Synthesis and characterization of C_{13} to C_{18} stationary phases by monomeric, solution polymerized, and surface polymerized approaches[☆]

Catherine A. Rimmer[a,*], Lane C. Sander[a], Stephen A. Wise[a], John G. Dorsey[b]

[a]*Chemical Science and Technology Laboratory, Analytical Chemistry Division, National Institute of Standards and Technology, 100 Bureau Drive, Stop 8392, Gaithersburg, MD, 20899-8392, USA*
[b]*Florida State University, Department of Chemistry and Biochemistry, Tallahassee, FL 32306-4390, USA*

Abstract

A series of bonded phases were synthesized from consecutive length alkylsilanes ranging from C_{13} through C_{18}, with three different bonding chemistries (monomeric, solution polymerized, and surface polymerized) at each phase length. The phases were characterized in terms of methylene selectivity, shape selectivity, and band broadening. No significant chromatographic differences were found to result from the synthetic routes, except that the different bonding chemistries provide a different range of bonding densities. For bonding densities ranging from 2 to 8 $\mu mol/m^2$, a linear increase in methylene selectivity was observed with increasing bonding density. Over the narrow range of bonded phase lengths investigated, shape selectivity is more dependent on density than phase length.
© 2003 Elsevier B.V. All rights reserved.

Keywords: Stationary phases, LC; Methylene selectivity; Shape selectivity; Selectivity; Band broadening

1. Introduction

Stationary phase design and characterization have been central to the development of liquid chromatography (LC), and alkyl-modified silica sorbents have dominated reversed-phase liquid chromatography (RPLC) technologies. Numerous studies have examined the effects of bonded phase length, bonding density, and bonding chemistry in efforts to better understand RPLC separation mechanisms [1,2].

Although octadecylsilyl (C_{18}) phases comprise

most commercial stationary phases used in RPLC, these phases exhibit a wide range of chromatographic characteristics based on differences in the substrate and chemical bonding methods. The properties of chromatographic silica substrates often differ markedly, whether by design or chance. Perhaps the most significant physical properties of silica relevant to chromatography include the surface area, pore size and pore size distribution, particle size and particle size distribution, porosity, and chemical composition [3].

The method by which the bonded phase is attached to the substrate may potentially affect the chromatographic characteristics of the phase. Monofunctional silanes (i.e., monochloro or monoalkoxy

---

silanes) are widely used in the production of reversed-phase sorbents. Monomeric surface modification approaches necessarily result in single-bond linkages between the organosilane and the silica surfaces. Such surface modification reactions are usually carried out with excess silane and are sterically limited. The maximum surface density that can be achieved for monomeric phases is about 4 μmol/m^2, which represents about one-half of the surface silanols available for reaction. Trifunctional silanes offer additional flexibility in the preparation of reversed-phase sorbents. Trifunctional silanes react with water to form polymers, which in turn may react with the silica surface. This polymerization can be viewed as a pre-assembly which facilitates compact spacing of the alkyl chains. The reaction is strongly dependent on the levels of water and silane, and surprisingly, on the pore diameter of the silica substrate [4–6]. Stationary phases prepared by solution polymerization may have surface densities of up to 6 μmol/m^2 [7]. Solution polymerized phases exhibit enhanced selectivity toward shape-constrained solutes; however, relatively few commercial columns are prepared by this approach. If water is added to the silica prior to the addition of the silane, polymerization occurs on the surface of the substrate. Wirth and Fatunmbi [8,9] described this reaction for carefully hydrated silica as a self-assembled monolayer (SAM). These phases provide the highest surface coverages, and they can approach the theoretical limit of 8 μmol/m^2. Nuclear magnetic resonance (NMR) studies have shown that the alkyl chains are not as ordered as SAMs on gold, as the surface of the silica is not flat [10]. Even higher "calculated" surface densities result for surface polymerization reactions that utilize more water, although physically such phases cannot exist as monolayers [11]. Phases prepared by surface polymerization exhibit a high degree of shape recognition, but also suffer from marked peak asymmetry and band broadening [11,12].

The influence of bonding chemistry on chromatographic performance has been investigated for C_{18} chain length stationary phases. The synthetic approach used for surface modification was found to dramatically influence column retention, selectivity, and efficiency. For nonpolar solutes, the differences have been attributed largely to the levels of alkyl chain density that can be achieved by each approach, rather than the nature of the bond linkages [11]. In general, the retention of nonpolar solutes (sometimes referred to as "column hydrophobicity" or "column strength") increases with increased bonding density. Exceptions to this trend have been observed for stationary phases with very high bonding densities, for which absolute retention may actually decrease [11]. It has been hypothesized that this effect may be due to pore blockage or other restrictions that limit solute accessibility to all parts of the stationary phase [4].

Perhaps of more significance are changes in column selectivity that result from different surface modification approaches. Methylene selectivity ($\alpha_{\text{methylene}}$) is a measure of the ability of a column to separate alkyl homologs, as is defined by the selectivity factor $k'_{\text{ethylbenzene}}/k'_{\text{toluene}}$, or by the slope of the line fit to a plot of ln k' vs. homolog number (methylene selectivity $= e^{\text{slope}}$). Various aspects of methylene selectivity related to stationary phase morphology have been investigated [13–17]. Sentell and Dorsey synthesized monomeric phases with bonding densities that ranged from 1.7 to 4.1 μmol/m^2 and evaluated methylene selectivities using a series of alkylbenzene homologs [13]. They concluded that over this bonding density interval, methylene selectivity is not influenced by bonding density, and the degree of phase ordering incurred with high bonding density phases does not affect the "nonspecific hydrophobic interactions" that cause methylene selectivity [13]. Lochmüller and Wilder have shown that the methylene selectivity of solutes in RPLC is comparable to those for partitioning between liquid n-alkanes and methanol–water [14].

The influence of phase length on shape selectivity has also been investigated. Sander and Wise found that stationary phases with chain lengths shorter than C_{18} provided little shape recognition [18], while longer phases provided increased selectivity [19], especially for large carotenoid isomers [20]. It has been hypothesized that the shorter chain phases do not provide deep enough "slots" to differentiate between the different solute shapes [21].

The influence of bonding chemistry on the relative retention of shape constrained isomers and related compounds (referred to as "shape selectivity" or "steric selectivity") has been studied in considerable

detail [6,11,22,23]. At room temperature, monomeric phases offer little ability to separate solutes on the basis of shape [11,22,23]. Solution polymerized phases exhibit a greater degree of alkyl phase order and have increased shape selectivity, and surface polymerized phases show the highest degree of shape recognition [11].

Considerable effort has been expended in the study of stationary phases with alkyl chain length effects in LC. In the literature, the influence of bonded phase length on methylene selectivity appears to be somewhat contradictory. Horvath and Melander found that there are no significant changes in methylene selectivity with bonded phase length beyond a phase length of C_8 [15]. In contrast, Berendsen and de Galen found that there is a "critical phase length" between C_6 and C_{10}, above which retention does not increase for small aromatic probes [16]. This was supported by work by Lochmüller and Wilder, who found that the methylene selectivity increased until the phase length reached C_{10} [14]. Krstulovic et al. found that the selectivity increased with increasing bonded phase length provided that the phase length exceeded the solute length [17].

Octadecylsilyl (C_{18}) phases comprise most commercial stationary phases used in RPLC. Initially, the utilization of octadecylsilane in stationary phases synthesis was probably based on convenience since these reagents were relatively inexpensive and readily available. Investigations of alkyl chain length effects in RPLC have utilized even chain length silanes for similar reasons—odd alkyl chain length silanes are not commercially available and the corresponding odd chain length terminal alkenes used to synthesize these silanes are expensive. Because differences have been observed between certain nonchromatographic properties of odd and even chain length systems, investigations of chromatographic behavior may also be warranted. Spectroscopic evidence suggests that densely packed odd length alkyl chains are oriented differently relative to the surface than even chains [24]. In addition, it has been shown that odd/even alkyl chain length differences affect the reactivity of terminal methyl groups in alkenes [25]. Since the odd length chains have not been used for chromatographic purposes, it is unknown if the orientation of the terminal methyl group effects the separation and selectivity of the phase.

This study builds on previous studies of the influence of phase length and bonding density on methylene and shape selectivities, and provides a systematic investigation of the variables for C_{13} to C_{18} chain length phases prepared by monomeric, solution polymerized, and surface polymerized surface modification chemistries. Does the bonding chemistry change the chromatographic characteristics of the stationary phase, or are these differences merely a result of the different bonding densities achieved through the different synthetic approaches?

2. Experimental[1]

2.1. Materials and instrumentation

Reagents for the synthesis of the silanes and stationary phases were obtained from the following sources: dimethyloctadecylchlorosilane and octadecyltricholorosilane were obtained from United Chemical Technologies (formerly Huls America, Bristol, PA, USA); monochlorosilane, trichlorosilane, hexadecene, tetradecene, tridecene, and hexachloroplatinic acid were purchased from Aldrich (Milwaukee, WI, USA); heptadecene and pentadecene were obtained from TCI America (Portland, OR, USA). All phases were synthesized on YMC Sil-200-S3 spherical silica, lot 1908, 3 μm particles with an average pore diameter of 231 Å and a nominal surface area of 177 m^2/g. Bonding densities were calculated from the carbon determinations performed by Atlantic Microlabs (Norcross, GA, USA) after drying the phases in a vacuum oven for 1 h at 100 °C. Shape selectivity was evaluated with Standard Reference Material (SRM) 869a "Column Selectivity Test Mixture for Liquid Chromatography", obtained from the Standard Reference Ma-

---

[1]Certain commercial equipment, instruments, or materials are identified in this report to specify adequately the experimental procedure. Such identification does not imply recommendation or endorsement by the National Institute of Standards and Technology, nor does it imply that the materials or equipment are necessarily the best available for the purpose.

terials Group [National Institute of Standards and Technology (NIST), Gaithersburg, MD, USA]. Methylene selectivity was tested with a homologous series of alkylbenzenes from toluene to nonadecylbenzene. HPLC-grade solvents were used in all chromatographic separations.

2.2. Silane synthesis

The C_{13} through C_{17} silanes used in this study were synthesized through the catalytic hydrosilylation of a terminal alkene as described by Berendsen et al. [26]. Approximately 0.1 mol of the alkene was heated and stirred under nitrogen with approximately 1 mg of the hexachloroplatinic acid catalyst until the catalyst was dispersed in solution. After cooling the reaction vessel in an ice bath, a slight molar excess of dimethylmonochlorosilane or trichlorosilane was added to the reaction mixture over a period of approximately 10 min. The solution was allowed to stir under nitrogen at room temperature for approximately 1 h after the addition of the silane. The product was then vacuum distilled to remove the alkylsilane of interest from the catalyst.

2.3. Bonded phase synthesis

The monomeric phases were prepared by adding a molar excess of the appropriate monochlorosilane to a solution of xylene and unmodified chromatographic silica. In addition, dimethylaminopyridine [27] was added to the reaction mixture to act as a scavenger base. The mixture was refluxed for 24 h, filtered hot, and washed with two 50-ml portions each of hot xylene, acetone, methanol, water, acetone, and pentane. A small portion of the stationary phase was removed and dried for carbon analysis; the remaining portion was stored in isopropanol until it was packed.

The solution polymerized phases were synthesized through the solution polymerization of trichloroalkylsilanes in the presence of water [7]. Untreated silica was dispersed in xylene, and a slight molar excess of the appropriate trichloroalkylsilane along with a specific volume of water were added to the mixture. The mixture was heated at reflux for 4 h, filtered hot, washed, and stored in the same manner as the monomeric phases.

The surface polymerized phases were prepared through the surface polymerization of the trichloroalkylsilane [28]. In this procedure, the silica was dried at 100 °C for approximately 24 h, and then hydrated for 3 h by exposure to air saturated with water at 23 °C (±2 °C) [28]. This humidified silica was added to xylene containing a slight molar excess of the trichloroalkylsilane. The reaction was allowed to stand at room temperature for approximately 16 h with occasional resuspension of the stationary phase in solution. It was then refluxed for approximately 1 h, washed and stored in the same manner as the monomeric phases.

2.4. Chromatography

Each stationary phase was slurry packed in a 15 cm×4.6 mm I.D. stainless steel column. An acetonitrile–water (85:15, v/v) mixture was used for the separation of SRM 869a, at a flow-rate of 1.5 ml/min and temperatures ranging from 0 to 40 °C in 10 °C increments. The temperature was controlled with a column jacket and circulating water bath. The majority of the separations of the homologous series of alkylbenzenes utilized an acetonitrile–water (85:15, v/v) mixture, at 1.5 ml/min at 25 °C. In some cases, the mobile phase strength and temperature were changed for the investigation of trends in methylene selectivity; these cases are noted within the text. The determination of void time is a controversial matter [29], however, for this comparison of bonded phase selectivity, the solvent disturbance method was found to provide reasonable results with acceptable error. Small variations in k' and/or α values that may result from the determination of the void time are not expected to affect the interpretation of the observed trends. A manual injector was used, for the injection of 5 µl of each sample. Detection, in all experiments, was performed at 254 nm.

3. Results

3.1. Synthesized stationary phases.

All of the stationary phases were submitted for carbon analysis, and bonding densities were calculated as previously described [26]. Carbon analysis

results and bonding densities are summarized in Table 1. The bonding densities of the monomeric phases are virtually identical, with the exception of the C_{16} phase and one of the C_{18} phases. Due to the reduced shape selectivity and bonding density of the original monomeric C_{18} phase, the synthesis was repeated, resulting in a phase with a bonding density comparable to the other monomeric phases. Similarly, the surface polymerized phases are very close in bonding density, with the exception of the C_{14} phase and one of the C_{16} phases. In this case, the C_{16} synthesis was repeated and the C_{14} phase was used for comparison with monomeric phases. The low density surface polymerized phases were due to low yield hydrosilylation reactions rather than the attachment of the trifunctional silanes to the silica surface. Finally, the solution polymerized phases show the greatest degree of variation; however, at each chain length bonding densities for the solution polymerized phases are intermediate to the monomeric and surface polymerized phases. The variety of bonding densities at each chain length facilitates comparison

of the effects of chain length and bonding chemistry on the chromatographic performance of the phases.

3.2. Methylene selectivity

A homologous series of alkyl benzenes from toluene though nonadecylbenzene was injected on each column. Column temperature was carefully controlled with a circulating fluid bath and a column jacket. Two methods were used to calculate the methylene selectivity. In the first method, the natural log of the retention factor was plotted versus the homolog number; the slope of the resulting straight line was ln (methylene selectivity). Methylene selectivity was also calculated for each successive pair of the homologous series as k'_{n+1}/k'_n and the results for the series were averaged. The two methods provided similar results.

Methylene selectivity was found to vary only over a narrow range for the various stationary phases (i.e., 1.28 to 1.71). In general, methylene selectivity was observed to increase with decreasing temperature and

Table 1
Summary of physical and chromatographic characteristics of stationary phases synthesized for this study

Bonding chemistry	Chain length	Bonding density (μmol/m^2)[a]	Methylene selectivity (from slope of ln k' vs. homolog No.)[b]
Monomeric[c]	18	1.19	1.28
Monomeric	18	3.59	1.42
Monomeric	17	3.56	1.42
Monomeric	16	2.11	1.31
Monomeric	15	3.48	1.39
Monomeric	14	3.64	1.38
Monomeric	13	3.63	1.42
Solution polymerized	18	5.11	1.48
Solution polymerized	17	5.50	1.47
Solution polymerized	16	4.59	1.39
Solution polymerized	15	5.60	1.39
Solution polymerized	14	4.21	1.32
Solution polymerized	13	5.92	1.38
Surface polymerized	18	7.97	1.71
Surface polymerized	17	7.28	1.53
Surface polymerized[c]	16	2.73	1.33
Surface polymerized	16	7.14	1.50
Surface polymerized	15	7.00	1.44
Surface polymerized	14	1.90	1.28
Surface polymerized	13	7.41	1.33

[a] Bonding density measurements are based on a single surface area measurement reported by the manufacturer.

[b] The standard deviation of the mean is less than 0.001 for the measurement of methylene selectivity.

[c] The synthesis was repeated due to the low bonding density of the phase. Both phases were used in the study.

with decreasing mobile phase strength. A slight decrease in methylene selectivity was observed with decreasing bonded phase length.

Interestingly, at constant temperature, mobile phase composition, and bonded phase chain length, methylene selectivity was observed to increase with bonding density, regardless of bonding chemistry. An example of this trend is illustrated in Fig. 1 for the C_{16} and C_{18} stationary phases. In a previous study carried out with monomeric stationary phases, bonding density was not observed to influence methylene selectivity [13]. This conclusion is perhaps not unexpected given the weak dependence of methylene selectivity on bonding density and the narrow range of bonding densities that were studied.

3.3. Shape selectivity

The ability of a phase to separate solutes based on shape is often described as "shape selectivity". SRM 869a, "Column Selectivity Test Mixture for Liquid Chromatography" was used to determine shape selectivity [30]. The test mixture is comprised of benzo[a]pyrene (BaP), tetrabenzonaphthalene (TBN), and phenanththrophenanthrene (PhPh). SRM 869a has been utilized extensively with C_{18} phases.
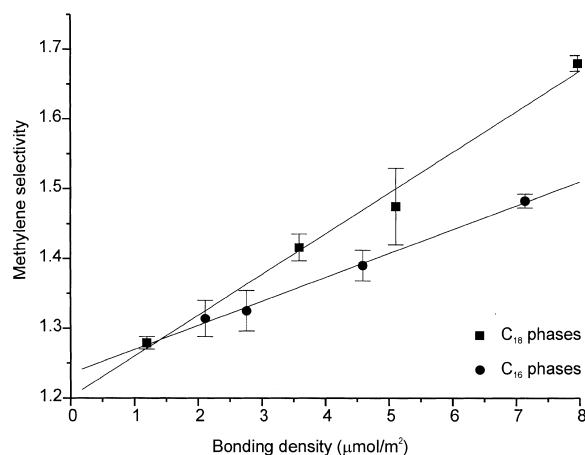

Fig. 1. Methylene selectivity plotted as a function of bonding density for C_{16} and C_{18} columns. Data are based on five replicate injections of alkylbenzene homologs separated at 25 °C, at 1.5 ml/min, in acetonitrile–water (85:15) mobile phase. Error bars represent ±1 standard deviation. ■ C_{18} phase, ● C_{16} phase.
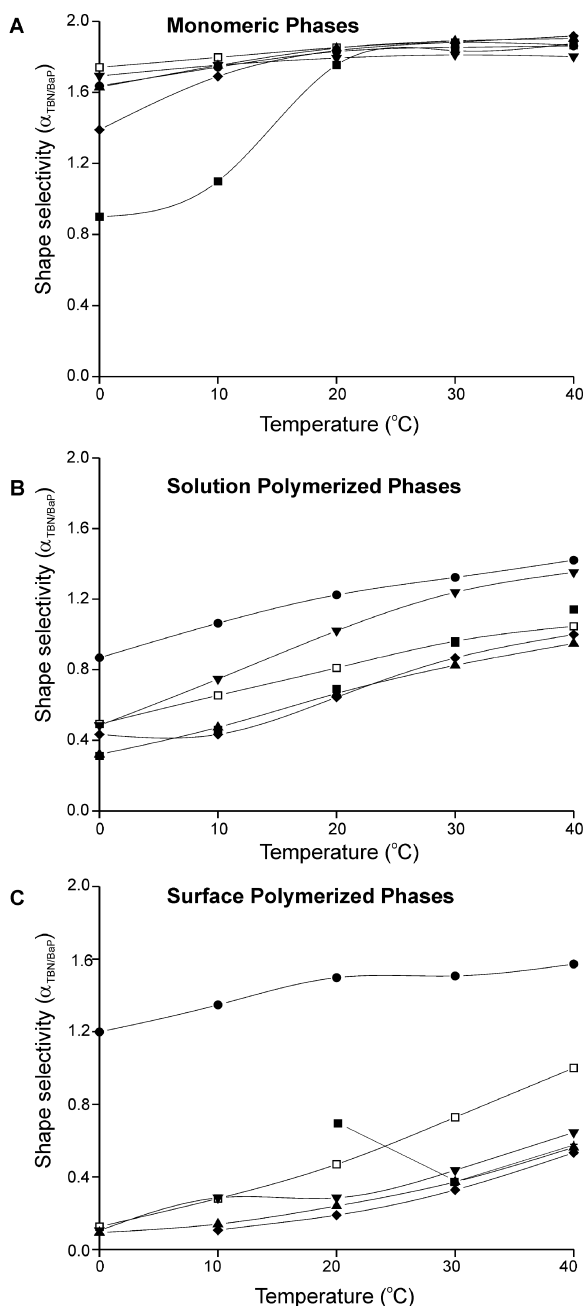

Fig. 2. Selectivity factor ($\alpha_{TBN/BaP}$; i.e., shape selectivity) plotted as a function of temperature for different stationary phases. (A) monomeric phases, (B) solution polymerized phases, and (C) surface polymerized stationary phases. Data represent the mean of three replicate injections. The RSD was found to be less than 1% for all measurements. □ C_{13}, ● C_{14}, ▲ C_{15}, ▼ C_{16}, ♦ C_{17}, ■ C_{18}.
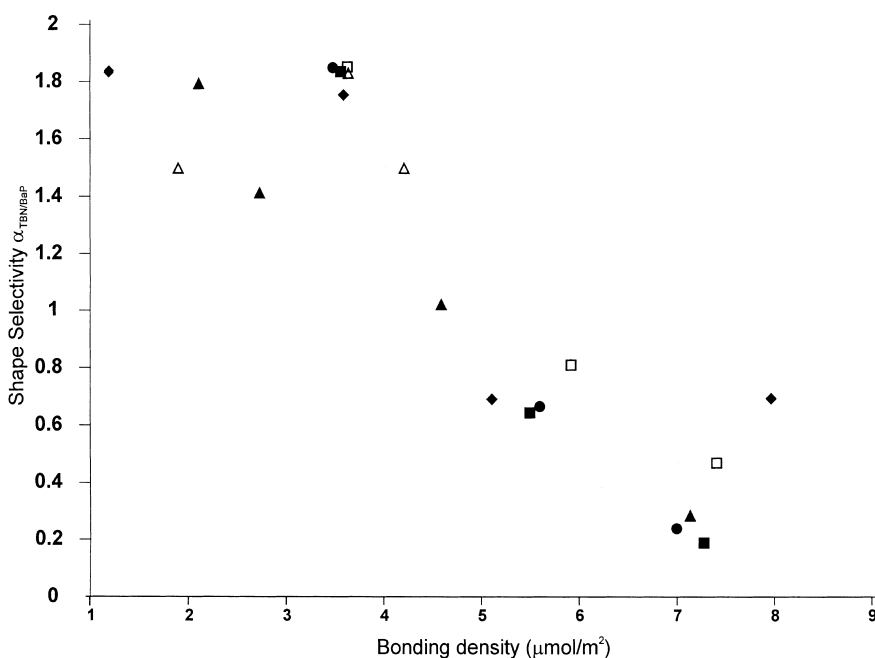
Fig. 3. Dependence of shape selectivity on bonding density at 20 °C. ♦ C_{18}, ■ C_{17}, ▲ C_{16}, ● C_{15}, △ C_{14}, □ C_{13}.

Values of $\alpha_{TBN/BaP} > 1.7$ are indicative of "mono-meric-like" phases with low shape selectivity; and phases with $\alpha_{TBN/BaP} < 1.0$ are indicative of "poly-meric-like" phases that exhibit the ability to dif-ferentiate between shape constrained solutes [12,30].

Increased shape recognition has been attributed to the degree of chain ordering in the phase [31]. The well-ordered chains form slots, which discriminate planar and non-planar solutes [21]. Stationary phase order and shape recognition have been shown to increase with increased stationary phase bonding density. It has also been shown that temperature can have a significant effect on phase ordering. By decreasing the column temperature from 20 to 0 °C, a monomeric C_{18} phase can take on attributes of a polymeric C_{18} phase [23].

Changes in shape selectivity ($\alpha_{TBN/BaP}$) are shown as a function of temperature for C_{13} to C_{18} mono-meric, polymeric, and self-assembled monolayer phases in Fig. 2. The different phase lengths are also represented at each type of bonding chemistry. Dramatic changes in selectivity are evident for the

monomeric C_{18} phase, with changes in column temperature (Fig. 2a). The shape of this curve is similar to that found in previous work [32]; however, the changes observed for the shorter alkyl chain length phases are unexpected. At low temperature the C_{17} phase data is similar to, but less pronounced than the C_{18} data. The change in the temperature dependence of shape selectivity with alkyl chain length is striking. The bonding densities for the C_{18} and C_{17} monomeric phases are nearly identical (3.59 vs. 3.56 μmol/m^2, respectively) and yet large differ-ences in shape selectivity ($\alpha_{TBN/BaP}$) are apparent at subambient temperatures. For the temperature inter-val studied, C_{16} and shorter length monomeric phases exhibit essentially the same minimal tempera-ture dependence for shape selectivity (i.e., little or no shape recognition). More regular changes in shape selectivity with temperature are observed for the solution polymerized and surface polymerized stationary phases. Both solution polymerized and surface polymerized stationary phases exhibit nearly linear relationships between shape selectivity

($\alpha_{TBN/BaP}$) and temperature for the temperature interval investigated. Increased shape recognition is also observed for increased bonding density for stationary phases of the same alkyl chain length as shown in Fig. 3.

As the shape selectivity is dependent on the bonding density of the phase, it is not surprising that surface polymerized phases exhibit the highest degree of shape recognition, monomeric phases show the lowest degree of shape recognition, and solution polymerized phases are intermediate. Along with increased shape selectivity, surface polymerized phases exhibit increased retention, peak broadening, and asymmetry, which limits their usefulness. This is

illustrated with separations for a series of C_{17} phases in Fig. 4 (see Table 2 for peak statistics). Fig. 4 also demonstrates the effect of tightly packed alkyl chains on chromatographic retention. The retention of phenanthrophenanthrene and tetrabenzonaphthalene decreased on the surface polymerized phases when compared with the monomeric and solution polymerized phases. In contrast, the retention of benzo[a]pyrene increased with increasing bonding density. It is hypothesized that due to the density of the phase the non-planar solutes partition less into the bonded phase. However, planar solutes, such as benzo[a]pyrene are able to access the "slots" between the alkyl chains.
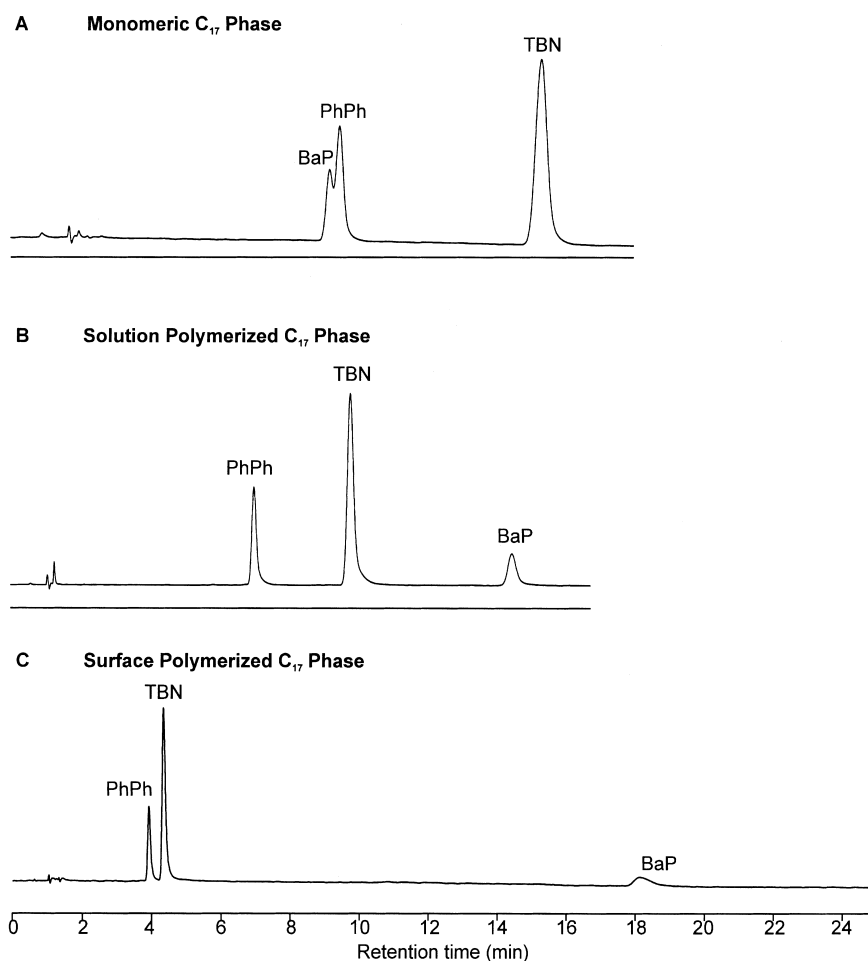

Fig. 4. Separation of Standard Reference Material 869a on C_{17} phases.

Table 2
Comparison of the chromatographic characteristics of monomeric, solution polymerized, and surface polymerized C_{17} phases[a]

	Monomeric	Solution polymerized	Surface polymerized
Bonding density (μmol/m^2)	3.56	5.50	7.28
$\alpha_{TBN/BaP}$	1.67 ± 0.002	0.64 ± 0.004	0.19 ± 0.002
A_s[b]	1.07 ± 0.02	1.32 ± 0.05	1.57 ± 0.15
N (column)[c]	$10\,000\pm200$	$13\,000\pm300$	$10\,000\pm500$

Measurements are an average of three replicate injections and are reported as ±1 standard deviation.

[a] Mobile phase acetonitrile–water (85:15), flow 1.5 ml/min, temperature 20 °C.

[b] Asymmetry was calculated as $A_s = R_{w10\%}/L_{w10\%}$, where $R_{w10\%}$ and $L_{w10\%}$ are the right and left peak widths measured at 10% of the peak height (calculated for TBN).

[c] The approximate number of theoretical plates was calculated as $N = 16(t_R/w_b)^2$, where t_R is the retention time and w_b is the peak width at baseline (calculated for TBN).

In each case, the efficiency of the surface polymerized phase was significantly lower than the efficiency of the corresponding monomeric and solution polymerized phases. This is to be expected since the surface polymerized phases have much higher alkyl ligand densities and reduced mass transfer kinetics. Surprisingly, the efficiencies of all of the solution polymerized phases were slightly higher than the monomeric phases. While this small difference may be due to variations in column packing, it does indicate that solution polymerized phases do not yield significantly lower efficiency separations than monomeric phases.

4. Conclusions

The performance of stationary phases in RPLC is influenced in different ways by the bonding chemistry, surface coverage, and alkyl chain length of the sorbent. For stationary phases ranging in length from C_{13} to C_{18}, retention behavior towards nonpolar solutes is most strongly influenced by the density of the alkyl chains at the silica surface. Column selectivity toward shape-constrained solutes increases with increased surface coverage; however, column efficiency and peak shape are degraded for densely loaded stationary phases. The approach used in bonded phase synthesis places limits on the density of the alkyl chains that can be achieved. Dramatic changes in shape selectivity that occur as a function of temperature are evident for moderately loaded (monomeric) alkyl phases that differ by only one carbon in length.

References

[1] L.C. Sander, S.A. Wise, CRC Crit. Rev. Anal. Chem. 18 (1987) 299.
[2] J.G. Dorsey, W.T. Cooper, B.A. Siles, J.P. Foley, H.G. Barth, Anal. Chem. 68 (1996) 515R.
[3] A. Berthod, J. Chromatogr. A 549 (1991) 1.
[4] L.C. Sander, S.A. Wise, J. Chromatogr. 316 (1984) 163.
[5] K.K. Unger, Porous Silica, Elsevier, Amsterdam, 1979.
[6] H. Engelhardt, N. Nikolov, M. Arangio, M. Scherer, Chromatographia 48 (1998) 183.
[7] L.C. Sander, S.A. Wise, Anal. Chem. 56 (1984) 504.
[8] M.J. Wirth, H.O. Fatunmbi, Anal. Chem. 64 (1992) 2783.
[9] M.J. Wirth, H.O. Futunmbi, Anal. Chem. 65 (1993) 822.
[10] A. Badia, W. Gao, S. Singh, L. Demers, L. Cuccia, L. Reven, Langmuir 12 (1996) 1262.
[11] L.C. Sander, S.A. Wise, Anal. Chem. 67 (1995) 3284.
[12] L.C. Sander, M. Pursch, S.A. Wise, Anal. Chem. 71 (1999) 4821.
[13] K.B. Sentell, J.G. Dorsey, J. Chromatogr. 461 (1989) 193.
[14] C.H. Lochmüller, D.R. Wilder, J. Chromatogr. Sci. 17 (1979) 574.
[15] W.R. Melander, Cs. Horvath, Chromatographia 15 (1982) 86.
[16] G.E. Berendsen, L. de Galan, J. Chromatogr. 196 (1980) 21.
[17] A.M. Krstulovic, H. Colin, A. Tchapla, G. Guiochon, Chromatographia 17 (1983) 228.
[18] L.C. Sander, S.A. Wise, Anal. Chem. 59 (1987) 2309.
[19] S. Strohschein, M. Pursch, D. Lubda, K. Albert, Anal. Chem. 70 (1998) 13.
[20] C.M. Bell, L.C. Sander, J.C. Fetzer, S.A. Wise, J. Chromatogr. A 753 (1996) 37.
[21] S.A. Wise, L.C. Sander, J. High Resolut. Chromatogr., Chromatogr. Commun. 8 (1985) 248.
[22] L.C. Sander, S.A. Wise, LC·GC 8 (1990) 378.
[23] L.C. Sander, S.A. Wise, J. Sep. Sci. 24 (2001) 910.
[24] R.G. Nuzzo, L.H. Dubois, D.L. Allara, J. Am. Chem. Soc. 112 (1990) 558.
[25] V.J. Angelico, S.A. Mitchell, V.H. Wysocki, Anal. Chem. 72 (2000) 2603.

[26] G.E. Berendsen, K.A. Pikaart, L. de Galan, J. Liq. Chromatogr. 3 (1980) 1437.

[27] K.B. Sentell, K.W. Barnes, J.G. Dorsey, J. Chromatogr. 455 (1988) 95.

[28] M. Pursch, D.L. Vanderhart, L.C. Sander, X. Gu, T. Nguyen, S.A. Wise, J. Am. Chem. Soc. 122 (2000) 6997.

[29] C.A. Rimmer, C.R. Simmons, J.G. Dorsey, J. Chromatogr. A 965 (2002) 219.

[30] SRM 869a, Column Selectivity Test Mixture for Liquid Chromatography (Polycyclic Aromatic Hydrocarbons). Certificate of Analysis, NIST, Gaithersburg, MD, 1998.

[31] M. Pursch, L.C. Sander, K. Albert, Anal. Chem. 68 (1996) 4107.

[32] L.C. Sander, S.A. Wise, Anal. Chem. 61 (1989) 1749.