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REVIEW ARTICLE

NEWER STATIONARY PHASES FOR REVERSE PHASE-LIQUID CHROMATOGRAPHIC ANALYSIS

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ABSTRACT

Reverse-phase liquid chromatography has been predominantly used for separation of analytes in the pharmaceutical industry. RP-HPLC consists of polar mobile phase and non-polar stationary phase. In reverse phase-liquid chromatography (RP-LC), the stationary phase is the layer of hydrophobic groups bonded to silica solid support materials. Most commonly used stationary phases in RP-LC are C8 and C18, which offer efficient separation of few selective polar compounds. But, these phases are not suitable for separation of complex mixtures in exotic buffer systems, extreme pH conditions and with complex mobile preparations. Hence, chromatographic retention and separation of polar compounds continues to be a challenging analytical problem. As a solution to this problem, newer stationary phases have been discovered and employed commercially. They offer the flexibility to use simpler mobile phases thereby avoiding ion-pair reagents, exotic buffer systems, extreme pH conditions and complex mobile preparations. The newer stationary phases developed in recent years are Polar-embedded alkyl phases, Fluorinated phases, Alkyl C30 phases, Hydrophilic interaction chromatography (HILIC) phases, Nanomaterial based, Type-C and Monoliths. This review covers the extensive experimental work done by various scientists on the newer stationary phases. It can be used by other researchers for further studies regarding separation science.

KEYWORDS: Stationary Phase, RP-LC, HILIC, Monoliths.

INTRODUCTION:

Stationary phase is the part of the Chromatographic system through which the mobile phase flows where distribution of the solutes between the phases occurs. It is mainly responsible for retaining the sample component in the column. In reverse phase liquid chromatography, molecules are bound to the hydrophobic matrix in an aqueous buffer (polar) and eluted from the matrix using a gradient of organic solvent (non-polar) [1].

RP-LC has following advantages [2]:

- The method has a very broad scope that allows sample with wide range of popularity to be separated. There is the possibility of using many different bonded phases, producing a very flexible system;
- The method uses relatively inexpensive mobile phase, and equilibration of the mobile phase with the column is rapid;
- It can be applied to the separation of ionic and ionisable compounds by the use of ion pairing or ion suppression techniques;
- The mode is generally experimentally easier, faster and more reproducible than other HPLC modes.

Most commonly used stationary phases in RP-LC are C8 and C18 for separation of few selective polar compounds. These phases are not suitable for separation of complex mixtures in exotic buffer systems, extreme pH conditions and with complex mobile preparations. So chromatographic retention and separation of polar compounds continues to be a challenging analytical problem. A number of approaches have been developed for retention of polar compounds, but many are limited in their applicability or have other serious drawbacks. For example, polar compounds can be derivatized to make them amenable to RP methods, but this is often time consuming. Newer 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.

SELECTION OF STATIONARY PHASES:

Selection of stationary phase, requires consideration of stationary phase chemistry, retention capacity and particle size. Identifying the best stationary phase for separation is the most critical step of column selection and decision should be based on sample solubility and chemical differences among the compounds of interest.

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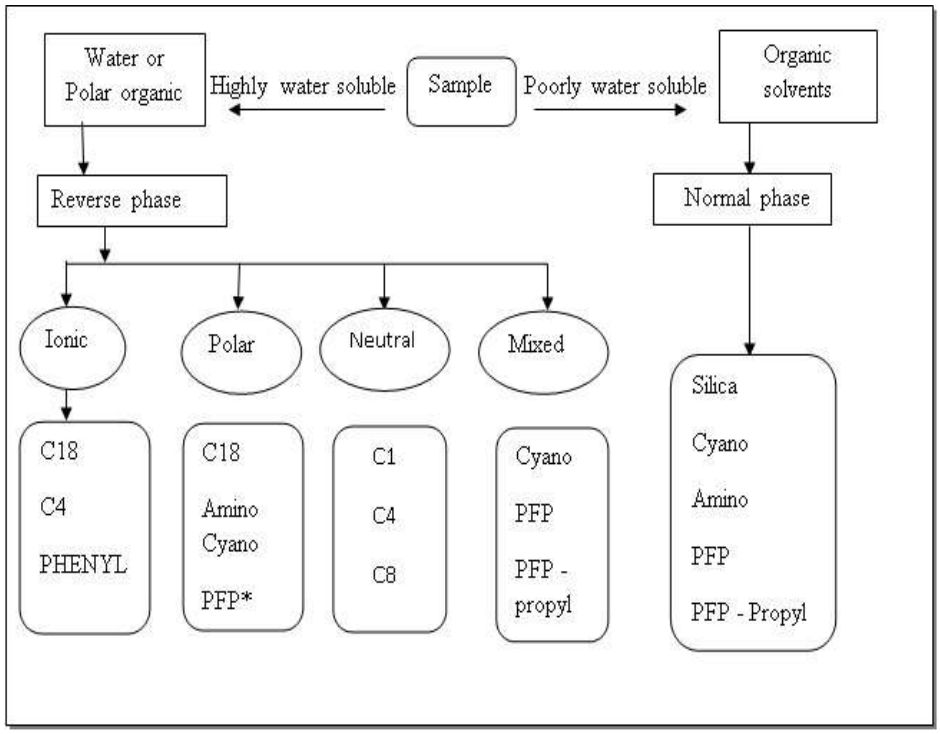

***PFP:** Pentafluorophenyl

Figure 1: Selection of stationary phase

CLASSICAL STATIONARY PHASES FOR RP-LC:

The use of silica based reversed phase sorbents is still predominant in pharmaceutical laboratory. The historical disadvantages of these packings has been chemical stability where eluents with pH value above 7 were not recommended due to dissolution of silica, and below pH 2, due to cleavage of siloxane linkages [3]. The most popular alternative to silica-based sorbents is fully polymeric reverse-phase poly (styrene-divinylbenzene) which is stable over a pH range of 1-13 and behaves as like a high carbon content C18 column [4-5]. Earlier, C18 or other alkyl group based columns were most commonly used chemistries for reversed phase chromatography. Classical stationary phases for reverse phase separation include Alkyl based stationary phase (C18, C14, C8), Phenyl and Poly (styrene-divinylbenzene) based.

Alkyl based Stationary Phases:

There are several alkyl based stationary phases (C18, C14, C12, C8, C4, and C2) available on the basis of polarity of analytes of interest [6].

➢C18 - Very hydrophobic, retentive and stable phase, first choice for most separation.

➢C8 - Preferred for lower organic mobile phase application for more polar solutes. C8 has similar selectivity as C18 but is much less retentive.

➢C4 and C3 - Less retentive then C8 and C18, mostly used for protein separation on wide-pore supports.

Early column packing's were based on C18 because C18-based silanes were readily available at that time and reasonable in cost. C18 silanes were used commercially for other purposes and were supplied by several sources. Another reason for the popularity of C18 is the relatively high organic content that can be reacted onto silica supports. This was especially important for pellicular and superficially porous silica supports that were used early in the history of HPLC. These materials had relatively low surface areas, which limited solute retention, so a high organic content was often required for desired retention of lightly held solutes. Another advantage of the long-chain C18 ligand is its better stability at both low and higher pH, compared to shorter chain ligands. However, there are some disadvantages to C18 bonded phases packings. Densely bonded packings can exhibit phase collapse when mobile phases contain a high aqueous component. For some solutes, shorter bonded phases can show slightly higher column efficiency. Also, column packings with shorter functional groups can re-equilibrate more rapidly after a gradient elution separation.

Considering the retention factors of several hydrophobic analytes, the hydrophobic selectivity, and the degree of coverage, commercially available columns can be divided into more hydrophobic and more polar phases [7]. (Table 1)

Table 1: Examples of hydrophobic and more polar RP-phases

Hydrophobic RP-phases	Polar RP-phases
Gromsil CP	Synergi POLAR RP
SMT OD C18	HyPURITY Advance
Nucleosil AB	Platinum EPS
Luna	Fluofix INW
Prodigy	Nucleosil Protect 1
Zorbax Extend	Zorbax SB C8
Zorbax ODS	Lichrospher Select B
Synergi MAX RP	Superspher Select B
Symmetry C18	Supelcosil ABZ plus
Hypersil BDS	Zorbax Bonus RP
HyPURITY C18	Lichrosorb

Phenyl bonded stationary phase:

Phenyl bonded stationary phase is used for medium polarity components and exhibit unique selectivity for aromatics [8].

Cyano:

This type of stationary phase was introduced in 1970 in separation science and used for separation of both polar and non-polar compounds [9]. It is used for selective separation of flavonoids, extraction of polar compounds from non-polar samples as well as the analysis of samples containing analytes with a wide range of hydrophobicity.

NEWER STATIONARY PHASE FOR RP-LC:

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 polar-embedded alkyl, fluorinated and alkyl C30 phases.

These types of stationary phases separate compounds based upon selective stationary phase interactions such as steric recognition, charge transfer or **π-π** interactions. Newer 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.

A number of newer stationary phases have been developed, these phases are:

A. Polar-embedded alkyl phases:
B. Fluorinated phases
C. Alkyl C30 phases
D. Hydrophilic interaction chromatography (HILIC) phases
E. Nanomaterial based
F. Type-C
G. Monoliths based

A. Polar-embedded alkyl phase:

In recent years, several stationary phases have been commercially introduced that use polar-embedded groups [10]. 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".

Jeannie Horak and Wolfgang Lindner [11] made sulfonyl/sulfonic acid-embedded reversed phase materials. A new sulfonyl-embedded reversed phase material with sulfonic acid moieties (SO*X*-RP) was prepared by a simple oxidation of two silica-based sulfur-embedded RP-phases (S-RP). They also compared SOX-RP phases with their corresponding S-RP phases as well as with two commercial carbonyl-containing RP phases (CO-RP) with amide and urea-embedding. It was found that SO*X*-RP phases exhibit exceptionally high; planar recognition ability for polyaromatic analytes at comparable retention times to the investigated CO-RP phases and highly reduced retention compared to the parent, non-oxidized S-RP-materials.

The polar-embedded concept has many advantages, *e.g.:* the stationary phase maintains a reversed-phase character, the phases provide a different selectivity compared with alkyl phases, particularly with polar analytes, the phases can be used in low percentages of organic solvent and even in 100% water without dewetting. This feature is especially useful for polar compound retention and leads to improved chromatographic performance (stable and reproducible retention) and faster gradient regeneration and silanol activity is suppressed, which leads to better peak shape

and decreased tailing of basic compounds, particularly at intermediate pH values.

The mechanism for the improved performance of polar-embedded alkyl phases has not been studied well nor have any systematic comparisons of the various types of polar-embedded functionalities been published. Researchers are engaged in considerable speculation about how they work relative to standard alkyl phases [12]. In highly aqueous mobile phases, these polar-embedded phases certainly wet more easily because of their hydrogen-bonding ability with water, and the contact angle between the surface and water could drop to less than 90° at which the water could penetrate the porous surface freely.

B. Fluorinated Stationary Phases:

Fluorinated alkyl phase was first used commercially by Yamamoto and Rokushika [13] while Study of retention behaviour was carried out by Sadek and Carr [14] for separation of complex mixtures including tocopherols, alkyl-substituted aromatics and texans.

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 [15]. Fluorinated stationary phases commercially available are with either alkyl chain or phenyl bonded groups. (Figure 2)

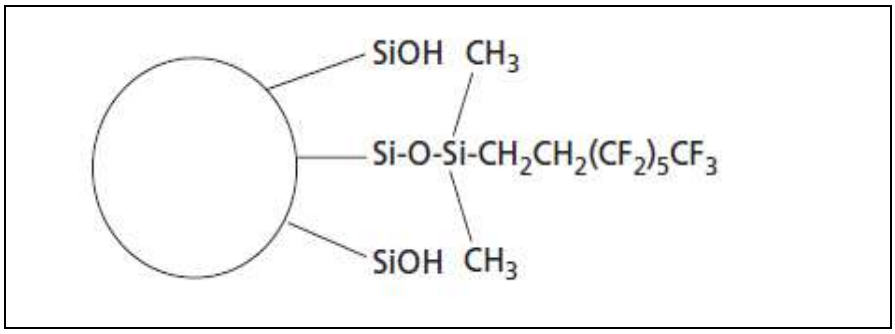

Figure 2: Typical structure of an alkyl fluorinated stationary phase based on perfluorooctyl- 1H, 1H, 2H, 2H-dimethylchlorosilane bonding reagent.

Fluorinated alkyl phases have been shown to increase selectivity for the geometrical isomers of substituted phenol. They are available in different alkyl chain lengths as well as straight and branched chain configurations. Many of the commercially available alkyl-chain fluoro phases contain both fluorinated and un-fluorinated methylene units [16]. These un-fluorinated 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 [17].They are alternative to traditional C8 and C18 phases[18-20].

C. Alkyl C30 Phases:

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 [21].

A patent has been issued to Nomura Chemical Co. for the application of C30 phases with highly aqueous mobile phases while Majors and Przybyciel have compiled a list of commercially available alkyl C30 phases [22].

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] (Figure 3).

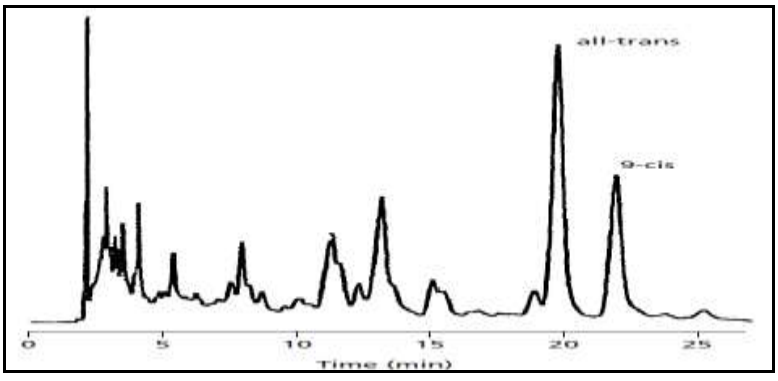

Figure 3: HPLC separation of carotenoids. Column: ProntoSIL C30, Dimension: 4.6 × 250 mm; Mobile phase: 80% methanol and 20% TBME; Flow-rate: 1.4 mL/min; Temperature: 20°C. (Courtesy of MAC-MOD Analytical Inc., Chadds Ford, Pennsylvania, USA.)

C30 phases have been shown to be more resistant to phase collapse under high aqueous 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 68–69ºC higher than the typical operating temperatures of HPLC columns (30-40ºC), and at these temperatures the solid C30 chains may be unable to collapse.

D. Hydrophilic interaction chromatography (HILIC):

Earlier, hydrophilic interaction chromatography (HILIC) has been receiving attentions as a useful alternative for compounds too polar to separate in reversed phase (C18). The term "hydrophilic" (in HILIC) refers to the affinity to water. HILIC can be characterized as the chromatographic technique using a normal stationary phase in combination with an RP mobile phase, containing more than 50% organic solvent in water. Resulting increase in retention of strongly polar compounds, for which it offers different selectivity compared to the traditional reverse phase chromatography [24]. In water rich mobile phases, HILIC may show better separation efficiency (lower HETP) for strongly polar compounds than reversed-phase, due to less viscous organic-rich mobile phases [25]. Another reason for increasing popularity of HILIC is its excellent suitability for coupling to mass spectrometry (LC/MS). In past time, several conventional stationary phases for HILIC, based on amine, amide, diol-types, and silica have been reported in literature [26-30].

Randon J et al. [31] reported use of Zirconia based monoliths in hydrophilic-interaction chromatography for original selectivity of xanthenes. Zirconium alkoxide was used for preparation of Zirconia based monoliths. Separation of three dimethyl xanthine isomers, theophylline, theobromine and paraxanthine was carried out, which are otherwise very difficult to separate in RP-LC with classical C18 stationary phases. The three isomers were easily separated in HILIC mode on a zirconia based stationary phase (Figure 4).

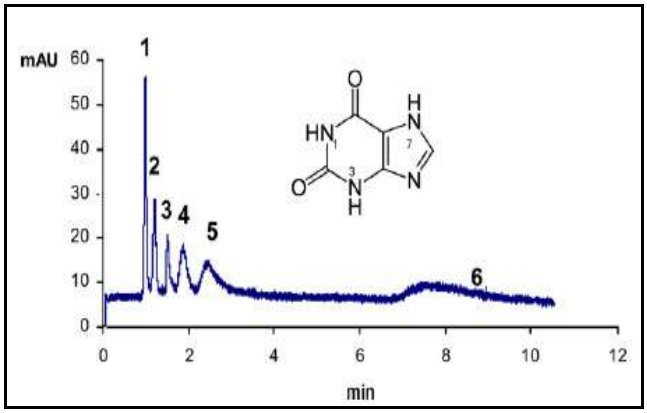

Figure 4: LC separation of xanthines on zirconia monolithic capillary columns (8.5cm length, 75µm I.D. Mobile phase MeCN/Tris 1mM pH 7.5 90/10; UV detection 254 nm, u = 0.14cms−1). Compounds: (1) Naphthalene; (2) Caffeine; (3) 7-hydroxyethyl theophylline (etofylline); (4) 1, 3-dimethylxanthine (theophylline); (5) 3, 7-dimethylxanthine (theobromine); (6) 1, 7-dimethylxanthine (paraxanthine).

A new imidazoline based stationary phase for HILIC has been developed by Li Y and co-workers [32] for selective separation of aromatic compounds as shown in Figure 5, separation of aromatic compounds was carried out by using of following chromatographic condition- Mobile phase:ACN/10 mM ammonium formate aqueous solution (30/70, v/v); UV detection:254 nm.

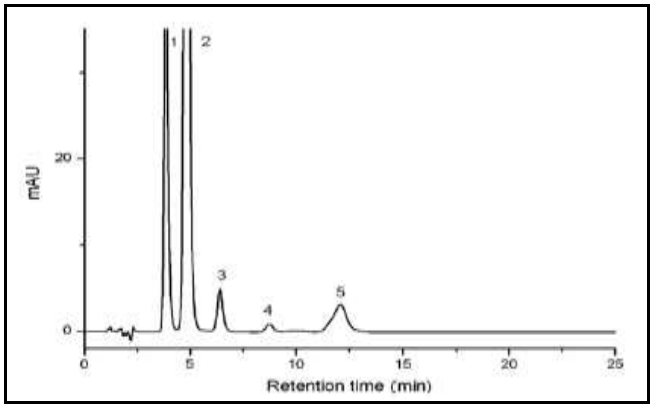

Figure 5: Separation of aromatic compounds [(1) Phenylamine, (2) Acetophenone, (3) Benzene, (4) Toluene and (5) Dimethylbenzene on imidazole column: Reprinted with permission from Ref. [32]

E. Nanomaterials in LC:

Material having particle size of less than 100 nm in at least one dimension are come into the category of nanomaterials. Nanoparticles (NPs) have received much attention in the time due to their novel chemical, physical and electrical properties [33].

NPs have great impact on separation science. They provide unique opportunities for the development of higher performance separation techniques that utilize NPs which possess a large surfaceto-volume ratio. NPs include silica NPs (SiNPs), gold NPs (AuNPs), titanium-dioxide, NPs (TiO2NPs), carbon NPs, polymer NPs, molecularly imprinted polymers, molecular micelles, and dendrimers. Nanotechnology has significantly accelerated the development of many fields of science [34].

NPs are used to improve the chemical stability, selectivity and separation efficiency of stationary phase in liquid chromatography. A few of NPS based materials used for separation of complex mixtures are described below:

Fullerene (C_{60}) based stationary phase:

C_{60} is a carbon allotrope. In previous investigations, it was clearly revealed that phenyl ligand bonded stationary phases can undergo effective π-π interactions with fullerenes [35]. It therefore appeared appropriate to develop fullerene-based stationary phases for the separation of solutes with phenyl moieties in their structures.

Earlier Jinno and co-workers, first investigated C_{60} based stationary phase [36-37]. Powdered solid C60 was packed into a fused-silica capillary by the slurry method with cyclohexane as the solvent. The retention behaviour towards various aromatic compounds indicated the possibility of C60 being a promising stationary phase.

Later on, Nagashima et al. [38-39] proposed a more versatile synthetic entry linking C60 with alcohols, phenols, and silica. On the basis of this work, Jinno et al.[40-42] synthesized C60 fullerene bonded silica and used it as stationary phase for HPLC (Figure 6). Application of this C_{60} based stationary phase for various polycyclic aromatic hydrocarbons (PAHs) was different from that of octadecylsilica (ODS) phases. C_{60} have unique molecular recognition capability and shape selectivity.

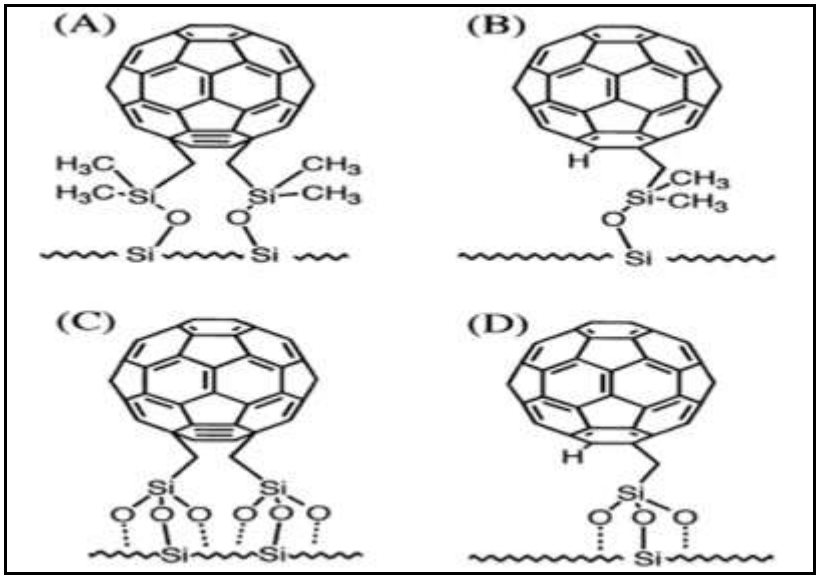

Figure 6: Structures of C60 bonded stationary phases. (Reproduced with permission from [41], Copyright 1997 Royal Society of Chemistry.)

Moreover, Stalling and co-workers [43-44] introduced polymer-based C60/C70 bonded phases for the highly selective separation of polychlorinated biphenyls (PCBs). A C60 bonded stationary phase was also reported by Chang et al. [45] showing interesting selectivity for quinines.

The fullerene C60-cryptand resin can be successfully used as a multifunctional LC packing material to separate not only anions and cations, but also neutral organic molecules due to the introduction of C60 into the novel stationary phase.

Single-walled carbon nanotube (SWCNT):

Carbon nano tubes (CNTs) have been used as stationary phases in HPLC. These were discovered in 1993 and first incorporated into an organic polymer monolithic stationary phase for μ-HPLC. The interest of researchers on SWCNTs is due to its high surface area, high mechanical strength, but ultra-light weight, rich electronic properties, and excellent chemical and thermal stability [46].

SWCNT is formed when one single layer of graphite is folded onto itself and the resulting edge is joined, with

high aspect ratio, lengths from several hundred nanometers to several micrometers, and diameters of 0.4–2 nm [47].

It was possible to separate PCB isomers and terpenes (linalool, geraniol, thymol, and a-terpineol) in HPLC by immobilizing functionalized SWCNTs (SWCNT-NH_2) on an aminopropyl silica surface. PCB congeners and terpenes showed good separation on functionalized CNT stationary phase due to the selective dipole-dipole and charge-transfer interactions [48].

Figure 7 shows the chromatograms of a reversed-phase test mixture separated on the control monolith and the poly (VBC-EDMA-SWCNT) monolith. It can be seen incorporation of SWCNT used to enhance chromatographic retention of small neutral molecules with strongly hydrophobic characteristics in reversed-phase HPLC, due to the hydrophobic interaction between analytes and SWCNT.

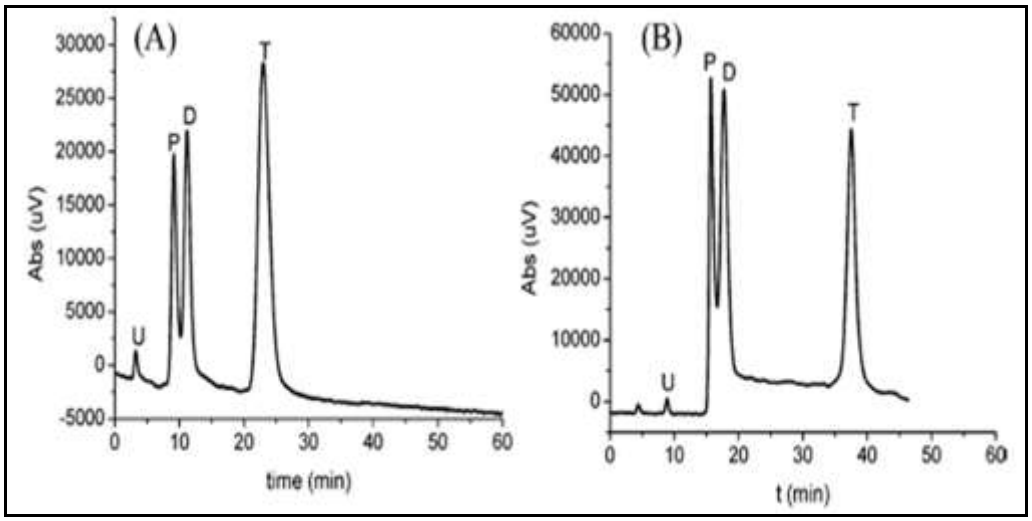

Figure 7: Chromatograms of a reversed-phase test mixture separated on (A) control monolith and (B) poly (VBC-EDMA-SWNT) monolith under isocratic elution conditions (50% aqueous acetonitrile + 0.1% (v/v) TFA). Flow rates: (A) 0.5 mL/min (permeability, 0.32 darcy); (B) 0.4 mL/min (permeability, 0.26 darcy). Both columns dimension: 0.075 mm (diameter) × 6400 mm (length); temperature: 25 ^{0}C; UV detection at 214 nm. Peaks: U, uracil; P, phenol; D, N, N-diethyl-m-toluamide; T, toluene. (Reproduced with permission from 49) Silica nanoparticles (SiNPs):

Recently, Cintron and Colon [50] utilized simple one-step sol-gel to synthesize uniform, spherical organo-silica nanoparticles with an average diameter of 670 nm containing octadecyl moieties by using tetraethoxysilane (TEOS) and octadecyltriethoxysilane (C18-TEOS) as precursors under basic conditions.

SiNPs could be used as packing material for capillary LC [51]. It generated lower drifts and baseline noise to provide good repeatability of liquid delivery. SiNPs as packing material in LC has great efforts in separation science as they reduce analysis time and improve separation efficiency.

F. Type C:

TYPE C silica is a relatively new chromatographic material that has been finding ever-increasing use in the last few years. The properties exhibited by these stationary phases are often significantly different than the ordinary silica used for most commercial products. While all TYPE C phases can be utilized in the reversed-phase, organic normal phase and aqueous normal phase modes, there are some unique capabilities within each retention mode that have resulted in innovative method development strategies with great success.

TYPE C stationary phase is mainly based on silica-hydride material. Type C stationary phases are an entirely different material with a slightly more hydrophobic surface that does not generate a dense water layer at the particle/mobile phase interface. While the mechanism of separation is not yet completely understood, the actual retention and separation capabilities for hydrophilic compounds have been extensively demonstrated [52-56].

Mode of operation:

TYPE-C stationary phase work in two modes viz. *aqueous normal phase* and *Reversed phase.*

a) Aqueous Normal Phase:

Every TYPE-C stationary phase displays ANP properties, *i.e.* increased retention of hydrophilic species as the amount of the least polar component in the mobile phase increases. The mobile phase consists of water and typically either acetonitrile or acetone.

The retention of polar compounds in the ANP mode is best illustrated by some examples that utilize the Cogent Diamond Hydride™ (DH) stationary phase [Figure 8]. This phase, which has a small amount of carbon on the surface, has both high hydrophilic retention as well as

excellent peak shape over a wide range of polar compounds. An analytical problem of determination of melamine and its degradation product, cyanuric acid, which have been found to be contaminants in both human and animal food sources, has been tackled by using Type C ANP mode.

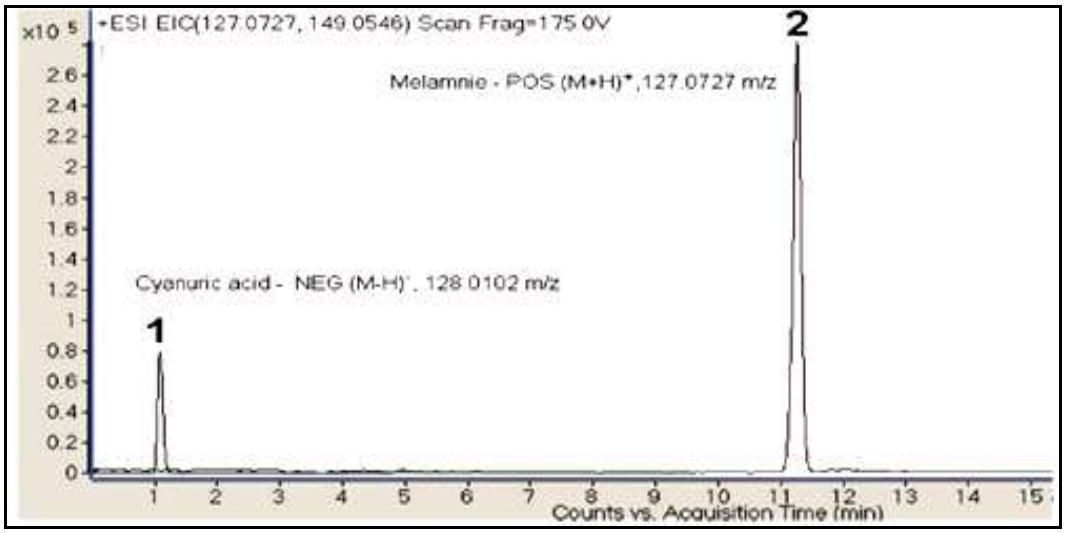

Figure 8: Separation of melamine and cyanuric acid on the Cogent Diamond Hydride column using an acetic acid mobile phase and a gradient from high to low concentration of acetonitrile in the mobile phase:
Mobile Phase: A: DI water + 0.1% acetic acid; B: Acetonitrile + 0.1% acetic acid. Gradient: 100% B to 50% B in 15 min; Column: 2.1 x 150 mm; Flow rate: 0.4 mL/min.
Sample concentration: Cyanuric acid 1.5 µg/ml and melamine 3 ug/ml. Detection: m/z 128 in the negative ion mode for cyanuric acid and m/z 127 in the positive ion mode on an Agilent 6210 MSD TOF spectrometer.

b) Reversed Phase:

All TYPE C stationary phases display some reversed-phase behaviour. Even the unmodified material can retain nonpolar compounds because the hydride surface is slightly hydrophobic. As the hydrophobicity of the stationary phase is increased by having greater surface coverage of bonded organic moieties, retention of nonpolar compounds increases as with all other reversed-phase materials.

For retention of hydrophilic compounds under RP conditions with TYPE C materials, mobile phases that typically contain 90-100% (v/v) water are used. Stationary phases based on silica hydride are especially suited to these conditions since they do not undergo "dewetting". Metformin, a drug used in the treatment of diabetes, was analysed on a Bidentate C18 column [57].

H. Monolithic stationary phase:

Monoliths are separation media that can be compared to a single large "particle" that does not contain inter-particular voids. As a result, all the mobile phase must flow through the stationary phase. This convective flow greatly accelerates the rate of mass transfer.

History of monoliths

Nobel Prize winner Richard Synge in 1952 envisioned "a continuous block of porous gel structure" [58]. First attempts to make "single-piece" stationary phase from highly swollen polymer gel and open-pore polyurethane foams during the late 1960s and the early 1970s were less successful. Interest in the monolithic formats has only been revived in the late 1980s when novel approaches towards true monoliths such as compressed hydrophilic gels, macroporous polymer discs, columns, tubes, as well as silica rods, have been developed [59]. A thorough theoretical treatment of mass transfer within monolithic materials has recently been developed by Liapis [60]. A comparison of morphologies characteristic of packed and monolithic columns is presented by Rozing [61]. Recently, Ekaterina P. et al. [62] developed a micro-bore titanium housed polymer monoliths for RP-LC for separation of small molecules. They silanised titanium with 3-trimethoxysilylpropyl methacrylate resulting in tight bonding of butyl methacrylate porous monolith to the internal walls, this provides stationary phase stability at column temperatures up to 110°C and at operating column pressure drops of >28 MPa. They separated mixture of small organic molecules on a prepared titanium housed monolithic column using an eluent of 60% acetonitrile–40% water and a column temperature of 110°C. The obtained chromatogram is shown as Figure 9 (a). The prepared column was also used for the separation of the mixture of Pesticides Figure 9(b).

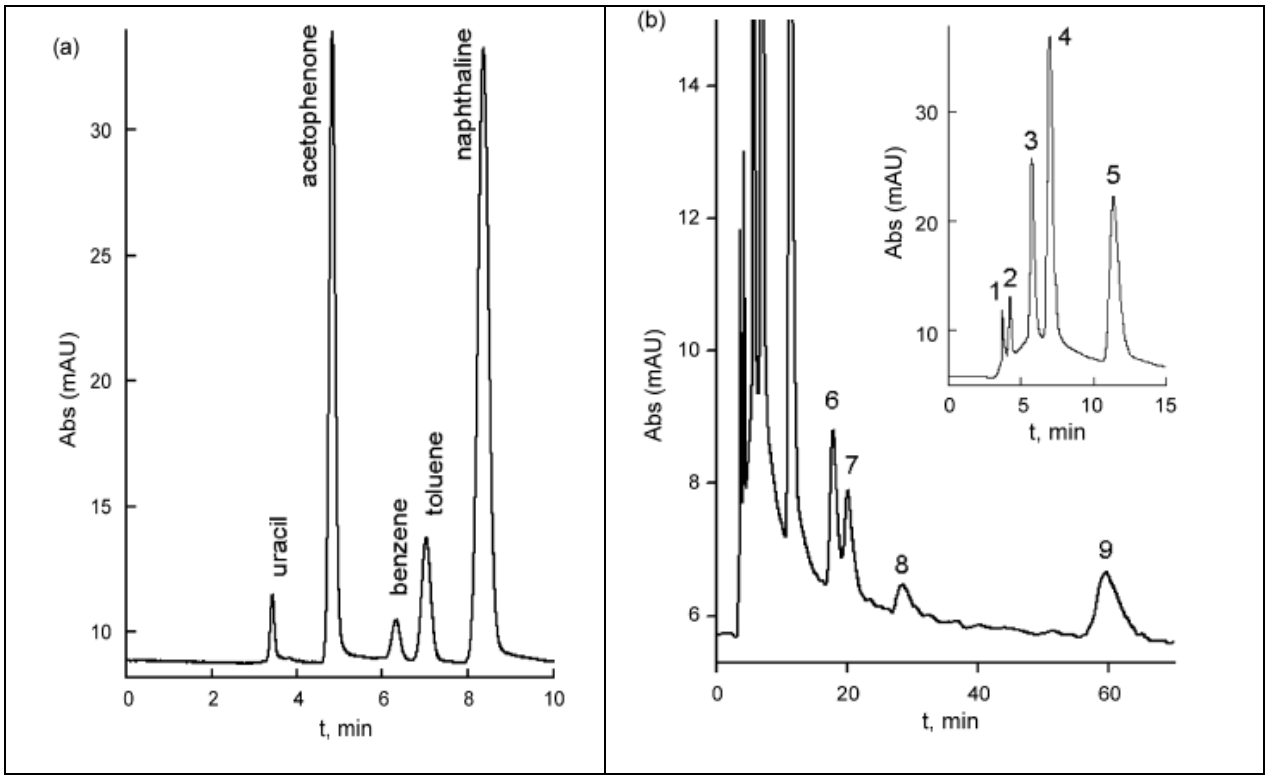

Figure 9: Separation of a (a) test mixture on a titanium housed butyl methacrylate–ethylene dimethacrylate polymer monolithic stationary phase. Column dimensions: 100mm×0.8mm I.D., Mobile phase = 60% ACN, 40% water, F=10µL/min, column temperature = 110°C, column backpressure = 19.6MPa and (b) the separation of pesticides: 1-Paraquat, 2-Aldicarb, 3-2-hydroxy-4-methoxy-benzene, 4-5-Chlorosalicylaldehyde, 5-Naphthalene, 6-Chlorpyrifos, 7-hexamethylbenzene, 8-Dieldrin, 9-DDT., Column: butyl methacrylate–ethylene dimethacrylate polymer monolith, 100mm×0.8mm i.d. Mobile phase = 60% CAN-40% water, F=10 µL/min, column temperature = 70°C. UV detection at 254 nm: Reprinted with permission from Ref [62].

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.

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.

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