Novel Phases for HPLC Separations

The author describes several commercially available novel phases for high performance liquid chromatography separations. Novel phases can provide an alternative and complementary separation for many analyses performed on C8 or C18 columns. Many of the separations discussed in the article could not be accomplished on C8, C18, or even phenyl phases. The author also includes a discussion on the mechanism of interaction for each of the novel phases.

Matthew Przybyciel
ES Industries, West Berlin, New Jersey, e-mail przybyciel@earthlink.net.

Reversed-phase high performance liquid chromatography (HPLC) is one of the most utilized forms of chromatography (1). The C8 and C18 stationary phases are the most widely used for reversed-phase HPLC. However, analysts occasionally encounter difficult separations for which selectivity, ruggedness, or reproducibility are not obtained easily using traditional C8 and C18 phases. These separations might require the use of selective or novel stationary phases such as pentafluorophenyl bonded to silica for the analysis of paclitaxel and related taxanes in the bulk drug (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).

Novel phases also can 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 also can be useful in preparative HPLC. Using a novel selective phase such as pentafluorophenyl, it might 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 quantitation considerably easier. Using this approach improvement in purity, isolation time, and solvent consumption can be realized for the isolation of minor components via preparative HPLC. Novel phases also offer the chromatographer the flexibility to use simpler mobile phases, thereby avoiding ion-pair reagents, exotic buffer systems, extreme pH conditions, and complex mobile preparations.

In this article, I will focus on five more popular novel chemistries — polar-embedded alkyl phases, fluorinated phases, phenyl hexyl, 2-(3 pyrenyl)ethyldimethylsilyl, and alkyl C30 phases.

Polar-Embedded Alkyl Phases

In recent years, a number of stationary phases have been introduced commercially that use polar-embedded groups (7). These polar groups generally are 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, originally were introduced commercially 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

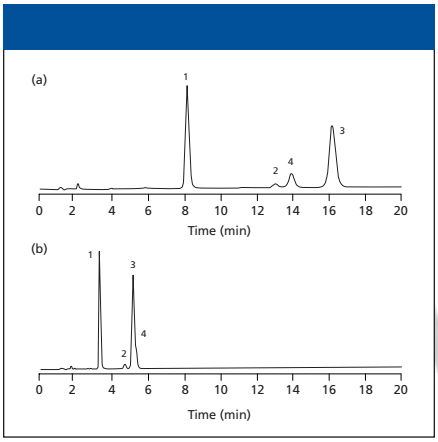

Figure 1: Separation of flavonols from *Ginkgo biloba* (12). Columns: (a) SymmetryShield RP8 and (b) Symmetry C8, 150 mm × 4.6 mm, 5 µm; mobile phase: 55% water, 35% acetonitrile, 10% 50 mM formic acid; flow rate: 1.2 mL/min; column temperature: 35 °C; detection: UV at 270 nm. Peaks: 1 = quercetin, 2 = kaempferol contaminant, 3 = kaempferol, 4 = isorhamnetin (Reproduced by permission of *Chromatographia*.)

analytes (10).

Many of the amide and carbamate phases exhibit lower hydrophobicity (11) and methylene selectivity (11,12) when compared to conventionally bonded C8 and C18 stationary phases. In addition, both the amide and carbamate phases have shown enhanced selectivity toward low molecular weight acids (11). Some of this retention behavior 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 mix modes of separation (12). However, the carbamate phases have been developed with one-step synthesis (13), thus eliminating unbonded amino groups. In addition, hydroysis of carbamate would result in a free carboxyl group 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 is commercially available from Waters (Milford, Massachusetts) showed enhanced selectivity for the separation of catechin standards for 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 is the separations of triazine pesticides on a commercially available C14 amide stationary phase (7). The retention and separation of these triazines is increased substantially using the C14 amide when compared to a conventionally bonded C8. 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 *Ginkgo biloba* (12). 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.

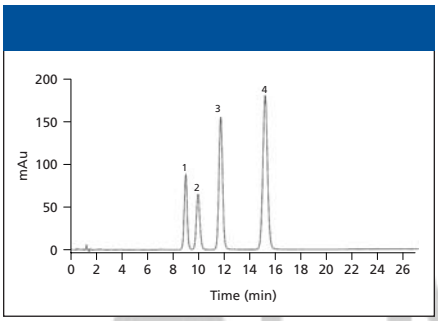

Figure 3: A separation of a mixture of nitronaphthalenes on a pentafluorophenyl bonded phase. Column: 150 mm × 4.6mm, 5 µm d_p FluoroSep-RP Phenyl; mobile phase: 55% methanol and 45% water; flow rate: 1.0 mL/min; temperature 30 °C; detection: UV absorbance at 254 nm; sample volume: 5 µL. Peaks: 1 = 1,5-dinitronaphthalene, 2 = 1,8-dinitronaphthalene, 3 = 1-nitronaphthalene, 4 = 1,3-dinitronaphthalene. (Courtesy of ES Industries.)

Fluorinated Stationary Phases

Perfluorinated and fluorinated stationary phases have shown novel selectivity for several compounds classes and as an alternative to traditional C8 and C18 phases (2,3,14). Table I is a partial list of perfluorinated 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 these alkyl-chain fluoro phases contain both fluorinated and unflourinated methylene units (see Figure 2) (15). These unfluorinated methylene units generally are found at the base of alkyl chain closest to the surface of the silica and can 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 the molecular interaction

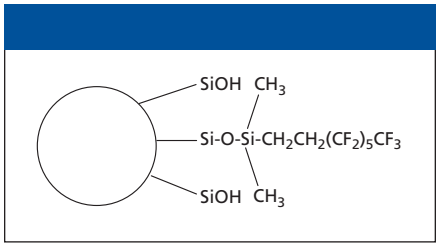

Figure 2: Typical structure of alkyl-fluorinated stationary phase based upon perfluorooctyl-1H, 1H, 2H, 2H-dimethylchlorosilane bonding reagent.

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 basic compounds such as nortriptyline. The use of neutral molecules can provide for an unconfounded explanation for the retention mechanism and allow for a clearer understanding of the role of the bonded stationary phase (6).

Perfluorinated and fluorinated stationary phases have shown novel selectivity for several compounds classes and as an alternative to traditional C8 and C18 phases.

The computer simulation provided some insight as 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. In addition, at higher levels of methanol (90:10 [v/v] methanol–water), they observed that anthracene was 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

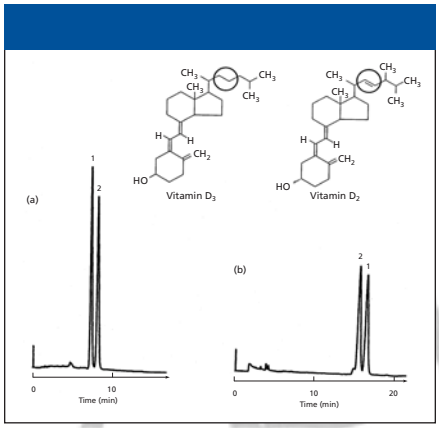

Figure 4: Vitamins D_2 and D_3. Columns: (a) Cosmosil 5PYE and (b) Cosmosil 5C18-MS-II, 150 mm × 4.6 mm, 5 µm d_p; mobile phase: 5% water, 95% methanol; flow rate: 1.0 mL/min; column temperature: 30 °C; detection: UV absorbance at 254 nm. Peaks: 1 = vitamin D_3, 2 = vitamin D_2. (Courtesy of Phenomenex.)

solutes differed from nonplanar aromatic solutes, and the alkyl fluorinated phase might display some shape and 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 the alkyl fluorinated phase are complex, and its eventual elucidation will require future investigations.

Perfluorinated and fluorinated phenyl phases generally contain a pentafluorophenyl group and unfluorinated alkyl chain spacer. Typically, the pentafluorophenyl group is bound to the silica surface via this alkyl spacer chain, and in many cases, the unfluorinated alkyl chain is a propyl group. As with fluorinated alkyl phases, the unfluorinated alkyl space groups can serve to stabilize the bonded group (16). Pentafluorophenyl columns have been used to separate many complex mixtures, including tocopherols (14), alkyl-substituted aromatics (18), and taxanes (2).

The retention properties of the pentafluorophenyl have been studied by Sadek and Carr (6). They concluded that the pentafluorophenyl material could be useful for the separation of groups containing aromatic groups. Unfortunately, they were unable to provide any firm retention mechanism for explaining this behavior. 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 (5). 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 (19) have studied the retention behavior of monosubstituted and disubstituted nitronaphthalenes on the pentafluorophenyl phase. As part of that study, they prepared C18 and phenyl phases using the same base silica as their commercially available pentafluorophenyl. Only the pentafluorophenyl column was able to completely resolve the nitronaphthalene mixture, as shown in Figure 3. Additionally, the pentafluorophenyl column retained every component of the mixture longer than either the C18 or phenyl column. The retention behavior of the pentafluorophenyl phase for separation of the nitronaphthalenes might suggest some π–π interactions as well as other interactions such as possible charge-transfer or electrostatic modes. The elucidation for retention mechanism for pentafluorophenyl remains largely unexplained and will require future investigation.

Hexylphenyl- and Aromatic-Based Stationary Phases

Phenyl bonded phases have been utilized in reversed-phase HPLC for many years (20). These phenyl phases have been reported to

Table I: Some commercially available perfluorated HPLC columns

Product Name	Supplier	Surface Area (m^2/g)	Pore Size (Å)	Column Chemistry
Curosil PFP	Phenomenex (Torrance, California)	—	—	Perfluorophenyl
Discovery F5 HS	Supelco (Bellefonte, Pennsylvania)	—	120	Perfluorophenyl
Fluophase RP	Thermo Electron (Bellefonte, Pennsylvania)	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	ES Industries	350	60	Pentafluorophenyl alkyl chain
Phenyl	(West Berlin, New Jersey)			
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)	—	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 and 1000	Perfluorinated phenyl

exhibit some π–π as well as steric recognition interactions (6). Bonded phenyl phases have traditionally contained either ethyl or propyl spacer chains between the silica surface and the phenyl group. These bonded ethyl–propyl phenyl phases display some π–π interactions. However, for many separations, these phases behave similarly to alkyl C8 phases. To gain unique selectivity for a phenyl phase, a new version has been developed that utilizes a hexyl spacer chain. The hexylphenyl stationary phase was orginally developed by Blevins (21) and commercialized by Phenomenex (Torrance, California) (22). This phase is claimed to have the properties of both a phenyl and alkyl phase for many separations. A similar version is available commercially from Thermo-Hypersil-Keystone (Bellefonte, Pennsylvania). However, this phase is comprised of separately bonded phenyl propyl and alkyl hexyl groups.

Other aromatic ligands have been bonded to silica such as 2-(2-naphthyl)-ethyldimethylsilyl (5), 2-(3-pyrenyl)ethyl-dimethylsilyl (5), and fluorene (23). The 2-(3-pyrenyl)ethyldimethylsilyl bonded phase is produced commercially by Nomura Chemical Co. (Seto, Japan) and marketed by Phenomenex. It has been reported that the 2-(3-pyrenyl)ethyldimethylsilyl phase exhibits properties that are intermediate between graphitized carbon phases and alkyl phases (24). The 2-(3-pyrenyl)ethyl-dimethylsilyl phase can interact via π–π as well as steric recognition mechanisms. An example of π–π behavior can be seen in the vitamin D_3–D_2 separation shown in Figure 4. There is reversal in elution order in comparison to the alkyl C18 column, and the double bond contained in vitamin D_2 can interact with the 2-(3-pyrenyl)ethyldimethylsilyl phase via a π–π interaction.

Alkyl C30 Phases

Alkyl C30 phases are the longest chain of the monomeric reversed-phase HPLC phases currently available. The alkyl C30 has long been used for the unique separation of cis-trans carotenoid isomers in reversed-phase HPLC (25). This unique separation behavior has been attributed to the exceptional shape selectivity of the C30 phase. Solid-state nuclear-magnetic resonance investigations of C30 phases indicate that unique selectivity results from highly ordered alkyl chains enabling molecular shape recognition for carotenoids and tocopherols (26). Majors and Przybyciel have compiled a list of commercially available alkyl C30 phases (7).

The C30 phase also has been applied to separations using highly aqueous mobile phases (27). These phases have been shown to be resistant to phase collapse in such conditions and the unique behavior of the C30 phase may be attributable to the higher melting point of the alkyl C_{30} chains.

Conclusion

Several classes of novel stationary phases including polar-embedded, fluorinated alkyl, fluorinated phenyl, phenyl hexyl, 2-(3-pyrenyl)ethyldimethylsilyl, and alkyl C30 have been reviewed. Although these stationary phases are novel, they are available commercially 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 even 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.

References

(1) R.E. Majors, *LCGC* **15**(11), 1009–1015 (1997).

(2) L.K. Shao and D.C. Locke, *Anal. Chem.* **69**, 2008–2016 (1997).

(3) C. Emenhiser, G. Englert, L.C. Sander, B. Ludwig, and S.J. Schwartz, *J. Chromatogr., A* **719**, 333–343 (1996).

(4) C.H. Lochmuller and C.W. Amoss, *J. Chromatogr.* **108**, 85–93 (1975).

(5) N. Tanaka, Y. Tokuda, K. Iwaguchi, and M. Araki, J. Chromatogr. 239, 761–772 (1982).

(6) P.C. Sadek and P.W. Carr, *J. Chromatogr.* **288**, 25-41(1984).

(7) R.E. Majors and M. Przybyciel, *LCGC* **20**(7), 584–593 (2002).

(8) T.L. Ascah and B. Feibush, *J. Chromatogr.* **506**, 357–369 (1990).

(9) T. Walter, P.C. Iraneta, and M. Capparella, "Observations on the Wetting of Reversed-Phase HPLC Packings," HPLC '97, Birmingham, U.K., June 23–27, 1997.

(10) R.E. Majors, *LCGC*, **16**(3), 228–244 (1998).

(11) J. Layne, *J. Chromatogr., A* **957**, 149–164 (2002).

(12) U.D. Neue, Y.-F. Cheng, Z. Lu, B.A. Alden, P.C. Iraneta, C.H. Phoebe, and K. VanTran, *Chromatographia* **54**, 169–177 (2001).

(13) U.D. Neue, C.L. Niederlander, and J. Peterson, U.S. patent number 5,374,755, 20 December 1994.

(14) S.L. Richheimer, M.C. Kent, and M.W. Bernart, *J. Chromatogr., A* **677**, 75–80 (1994).

(15) T. Monde, T. Kamiusuki, T. Kuroda, K. Mikumo, T. Ohkawa, and H. Fukube, *J. Chromatogr., A* **722**, 273–280 (1996).

(16) A. Haas, J. Kohler, and H. Hemetsberger, *Chromatographia* **14**, 341–344 (1981).

(17) F.M. Yamamoto and S. Rokushika, *J. Chromatogr., A* **898**, 141–151 (2000).

(18) W. Ecknig, B. Trung, R. Radeglia, and U. Gross, *Chromatographia*, **16**, 178–182 (1982).

(19) M. Przybyciel and M.A. Santangelo, "Exploring the Capabilities of Perfluorinated Stationary Phases for HPLC," presented at the 54th Pittsburgh Conference on Analytical Chemistry and Applied Spectroscopy, Orlando, Florida, 9–14 March 2003.

(20) G.E. Berendsen and L. de Galan, *J. Liquid Chromatogr.* **1**, 403 (1978).

(21) D.D. Blevins, "The Synthesis and Characterization of Bonded Phase Chromatographic Absorbents," Ph.D. dissertation, Dept. Chemistry, Univ. Arizona, Tucson, Arizona, 1982.

(22) F. Ahmed and T. Hanai, U.S. patent 5,993,653, 30 November 1999.

(23) R. Brindle and K. Albert, *J. Chromatogr., A* **757**, 3–20 (1997).

(24) N. Tanaka, T. Tetsuya, K. Kimata, K. Hosoya and T. Araki, *J. Chromatogr.* **549**, 29–41 (1991)

(25) C. Emenhiser, G. Englert, L.C. Sander, B. Ludwig, and S.J. Schwartz, *J. Chromatogr., A* **719**, 333–343 (1996).

(26) M. Pursch, S. Strohschein, H. Handel, and K. Albert, *Anal. Chem.* **68**, 386–393 (1996).

(27) N. Nage and T. Enami, "Development of a New Tricontyl (C30) Bonded Silica for High Performance Liquid Chromatography: Application of High Throughput Separation and Sample Enrichment," presented at the 53rd Pittsburgh Conference on Analytical Chemistry and Applied Spectroscopy, New Orleans, Louisinana, 17–22 March 2002. ■

ADVANSTAR COMMUNICATIONS
Peer Review Only. All Rights Reserved. Advanstar Communications Inc. 2005