Novel Phases for HPLC Separations

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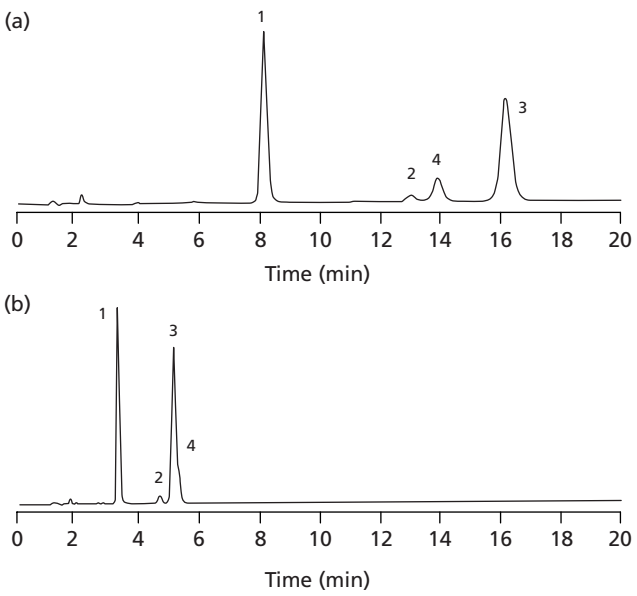
Reversed-phase high performance liquid chromatography (RP-HPLC) is one of the most used forms of chromatography.[1] The C8 and C18 stationary phases are the most widely used for RP-HPLC and, together with appropriate control of operational parameters such as solvent composition, pH, temperature and flow-rate, can enable many separations. However, analysts occasionally encounter difficult separations for which selectivity, ruggedness or reproducibility are not easily obtained using traditional C8 and C18 phases. These separations may require the use of more selective or novel stationary phases, such as pentafluorophenyl (PFP) bonded to silica for the analysis of paclitaxel and related taxanes in bulk pharmaceuticals[2] and the separation of geometrical isomers of Carotene.[3] These types of stationary phases separate compounds based upon selective stationary phase interactions such as steric recognition, charge transfer or π-π interactions.[4–6]

Figure 1: Separation of quercetin, kaempferol and isorhamnetin. Columns: (a) SymmetryShield RP8 and (b) Symmetry C8, 4.6 × 150 mm, 5 µm; mobile phase 55% water, 35% acetonitrile, 10% 50 mM formic acid; flow-rate: 1.2 mL/min; column temperature: 35 °C; detection: UV at 270 nm (reference 12). (Reproduced by permission of *Chromatographia*.)



Peaks: 1 = quercetin, 2 = contamination product of kaemferol, 3 = kaemferol, 4 = isorhamnetin.

Novel phases can also provide an alternative and complementary separation for many analyses performed on C8 or C18 columns. In many instances the elution order of solutes differs on novel phases thus providing enhanced selectivity for difficult-to-separate compounds. This complementary approach can aid in identification, proof of purity and quantitation. The change of elution order noted on any of the novel phases can also be useful in preparative HPLC. Using a novel selective phase, such as PFP, it may be possible to reverse an elution order and enable the elution of a minor component in front of a major component, thereby making collection and/or quantitation considerably easier. Using this approach improvements in purity, isolation time and solvent consumption can be realized for the isolation of minor components via preparative HPLC. Novel phases also offer the flexibility to use simpler mobile phases thereby avoiding ion-pair reagents, exotic buffer systems, extreme pH conditions and complex mobile preparations.

Although many types of chemically bonded novel phases have appeared in the literature and have been introduced by commercial organizations, coverage of all of them is beyond the scope of this present contribution. Instead we will focus on three more popular novel chemistries — polar-embedded alkyl phases, fluorinated phases and alkyl C30 phases.

Polar-Embedded Alkyl Phases

In recent years, several stationary phases have been commercially introduced that use polar-embedded groups.[7] These polar-embedded groups are generally incorporated in the alkyl ligand close to the surface silica. A variety of polar functional groups including amide, carbamate, urea and ether have been "embedded". The polar-embedded groups, in particular the amide, were originally chosen for their ability to deactivate silanol interactions with basic analytes.[8] Subsequently, polar-embedded phases have found application as stable phases for highly aqueous mobile phases[9] and have exhibited novel properties for polar analytes.[10]

Many of the amide and carbamate phases exhibit lower hydrophobicity[11] and methylene selectivity[11,12] when compared with conventionally bonded C8 and C18 stationary phases. In addition both the amide and carbamate phases have shown enhanced selectivity towards low molecular weight acids.[11] Some of this retention behaviour has been attributed

to the process used to make many amide phases. Some amide phases are produced via a two-step process in which an aminopropyl bonded phase is acylated to form an amide linkage.[8] In this process it is believed that not all aminopropyl groups are acylated and that unbonded amino groups can lead to mixed modes of separation.[12] However, the carbamate phases have been developed using a one-step synthesis[13] thus eliminating unbonded amino groups. In addition hydrolysis of a carbamate functionality results in a free carboxyl group and not a free amino group. These free carboxyl groups are unlikely to directly interact with low molecular acids. The enhanced selectivity of acidic analytes is most likely the result of the positive character of the carbamate group.[11] A carbamate C8 stationary phase commercially available from Waters (Milford, Massachusetts, USA) shows enhanced selectivity for the separation of catechin standards in the analysis of commercial preparations of *Camelia sinensis* extracts.[12] Catechins are functionalized with phenol groups and phenols have been shown to have longer retention on carbamate packings.[12]

Another example of a different selectivity comes from the separations of triazine pesticides on a commercially available C14 amide stationary phase.[7] The retention and separation of these triazines is substantially increased using the C14 amide when compared with a conventionally bonded C8 phase. A dramatic difference in both selectivity and retention with a classic bonded alkyl C8 phase and polar-embedded carbamate C8 phase is depicted in Figure 1, which shows the separation of the flavonols isolated from *Ginko biloba*.[12] Peak 2 is a contamination product of kaempferol. Retention is about three times longer on the carbamate phase when compared with the alkyl phase. In addition, the carbamate phase resolved kaempferol and isorhamnetin.

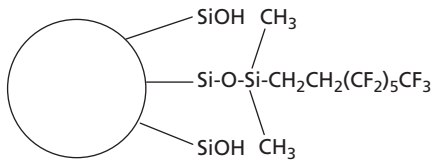
Fluorinated Stationary Phases

Perfluorintated and fluorinated stationary phases have shown novel selectivity for several compound classes and in many instances have proven useful as an alternative to traditional C8 and C18 phases.[2,3,14] Table 1 is a partial list of perfluorinated

Table 1: Some commercially available perfluorinated HPLC columns.

Product Name	Supplier	Surface Area (m^2/g)	Pore Size (Å)	Column Chemistry
Curosil PFP	Phenomenex (Torrance, California, USA)	—	—	Perfluorophenyl
Discovery F5 HS	Supelco (Bellefonte, Pennsylvania, USA)	—	120	Perfluorophenyl
Fluophase RP	Thermo Electron (Bellefonte, Pennsylvania, USA)	310	100	Perfluorohexyl straight chain
Fluophase WP	Thermo Electron	100	300	Perfluorohexyl straight chain
Fluophase PFP	Thermo Electron	310	100	Perfluorophenyl
Fluofix 120E	Thermo Electron	-—	120	Perfluorohexyl branched chain
Fluofix 300E	Thermo Electron	—	300	Perfluorohexyl
FluoroSep-RP Phenyl	ES Industries (Berlin, New Jersey, USA)	350	60	Pentafluorophenyl alkyl chain
FluoroSep-RP Octyl	ES Industries	450	60	Perfluorooctyl
Chromegabond LS	ES Industries	—	180	Perfluoroalkyl chain
FluoroSep-RP Phenyl HS	ES Industries	450	60	Pentafluorophenyl alkyl chain
FluoroSep-RP Propyl	ES Industries	120	300	Perfluoropropyl
MacroSep HPR	ES Industries	—	300	Perfluoroalkyl chain
Chromegabond PFP/T	ES Industries	350	60	Pentafluorophenyl propyl
FluoroSep-RP Phenyl Plus	ES Industries	—	120	Pentafluorophenyl alkyl chain
Allure PFP Propyl	Restek (Bellefonte, Pennsylvania, USA)	—	60	Pentafluorophenyl propyl
Ultra PFP	Restek	—	100	Perfluorophenyl
TAC-1	Whatman (Maidstone, Kent, UK)	—	159	Pentafluorophenyl propyl
Chromegabond FSP Plus	ES Industries	—	120	Perfluorinated phenyl alkyl chain
MacroSep Fluoroalkyl	ES Industries	—	1000	Short-chain perfluorinated alkyl
MacroSep FSP	ES Industries	—	300 & 1000	Perfluorinated phenyl

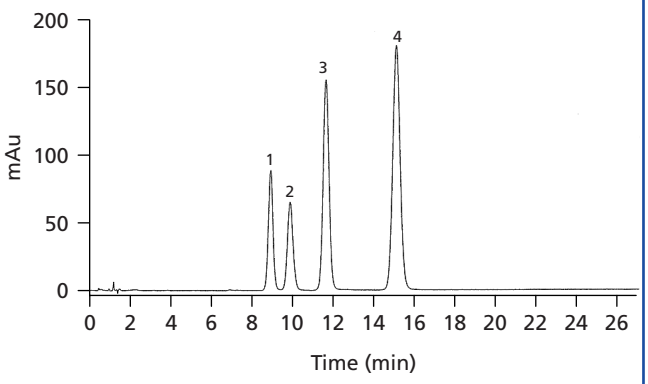
Figure 2: Typical structure of an alkyl fluorinated stationary phase based on perfluorooctyl-1H,1H,2H,2H-dimethyl-chlorosilane bonding reagent.



and fluorinated stationary phases commercially available with either alkyl chain or phenyl bonded groups. Such phases are available in different alkyl chain lengths as well as straight- and branch-chain configurations. Many of the commercially available alkyl-chain fluoro phases contain both fluorinated and unflourinated methylene units (see Figure 2).[15] These unfluorinated methylene units are generally found at the base of alkyl chain closest to the surface of the silica and may serve to stabilize the bonded alkyl chain.[16] Fluorinated alkyl phases have been shown to increase selectivity for the geometrical isomers of substituted phenols.[15]

Yamamoto and Rokushika[17] have studied fluorinated alkyl phases using computer simulation of molecular interaction energy based on a test set of solute molecules and various mobile-phase compositions. They prepared a C8 alkyl and two fluorinated phases bonded to the same base silica thus allowing for the direct comparisons between unfluorinated and fluorinated alkyl phases. Additionally, their solute test set comprised neutral molecules, thereby minimizing the influence of silanol group interactions. Silanol group interactions can dominate the reaction mechanism of many solutes, in particular those of basic compounds such as nortriptyline. The use of neutral molecules can provide for an unconfounded explanation of the retention mechanism and allow for a clearer understanding of the role of the bonded stationary phase.[18] The computer simulation provided some insight in to the retention mechanism of branched and linear fluorinated alkyl stationary phases. The authors observed that the fluorinated phases used in the study exhibited lower retention for both neutral *n*-alkanes and polyaromatics when compared with the C8 phases. In addition, at higher levels of methanol (90:10 (v/v) methanol–water solution) they observed that anthracene eluted before benzene on the branched fluorinated phase. Based on this chromatogram and the aromatic test probes they concluded that the interaction of planar and rigid aromatic solutes differed from non-planar aromatic solutes and the alkyl fluorinated phase may display some shape/size selectivity. Furthermore, they concluded that the retention of the aromatic test probes at higher methanol concentrations was obstructed by the methanol molecules solvating the solute, reducing the retention of larger aromatics. The retention characteristics of alkyl fluorinated phases are complex and their eventual elucidation will require future investigations.

Figure 3: Separation of a mixture of nitronaphthalenes on a pentafluorophenyl (PFP)-bonded phase. Column: 150 × 4.6 mm, 5 µm d_p FluoroSep-RP Phenyl; mobile phase: 55% methanol and 45% water; flow-rate: 1.0 mL/min; temperature 30 °C; detection: UV absorbance at 254 nm; sample volume: 5 µL. (Courtesy of ES Industries.)



Peaks: 1 = 1,5-dinitronaphthalene, 2 = 1,8-dinitronaphthalene, 3 = 1-nitronaphthalene, 4 = 1,3-dinitronaphthalene.

Perfluorinated and fluorinated phenyl stationary phases are also commercially available (see Table 1). These phases generally contain a PFP group and unfluorinated alkyl chain spacer. Typically, the PFP group is bound to the silica surface via this alkyl chain spacer and in many instances the unfluorinated alkyl chain is a propyl group. As with fluorinated alkyl phases the unfluorinated alkyl space groups may serve to stabilize the bonded group.[16]

Pentafluorophenyl columns have been used to separate many complex mixtures including tocopherols,[14] alkyl-substituted aromatics[19] and taxanes.[2]

The retention properties of the PFP have been studied by Sadek and Carr.[18] They concluded that the PFP material could be useful for the separation of molecules containing aromatic groups. Unfortunately, they were unable to provide any firm retention mechanism for explaining this behaviour. However, they could not rule out π-π interactions, which have been reported to play an important role in the retention mechanism of highly conjugated aromatic solutes.[20] Sadek and Carr were cautious about proposing retention mechanisms for polar hydrogen-bond acceptor or donor solutes used in their study because of the possibility of silanophilic interactions.

Przybyciel and Santangelo[21] have studied the retention behaviour of monosubstituted and disubstituted nitronaphthalenes on the PFP phase. As part of that study they prepared C18 and phenyl phases using the same base silica. Only the PFP column was able to completely resolve the nitronaphthalene mixture (Figure 3). Additionally, the PFP column retained every component of the mixture longer than either the C18 or phenyl column. The retention behaviour of the PFP phase for separation of the nitronaphthalenes may suggest some π-π interactions as well as other mechanisms including charge-transfer or electrostatic modes. The elucidation of retention mechanism for PFP remains largely unexplained and will require future investigation.

Alkyl C30 Phases

Alkyl C30 phases are the longest chain of the monomeric RP-HPLC phases currently available. The alkyl C30 has long been used for the unique separation of cis-trans carotenoid isomers in RP-HPLC.[22] A chromatogram showing this separation is contained in Figure 4. This unique separation behaviour has been attributed to the exceptional shape selectivity of the C30

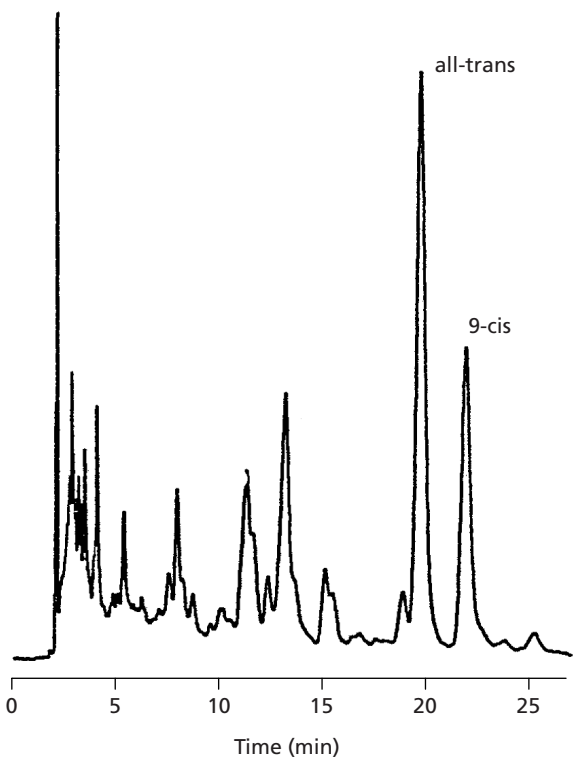
phase. Solid-state NMR investigations of C30 phases indicate that unique selectivity results from highly ordered alkyl chains enabling molecular shape recognition for carotenoids and tocopherols.[23] Majors and Przybyciel have compiled a list of commercially available alkyl C30 phases.[7]

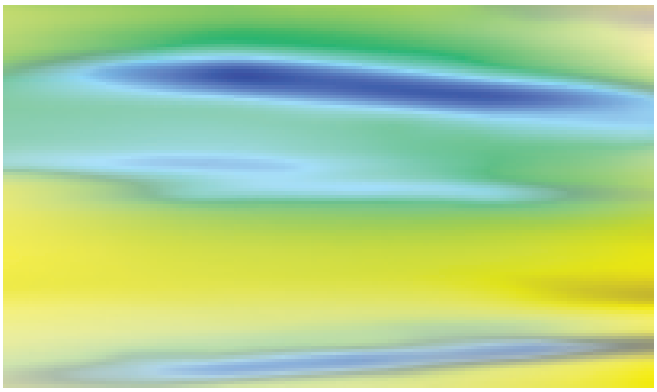
The C30 phase has also been applied to separations using highly aqueous mobile phases.[24] These phases have been shown to be resistant to phase collapse in such conditions and the unique behaviour of the C30 phase may be attributable to the higher melting point of the alkyl C30 chains. The melting point of alkyl C30 is 68–69 °C higher than the typical operating temperatures of HPLC columns (30–40 °C), and at these temperatures the solid C30 chains may be unable to collapse. The solid chains may then be available for chromatographic interactions. A patent has been issued to Nomura Chemical Co. for the application of C30 phases with highly aqueous mobile phases.[25]

Conclusion

Several classes of novel stationary phases including polar-embedded, fluorinated alkyl, fluorinated phenyl and alkyl C30 have been reviewed. Although these stationary phases are novel, they are commercially available from several column manufacturers. Each novel phase class has been used for a variety of separations and many of these separations have relied on a unique stationary phase–solute interaction. Many of the separations could not be accomplished on C8, C18 or phenyl phases. The actual mechanisms of interaction for many novel phases have been investigated, but a clear understanding of these interactions is still pending. Improvements, refinements and the introduction of new and better novel phases will result from efforts to further elucidate these retention mechanisms. Additionally, these studies will provide the chromatographer with the knowledge and tools necessary to recognize the potential for separations that go beyond C8 and C18 stationary phases.

Figure 4: HPLC separation of carotenoids. Column: ProntoSIL C30, 4.6 × 250 mm; mobile phase: 80% methanol and 20% TBME; flow-rate: 1.4 mL/min; temperature 20 °C. (Courtesy of MAC-MOD Analytical Inc., Chadds Ford, Pennsylvania, USA.)





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