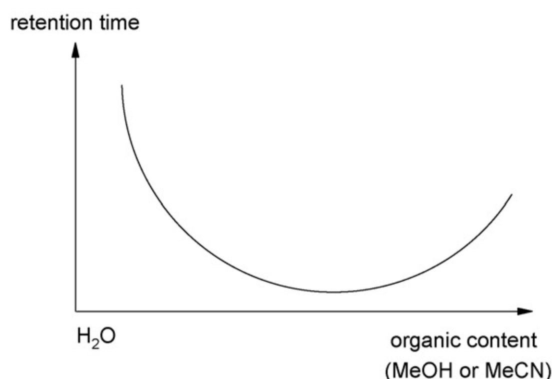


Fig. 1. "U-shape" retention of a basic compound on the fluorinated stationary phase.

Table 1
Some commercially available fluorinated phases

Brand name endcap	Provider	Bonding phase	Particle size (μm)		Pore size (Å)
Fluoro*Flash*	FTI[a]	C_8F_{17}	5	60	No
FluoroSep-RP Octyl	ES	C_8F_{17}	5	60	
Fluofix	Wako	C_6F_{13}-branched	5	120, 300	No, yes
	Thermo		5	100, 300	Yes
Fluophase RP/WP	Thermo	C_6F_{13}	5	100, 300	Yes
Tridecafluoro	Silicycle		3, 5	100, 120	
FluoroSep-RP Phenyl	ES	C_6F_5	3, 5	60	Yes
Fluophase PFP	Thermo		5	100	
Discovery F5 HS	Supelco		3, 5	120	
Pentafluorophenyl	Silicycle			100, 120	
FluoroSep-RP Propyl	ES	C_3F_7	5	300	
Fluorochrom	Silicycle	Non-specified	3, 5	100, 120	

[a] FTI—Fluorous Technologies, Inc.

possible explanation is that when the mobile phase has high organic content, the fluorinated phase is wetted and more free silanols are accessible by the polar solutes and shows hydrophobic ion-exchanger property. The "U-shape" retention has also been observed on some non-fluorinated reverse phases, but it is much less significant than on the fluorinated phases. The "U-shape" retention on fluorinated phases has special utilities for LC–MS analysis of basic and polar pharmaceutical and biological molecules [5,12]. These compounds have good retentions on the fluorinated phases at high organic content and low buffer concentrations, which are the favorable conditions for ESI-MS analysis.

3. Commercially available fluorinated stationary phases

Numbers of silica gel-based fluorinated phases such as C_8F_{17}, C_6F_{17}, C_6F_{13}-branched, C_6F_5, and C_3F_7 are commercially available (Table 1). Among them, the Fluoro*Flash* and FluoroSep-RP columns have the C_8F_{17} phase. The C_8F_{17} columns have stronger retention than the less fluorinated columns for separation of fluorinated solutes. In addition to analysis of fluorinated compound mixtures, C_8F_{17} columns have been used in fluorous mixture synthesis (FMS) for separation of small molecules and oligomers. Similar C_8F_{17} silica gels have been used for solid-phase extractions (F-SPE) of fluorous compounds [16].

4. Fluorinated phases for HPLC analysis

Fluorinated stationary phases including perfluoroalkyl- and perfluorophenyl-functionalized silica gels, and some polymer-supported fluorinated beads have been developed for HPLC analysis. A typical mobile phase is a MeOH–H_2O gradient, similar to that used in reverse-phase HPLC. Other solvents such as MeCN or THF can be used to replace MeOH for gradient elution.

4.1. Perfluoroalkyl phases

In 1980, the de Galan group reported the preparation of fluorinated phase (heptadecafluorodecy1)dimethylsilyl ($C_8F_{17}CH_2CH_2(Me)_2Si$–) for HPLC applications [3]. The utility of this C_8F_{17} column was demonstrated in the separation of a mixture of three herbicides: Mataven, Barnon, and Suffix. Mataven and Barnon both contain a fluorine atom ortho to a chlorine atom, whereas Suffix contains two chlorine atoms but no fluorine atom. On the C_8F_{17} column with a 65:35 MeOH–H_2O mobile phase, three compounds were well separated from each other in 10 min. This kind of resolution could not be achieved on a decyl (C_{10}) column (Fig. 2).

The de Galan group also employed the C_8F_{17} column with a 60:40 MeOH–H_2O mobile phase to separate a mixture containing

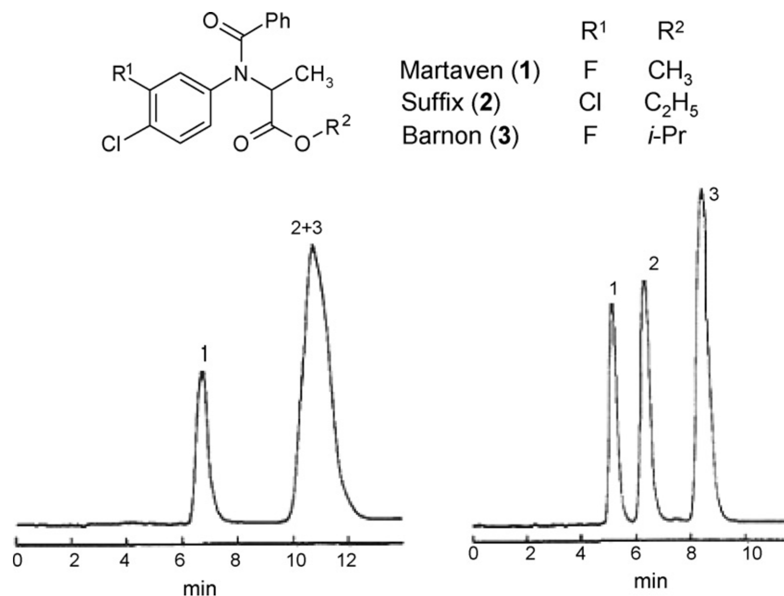


Fig. 2. Chromatograms of three herbicides on C_{10} (left) and C_8F_{17} (right) columns. From Ref. [3].

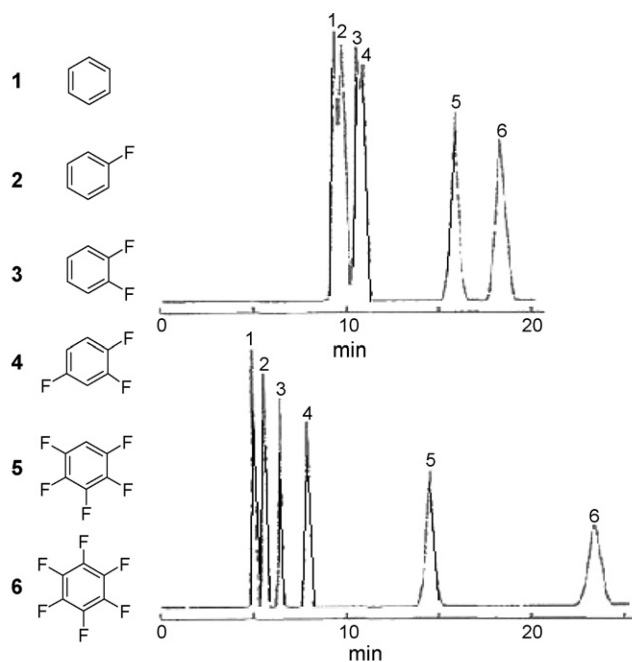


Fig. 3. Chromatograms of a 6-component mixture on C_{18} (top) and C_8F_{17} (bottom) columns. From Ref. [4].

benzene and five fluorinated analogs [4]. A nice baseline separation was achieved and the peaks were eluted in the order of increasing fluorine content (Fig. 3). Such a resolution could not be achieved on a C_{18} column. The different behavior of C_{18} and fluorinated columns indicated that for fluorinated molecules, these two systems are not iso-eluotropic; the fluorine–fluorine interaction is a dominant factor for separation of mixtures containing fluorinated compounds by the fluorinated phase.

To further elucidate the nature of the specific effects observed for both fluorinated and non-fluorinated molecules, 2,2,2-trifluoroethanol (TFE) as the organic modifier was added to the mobile phase [4]. With a mobile phase of TFE–water (40:60), the elution order of benzene and its fluorinated analogs on a C_{18} column was reversed. Trifluorobenzene was eluted first, followed by other solutes overlapped as a single peak. This work demonstrated that the fluorine–fluorine interactions could be controlled, either to enhance the separation of fluorinated molecules on a C_8F_{17} column, or to reverse the separation order by using a C_{18} column with TFE in the mobile phase.

Since the de Galan group's pioneering work, other groups have also made significant contributions to the development of other kind of perfluoroalkyl phases and also on mechanism studies [2].

4.2. Pentafluorophenyl phases

In addition to dispersive interactions available on traditional alkyl phases [2k], the pentafluorophenyl (PFP) phase also allows for dipole–dipole, π–π, charge transfer, and ion-exchange interactions [2a]. The PFP phase has lower fluorine content than some perfluoroalkyl columns such as C_8F_{17} and C_6F_{13}, and may not always provide best results for separation of fluorinated compounds. It has special selectivity for aromatic compounds. It was found that fluorobenzene, fluorotoluene, *p*-fluorophenol, and *p*-fluoroaniline were retained longer and with greater selectivity on the PFP column than on the phenyl column. In a similar study [17], the retention of the halogen-containing aromatics such as mono-, di- and trichlorobenzenes was enhanced on the PFP column. The PFP phase gave larger capacity factors for aromatics, halogenated aromatics, and polycyclic aromatic hydrocarbons than the phenyl phase, due to donor–acceptor complex formation [18]. A comparison of the PFP phase with the corresponding phenyl phase for the separation of a mixture of 9 vanillin analogs has been made [2a]. All components were separated on the PFP column within 20 min (Fig. 4).

Another important application of the PFP phase is for separation of polar compounds at high concentrations of organic modifiers in the mobile phase. The Clark group separated a mixture of 10 basic solutes using MeCN and a phosphate buffer as a mobile phase (Fig. 5) [19].

4.3. Polymer-based fluorinated phases

The development of polymer-based fluorinated phases could provide silanol-free packing materials. The Hirayama group reported the preparation of porous perfluorooctyl (C_8F_{17}) spherical polymer beads for HPLC packing [20]. The fluorinated beads were prepared by suspension copolymerization of heptadecafluorodecyl acrylate with divinyl monomers as a cross-linking agent. The fluorine content was easily adjustable by changing the monomer

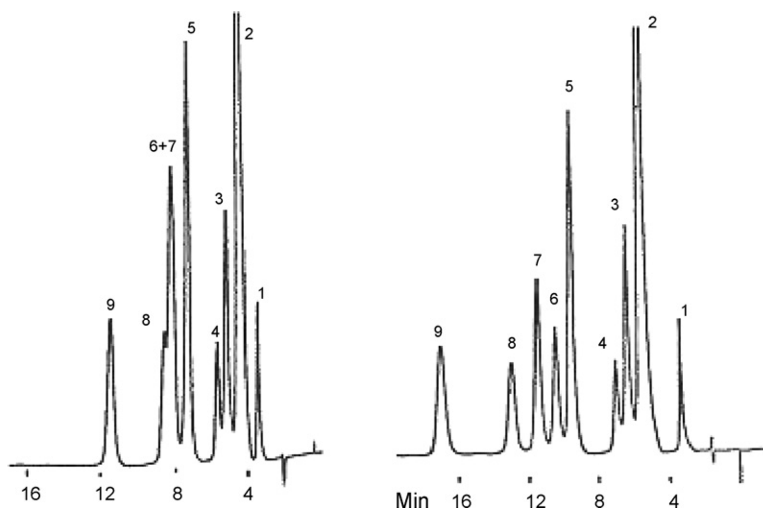


Fig. 4. Chromatograms of vanillin analogs on phenyl (left) and PFP (right) silica columns. Peaks: (1) vanillyl alcohol; (2) vanillic acid; (3) *p*-hydroxybenzoic acid; (4) syringic acid; (5) vanillin; (6) syringaldehyde; (7) acetovanillone; (8) acetosyringone; (9) ethylvanillin. From Ref. [2a].

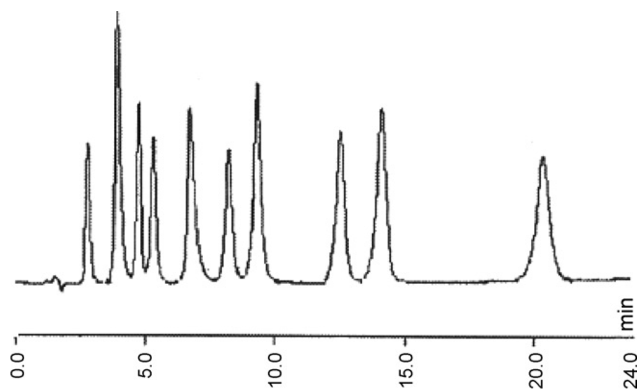


Fig. 5. Chromatogram of a 10-component basic mixture on a FluoroSep-RP (C_6F_5) column. The mixture includes (1) procainamide; (2) norpsudoephedrine; (3) procaine; (4) quinidine; (5) lidocaine; (6) alprenolol; (7) propranolol; (8) diphenhyldramine; (9) nortriptyline; (10) asmitriptyline. From Ref. [19].

ratio. The packing material showed efficient separation for fluorine-containing compounds including 5-fluorouracil and uracil, a mixture of fluorinated steroids. In addition, the fluorinated beads were found to be highly acid- and alkali-resistant (Fig. 6). They showed no abnormal adsorption for ionic compounds and no excessive retention of large hydrophobic compounds. These favorable properties are resulted from the low surface energy of the higher fluorine content.

In addition to direct polymerization of fluorinated monomers, fluorinated polymers can be prepared by the reduction of Teflon (polytetrafluoroethylene, PTFE) or polychlorotrifluoroethylene (PCTFE) using alkali-metal amalgams. The reduced particles can be further functionalized and used for HPLC packing. The Pearson group coated PCTFE with a trialkyl (C_8–C_{10}) methylammonium reagent and used it for ion-exchange separation of transfer RNA molecules [21]. Quaternary ammonium compound coated PCTFE particles provided better a separation for analysis of nucleosides, nucleotides, and DNA fragments [22–24]. The Williams group evaluated a proprietary fluorocarbon PTFE-like packing material for HPLC separation of peptides and proteins [25]. Gradient separations of various peptide and protein mixtures under acidic or basic conditions were evaluated (Fig. 7).

5. Fluorinated phases for fluorous mixture synthesis

Other than analytical scale HPLC, fluorinated phases have been used for large-scale separation. The most important application is for fluorous mixture synthesis [26].

In 2001, the Curran group introduced the concept of FMS in the synthesis of solution-phase libraries [7]. FMS is able to produce individual pure compounds without the effort of deconvolution. In FMS, a set of substrates is individually attached to a corresponding set of homologous fluorous tags. The mixture of the tagged substrates is employed for multistep reactions. The resulting

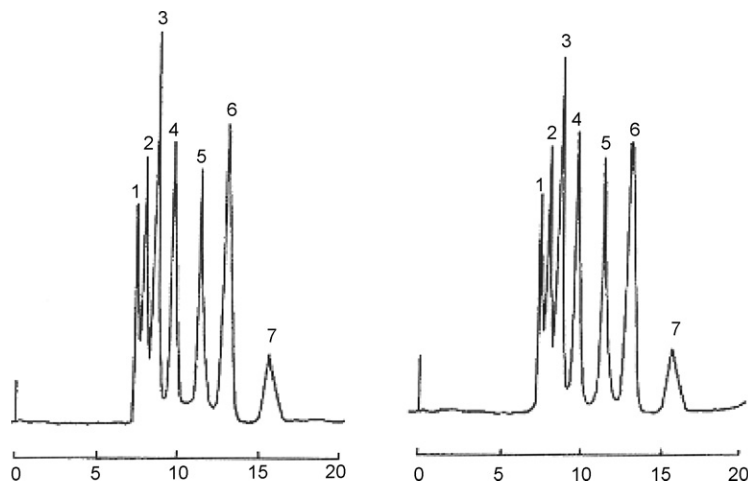


Fig. 6. Chromatograms of a 7-component fluorinated benzene mixture on a polymer C_8F_{17} column before (left) and after (right) treated with 0.1 M NaOH for 1 day. Peaks: (1) benzene; (2) fluorohenzene; (3) 1,2-difluorobenzene; (4) 1,2,4-trifluorohenzene; (5) 1,2,3,5-tetrafluorobenzene; (6) pentafluorobenzene; (7) hexafluorohenzene. From Ref. [20].

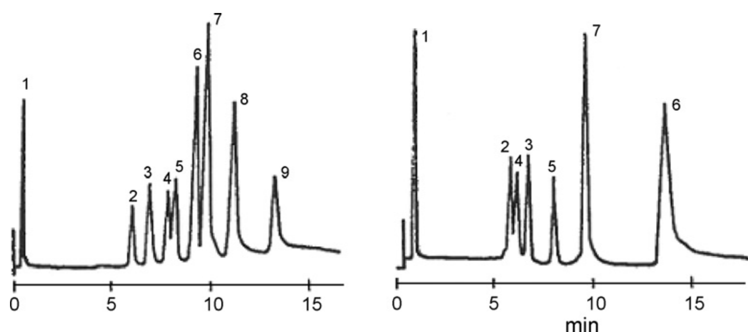


Fig. 7. Effect of mobile phase pH on separation of proteins on a polymer C_8F_{17} column with 0.1 M sulfuric acid, pH 1 (left) and 0.1% trifluoroacetic acid, pH 2 (right). Peaks: (2) enkephalin (leucine); (3) enkephalin (methionine); (4) insulin chain A; (5) angiotensin I; (6) ribonuclease; (7) insulin; (8) lysozyme; (9) carbonic anhydrase; (10) ovalbumin. From Ref. [25].

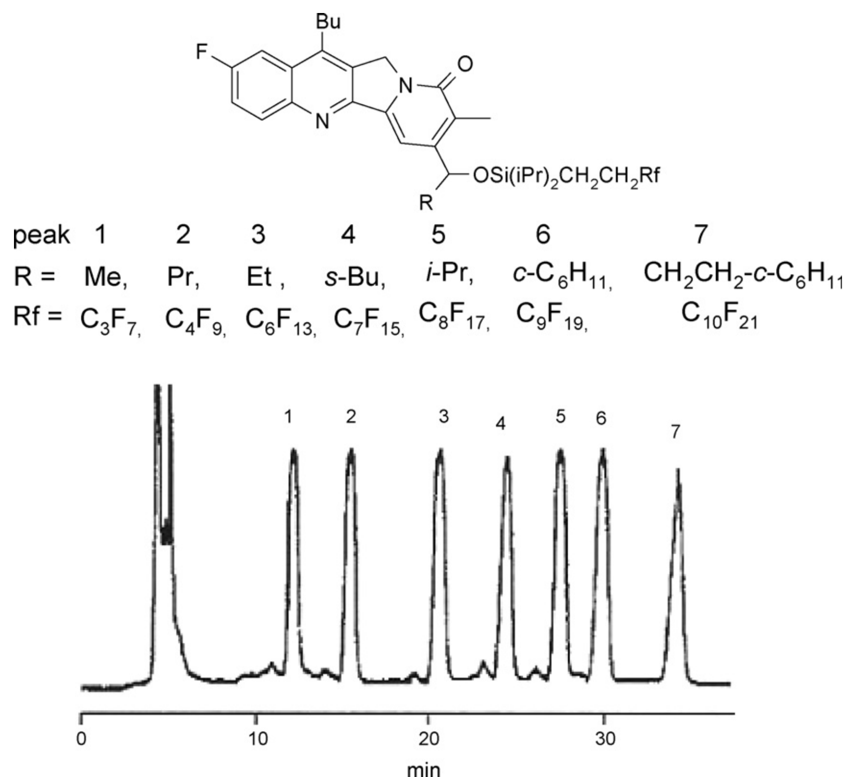


Fig. 8. Semiprep-scale HPLC demixing of a 7-component synthetic mappicine analogs on a FluroPhase-RP (C_6F_{13}) column. From Ref. [27].

tagged products are demixed by HPLC on a fluorinated phase to afford individual pure final products.

The power of FMS has been demonstrated in the preparation of 560 natural product mappicine analogs [27]. A 7-component mixture was used as the starting material for FMS to generate eighty mixtures after multistep synthesis. Each 7-component mixture was subjected to semiprep-scale HPLC to afford 560 demixed pure samples (Fig. 8).

Four analytical columns (1) Fluofix (C_6F_{13}-branched), (2) Fluophase-RP (C_6F_{13}), (3) Fluophase-PFP (C_6F_5), and (4) C_{18} columns have been evaluated for HPLC demixing of tagged mappicine analogs (Fig. 9) [27]. The Fluophase-RP and Fluofix columns have demonstrated comparably good separation. They both had a time window of 12 min between C_3F_7 and $C_{10}F_{21}$ peaks. The results indicated that the stationary phases have enough fluorine to induce fluorine-content-dominant separations. The Fluophase-PFP column had low resolution on C_7F_{15} and C_8F_{17} peaks. The small time window of 8 min indicated that the pentafluorophenyl phase does not have enough fluorine content, so it is sensitive to the polarity (or size) of the substrates. The reverse C_{18} column did not separate C_7F_{15}- and C_8F_{17}-tagged mappicines at all, because it only provided hydrophobic-based separation. The mismatch of tags and side chains (*s*-Bu/C_7F_{15} *vs.* *i*-Pr/C_8F_{17}) yielded poor resolution.

In another FMS to make a drug-like heterocyclic compound library, a new protocol using four Fluoro*Flash* (C_8F_{17}) columns was developed for parallel HPLC analysis [28]. Every round, four mixture samples each having five components was simultaneously injected into four columns, resulting in a total of 20 compounds in 5 min (Fig. 10). The efficiency of FMS was dramatically improved compared to single column analysis or demixing. Substrates bearing the same fluorous tag (Rf) but different substitution group (R^1) have similar retention times; they can be collected in the predictable time windows.

In addition to these two examples described above, fluorinated phases have been used in quasiracemic [29] and racemic [30] syntheses of natural product analogs and drug-like molecules. They have also been used for separation of FMS samples of oligo(phenylene vinylene)s and hybrid oligomers [31].

6. Other liquid chromatography applications

As a new class of stationary phases, fluorinated packing materials have been found very useful in the development of other chromatography-related applications such as supercritical fluid chromatography, micellar LC, electrochromatography, ion chromatography, and LC–MS.

6.1. Supercritical fluid chromatography

Packed column supercritical fluid chromatography is considered a good replacement for normal-phase liquid chromatography, especially for the separation of polar compounds. Compared to normal-phase HPLC, it has the advantages of using a greener mobile phase solvent, faster separation, higher efficiency, faster column re-equilibration, and a broad experimental variation for optimization. SFC resembles the HPLC except the mobile phase composition is different. A variety of packed LC columns can be directly used for SFC [32].

The Lee group employed a FluoroFix (C_6F_{13}-branched) column and Freon F113, methanol, or water as mobile phase modifiers to separate mixtures of perfluorinated polyethers (Z-Dol-2800) (Fig. 11) [33]. Approximately 80 components in the Z-Dol-2800 sample with an average molecular weight of 2800 were well resolved. The results demonstrated that the fluorinated phases have better selective retention than C_{18} for perfluorinated polymers.

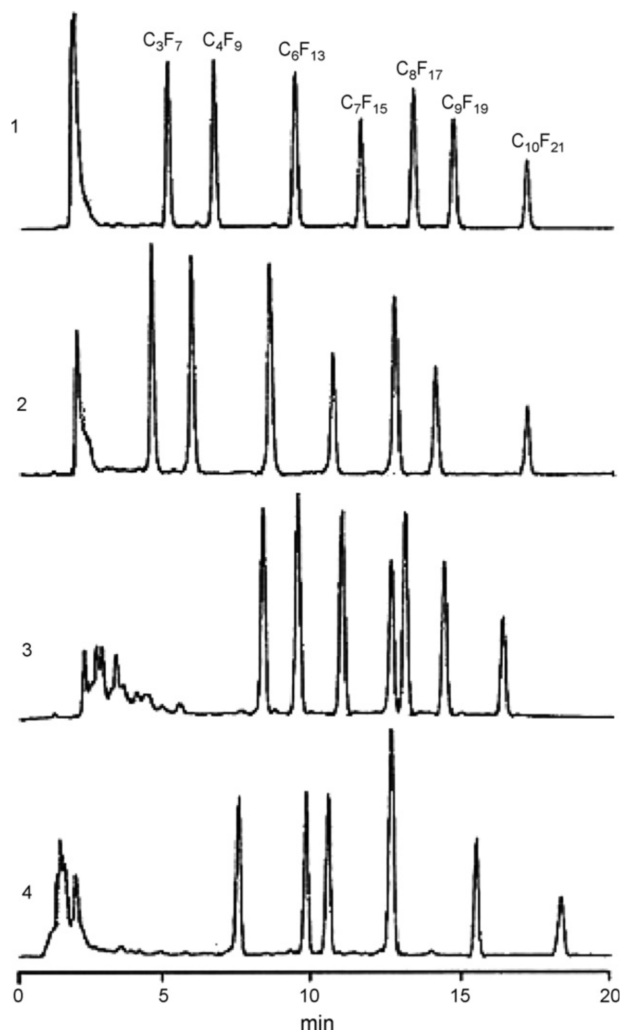


Fig. 9. Chromatgrams of tagged 7-component mappicines analogs (see Fig. 8 for structures) on four different columns (1) FluroPhase-RP (C_6F_{13}); (2) Fluorofix (C_6F_{13}-branched); (3) Fluorophase-PFP (C_6F_5), and C_{18}. From Ref. [27].

6.2. Micellar electrokinetic liquid chromatography

Electrokinetic micellar liquid chromatography is a powerful alternative to ion-pair chromatography for charged solutes [34]. It offers a number of advantages including simultaneous separation of ionic and non-ionic compounds, reproducible and predictable retention behavior, simultaneous enhancement of solvent strength and separation selectivity, and rapid gradient capability. However, compared to reverse-phase LC, MEKC has two major drawbacks: (1) excessive retention is observed for hydrophobic compounds due to the weak eluting power of micellar mobile phases when used with conventional porous stationary phases; and (2) reduced efficiency results due to one or more causes of slower mass transfer and flow anisotropy.

The Khaledi group used fluorinated phases to address the issues associated with MEKC [9]. Reduced separation times for amino acids (Fig. 12) and small peptides and stronger retention for the early eluting sulfonamides were observed using the C_8F_{17} column on which the surfactant is less adsorbed. The unique phenomenon of the simultaneous enhancement of solvent strength and selectivity that often occurs in the MEKC systems with the hydrocarbonaceous phases was also observed for the C_8F_{17} column. The Foley group further studied the effects of stationary phases and pore size on the efficiency of MEKC [35].

6.3. Open tubular electrochromatography

The open tubular electrochromatography offers the advantages of operating at low column backpressures and high efficiency for detecting low quantities of analytical samples. The Colon group developed a sol–gel process to prepare stationary phases for OTEC [36]. The sol–gel process is a low temperature procedure to prepare glasses, and can produce materials with high optical quality. The stationary phases made by the sol–gel process usually have high hydrolytic stability, high mass laudability, high surface area leading to a strong retention, high column efficiency, and a relatively easy preparation procedure. The sol–gel-derived fluorinated column has been prepared by hydrolyzing a mixture of a perfluorohexane (C_6F_{13})-containing silane and tetraethoxysilane followed by casting a thin film onto the inner walls of the fused-silica capillary. This fluorinated phase was tested for OTEC separating a mixture containing six model organofluorine compounds (Fig. 13) [11]. High column efficiencies (100,000–300,000 plates/m) for the organofluorine compounds were achieved. Corresponding C_8 phases prepared by either the sol–gel process or by conventional methods did not separate the model organofluorine mixture.

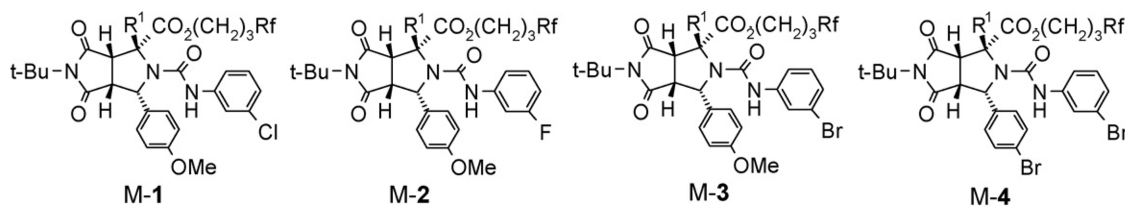
6.4. Ion chromatography

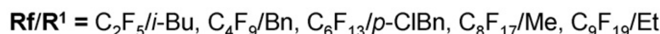
Ion chromatography is designed for analysis of anionic and cationic components. It has special utility for analysis of environmental water samples to determine common anions such as F^-, Cl^-, Br^-, I^-, NO_3^-, SO_4^{2-}, and HPO_4^{2-}. Reverse-phase C_{18}, graphic carbon, and cetyltrimethylammonuim-coated graphitized carbon columns have been developed for ion chromatography. Recently, the US Environmental Protection Agency recommended chemically suppressed IC as a method for the determination of anions in natural water.

Most reported methods use cationic surfactant for analysis of anions. The Helaleh group developed a unique method that employed C_{18} and graphic carbon columns coated with an anionic fluorine-containing surfactant [10]. The fluorine-containing surfactant is EF132 (*N*-[3-(perfluorooctanessulfonamide)propyl]-*N*,*N*,*N*-trimethylammonium iodide, $C_8F_{17}SO_2NHC_3H_3N^+(CH_3)I^-$). Because the fluorine-containing surfactant was strongly absorbed on the porous graphitic carbon surface, there was no need to add additional surfactant to the mobile phase. The column can be used for up to six months. The absorption of the surfactant on the silica gel, however, was found to be much weaker. Those two columns were tested for the separation of a standard mixture of five inorganic anions including Cl^-, Br^-, I^-, NO_3^-, and SO_4^{2-}. Fig. 14 shows the anion-exchange chromatogram obtained from a carbon column coated with EF132 and eluted with 0.26 mM sulfosalicylic acid. Compared to the uncoated C_{18} column, the fluorine surfactant-coated column had an unusual selectivity for I^- relative to that for SO_4^{2-}. It was also found that the monovalent ions were eluted before divalent ions. These results suggested that the separation was mainly based on an anion-exchange mechanism. The method developed for graphitized column coated with the fluorine-containing surfactant had good reproducibility and detection limits. It has been successfully applied to the analysis of anions in environmental water samples, including rain, river, and underground water.

6.5. Liquid chromatography–mass spectrometry

In the last decade, liquid chromatography–mass spectrometry has become an increasingly important tool for routine analysis in synthetic, pharmaceutical, and biotechnology labs [37]. Since the





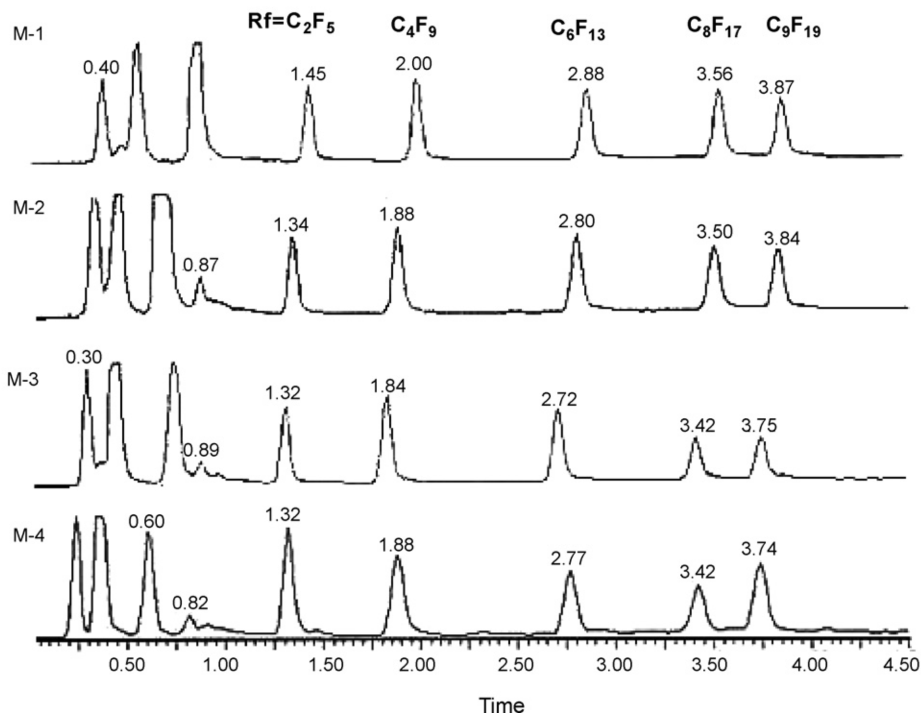


Fig. 10. Four-column parallel LC–MS of 4 FMS samples (M-1 to M-4). From Ref. [28].

direct analysis of basic pharmaceuticals by reverse LC has been difficult due to the secondary interaction of the solutes with the residue silanol on the silica gel, ion-paring and ion-suppression techniques have been developed to address the issue. However, the presence of ionic agents in the mobile phase decreases the MS detection limit in LC-ESI-MS analysis [38]. The discovery of ''U-shape'' retention for polar solutes on fluorinate phases has provided an ideal solution [12]. With high organic content in the mobile phase, the fluorinated phase has normal-phase characteristics and provides stronger retention to the polar solutes. The absence of ion-pairing or ion-suppressing agents in the mobile phase provides good sensitivity in ESI-MS. Because of the low surface energy of the fluorinated phases, the interaction between hydrophobic compounds with the packing materials is reduced. This characteristic reduces excessive retention of basic compounds

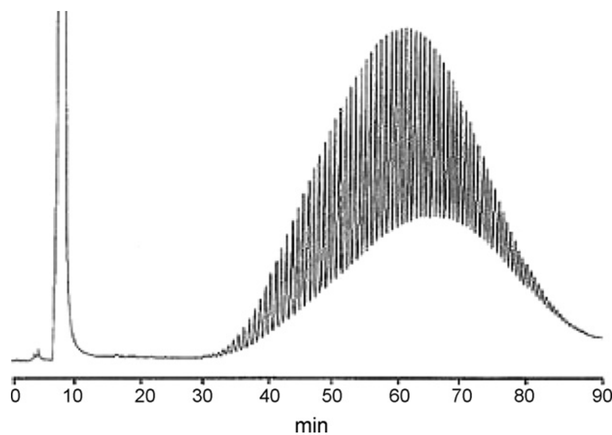


Fig. 11. SFC chromatogram of Z-Dol-2800 sample on the Fluorofix column. From Ref. [33].

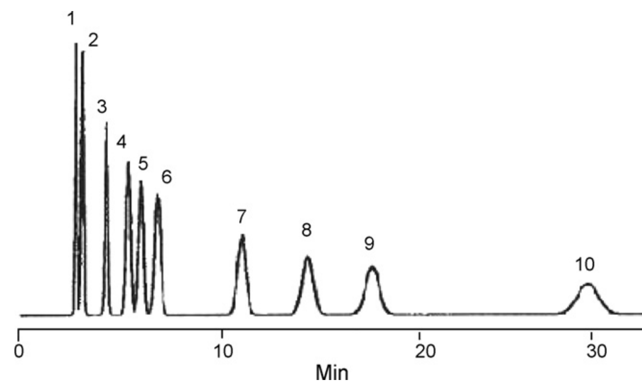


Fig. 12. MEKC chromatogram of a 10-component amino acid mixture on a C_8F_{17} column. Peaks: (1) Ala-Tyr; (2) Tyr; (3) Met; (4) Leu-Tyr; (5) Asp-Phe; (6) Trp; (7) Leu-Trp; (8) Phe-Phe; (9) Gly-Phe-Leu; (10) Lys-Phe. From Ref. [9].

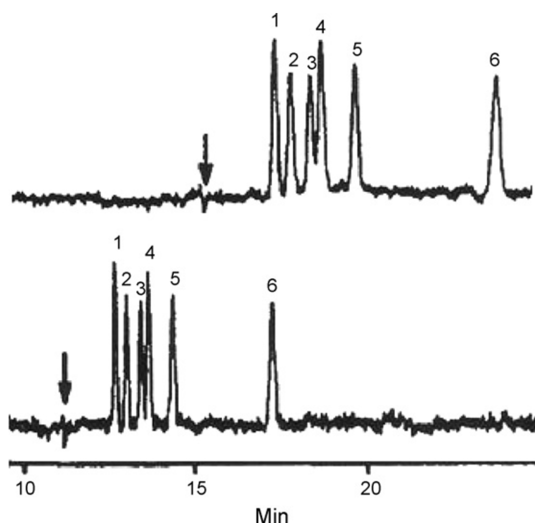


Fig. 13. Electrochromatograms of a 6-component test mixture by OTEC on a C_6F_{13} phase at separation voltages of 15 kV (top) and 20 kV (bottom). Peaks: (1) fluorohenzene; (2) 1,4-difluorobenzene; (3) 1,3-difluorobenzene; (4) 1,2-difluorobenzene; (5) 1,2,4-trifluorobenzene; (6) 1,2,3,5-tetrafluorobenzene. The arrow is the EOF marker. From Ref. [11].

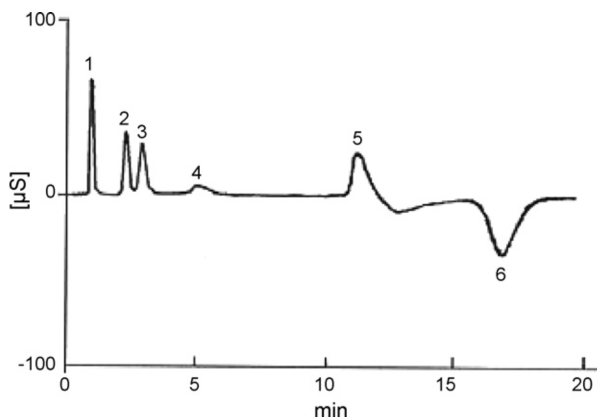


Fig. 14. IC chromatogram of common anions on grahitized carbon column coated with fluorinated surfactant. Peaks: (1) Cl^-; (2) Br^-; (3) $NO_3{}^-$; (4) I^-; (5) $SO_4{}^-$; (6) elution dip. From Ref. [10].

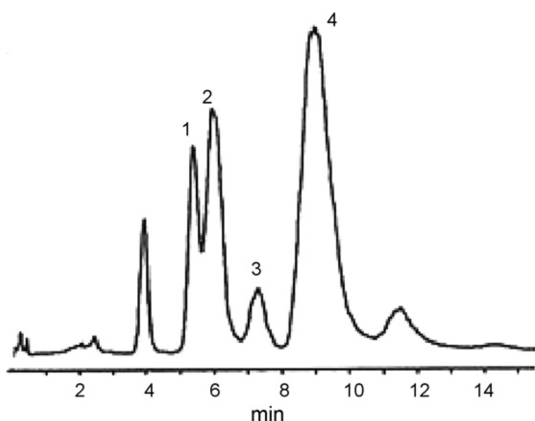


Fig. 15. Chromatogram for the LC-ESI-MS analysis of β-blockers on PFP phase. Peaks: (1) atenolol; (2) acebutolol; (3) alprenolol, (4) propranolol. From Ref. [6].

with high pK_a and strongly hydrophobic nature, so the fluorinated phase usually shows a better peak shape than the common C_8 and C_{18} phases.

The Needham group evaluated the silica gel-based C_6F_5 (PFP) phase for basic solutes using high MeCM concentration (90%), low buffer concentration (<10 mM), and no ion-pairing or in-suppression agents in the mobile phase [6]. For analysis of a model basic mixture containing β-blockers (Fig. 15), the PFP phase showed better peak shape than C_8 and C_{18} phases. More importantly, the ESI-MS signal is increased over 200% compared to that on the non-fluorinated carbohydrate phases. The PFP phase is stable and the results are reproducible.

7. Summary

The special chemical and physical properties of fluorocarbons have resulted in the development of a new class of LC stationary phases. The major utility of fluorinated phases include: (1) the resolution of organofluorine compounds based on fluorine content, (2) the analysis of polar pharmaceutical and biological samples based on the dual-functionality of fluorinated silica gels, and (3) the improvement of the performance of many kinds of LC applications. With better understanding of separation mechanisms, more silica gel- and polymer-based fluorinated phases could be developed to further enhance the chromatography technologies.

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