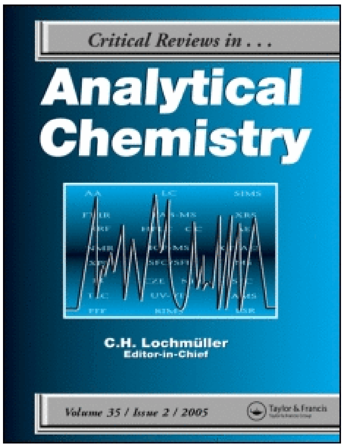

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RECENT ADVANCES IN BONDED PHASES FOR LIQUID CHROMATOGRAPHY

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RECENT ADVANCES IN BONDED PHASES FOR LIQUID CHROMATOGRAPHY

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I. INTRODUCTION

Over the past 10 years high performance liquid chromatography (LC) has evolved into a mature analytical technique. Most LC separations are currently carried out in the reversed-phase mode using 25-cm columns filled with 5 or 10 μm nonpolar bonded substrates. A smaller fraction of separations are carried out in the normal-phase mode with polar bonded phases. Although major changes in column technology have become less commonplace in recent times, refinements continue to be made. New bonded phases have been developed for the separation of specific classes of compounds and new substrates have been introduced that extend column lifetimes and permit use over wide pH intervals. Even though bonded phase usage is now common, solute retention mechanisms with these materials are not fully understood (but considerable progress has been made). To aid the study of retention mechanisms, novel approaches have been taken in the physical and chemical characterization of bonded phase sorbents.

This review will cover theoretical and practical aspects of bonded phase research carried out over the past 5 to 10 years. Several excellent reviews have been published encompassing earlier work.[1-10] More recently, Sander and Wise[11] have published a review dealing with bonded phase selectivity towards polycyclic aromatic hydrocarbons (PAH). The proceedings of Silanes, Surfaces, and Interfaces Symposium have been published[12] containing numerous papers on recent developments in the field of silane chemistry and surface characterization. Trends in column usage and new column introductions have been reported by Majors.[4,13-17] This paper will review research concerning LC substrates, bonded phase synthesis and novel bonded phases methods used in the characterization of these materials, and progress in the development of retention theory. An effort was made to include studies representative of the more important ongoing research in bonded phase technology. Not included in this review is work dealing with ion exchange chromatography, immobilized enzymes, size exclusion chromatography, or purely mechanical aspects of LC. A summary of the references cited dealing with bonded phase synthesis is tabulated by bonded phase type and authors in Appendix 1. A similar tabulation for bonded phase characterization techniques is presented in Appendix 2.

II. BONDED PHASE SYNTHESES

A. Monomeric Alkyl Syntheses

Recent surveys[13] of chromatographic practices have shown that over one half of chromatographic separations are performed in the reversed-phase mode on monomeric alkyl bonded phase columns. About one fourth of the separations are carried out in the normal-

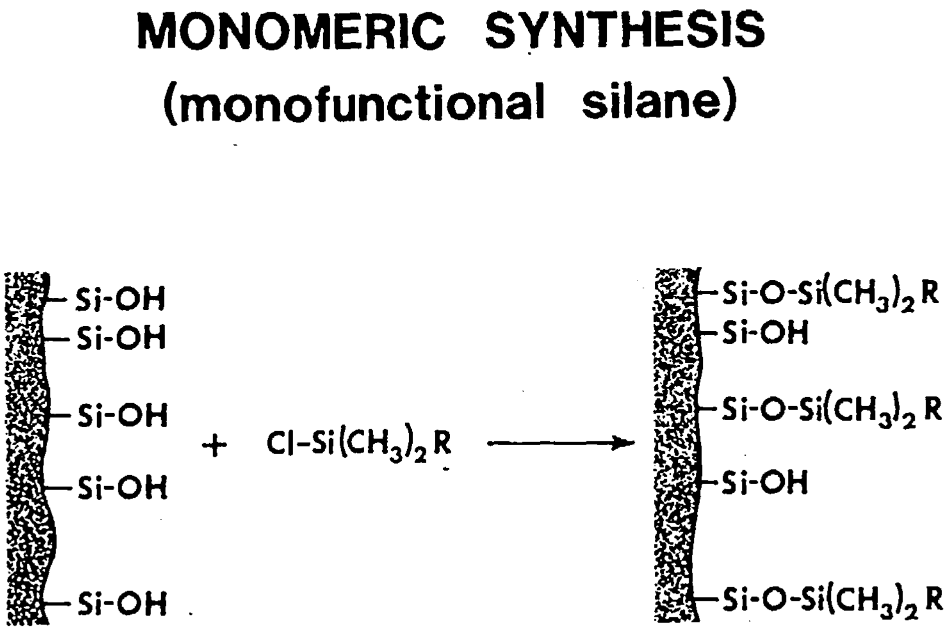


FIGURE 1. Monomeric bonded phase synthesis using monofunctional silanes. (From Sander, L. C. and Wise, S. A., *Advances in Chromatography*, Vol. 25, Marcel Dekker, New York, 1986, 139. With permission.)

phase mode, and the remaining separations are split between ion exchange and size exclusion chromatography. The popularity of alkyl phases can be attributed to the applicability of these phases to diverse separation problems. The synthesis of monomeric alkyl bonded phases has become routine in research, and most (if not all) commercial alkyl phases are monomeric in nature. For research purposes, homemade phases are often preferable to commercial phases. The proprietary nature of commercial columns does not lend itself to fundamental studies of retention processes. Homemade columns, on the other hand, can be synthesized to required specifications using known reagents and procedures. Thus, greater control can be achieved over the bonded phase parameters that ultimately influence solute retention. Phase differences among homemade columns can be more easily assessed than with commercial columns, and the resulting variations in retention can be attributed to these differences. In this way, retention mechanisms in reversed-phase LC are elucidated.

Monomeric alkyl phases are produced by the reaction of monofunctional silanes (e.g., monochloro or monoalkoxysilanes) with silanols on the silica surface as shown in Figure 1. Monomeric phases can also be prepared by the reaction of silica with di- or trifunctional silanes, if care is taken to exclude water from the reaction. Reaction of a monofunctional silane with silica results in only a single bond linkage for each silane molecule that reacts with the silica. With polyfunctional silanes, one or two bonds with the silica surface may be possible per molecule (Figure 2). Steric constraints of bond angles make it highly unlikely for three bonds to form at the surface using a trifunctional silane. Phases produced from di- and trifunctional silanes are sometimes incorrectly referred to as polymeric phases. However, when care is taken to exclude water the dominant properties of the resulting phase are similar to those for phases prepared with a monofunctional silane reagent. If trace quantities of water are present in the reaction slurry when using polyfunctional silanes, polymerization reactions may occur resulting in phases with heavier loadings and altered selectivity.

The scientific literature is rich with papers describing the synthesis of monomeric alkyl stationary phases, primarily C_{18} phases. A goal of many of these works appears to be to achieve phase surface coverage values greater than those reported in prior studies. The

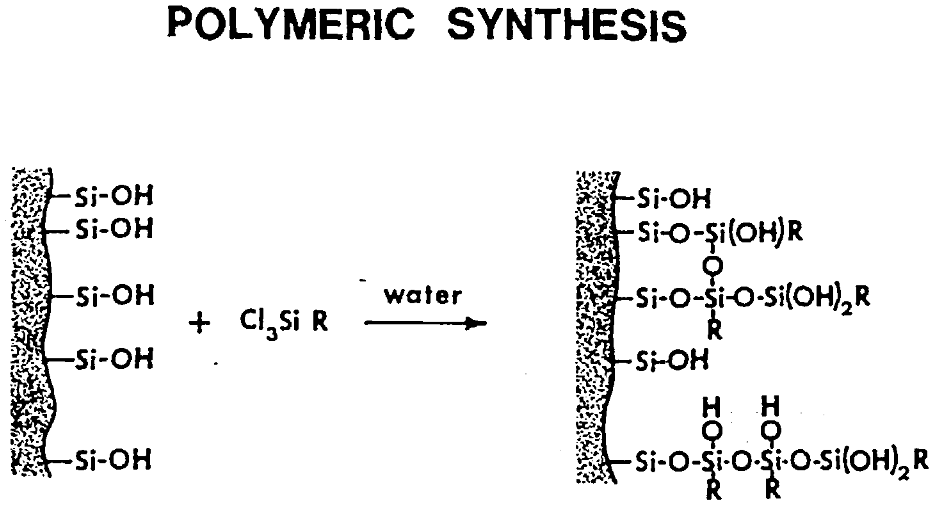


FIGURE 2. Polymeric bonded phase synthesis using polyfunctional silanes in the presence of water. Horizontal (cross-linking) and vertical polymerization reactions are illustrated. (From Sander, L. C. and Wise, S. A., *Advances in Chromatography*, Vol. 25, Marcel Dekker, New York, 1986, 139. With permission.)

rationale behind this reasoning is that by increasing phase surface coverage, a greater percentage of the silica silanols are either chemically modified or made inaccessible to interaction with solutes, and thus, tailing effects associated with silanols are reduced. Surface coverage values are usually reported in units of μmol/m^2 or groups/nm^2 (nm^{-2}). Berendsen and de Galan[18] derived expressions for the calculation of surface coverage values that account for the weight increase of silica due to the attachment of ligands and the loss of hydrogen in the silanization reaction (Equations 1 and 2):

$$N\,(\mu\text{mol/m}^2) = \frac{10^6\,P_c}{1200n_c \cdot P_c(M - 1)}\,\frac{1}{S} \tag{1}$$

$$N\,(\text{nm}^{-2}) = \frac{6.02 \times 10^5\,P_c}{1200n_c - P_c(M - 1)}\,\frac{1}{S} \tag{2}$$

where P_c is the percent carbon loading of the bonded phase, n_c is the number of carbon atoms in the silane reagent, S is the surface area of the unbonded substrate, and M is the molecular weight of the bonded silane ligand. Comparison of the two equations reveals that interconversion of units is easy:

$$N\,(\text{nm}^{-2}) = 0.6\,N\,(\mu\text{mol/m}^2) \tag{3}$$

The extent of surface modification in bonded phase syntheses is dependent upon the reaction conditions as well as the physical and chemical properties of the substrate and silane reagent. These parameters have been the subject of numerous investigations, many of which are described below. It is generally agreed that the surface silanol concentration for most forms of silica is about 8 μmol/m^2 (4.8 silanols per square nanometer). Due to steric constraints, only about one half of these groups can be chemically modified. Attached ligands shield neighboring unbonded silanols from reaction. This limit is affected by silane functionality, reactivity, and, of course, size. Higher surface coverages result from the reaction

Table 1
SURFACE COVERAGES OF *N*-ALKYLSILYL BONDED PHASES[20]

Chain length	Carbon (%)		Surface coverage	
	Direct	Endcapped	μmol/m²	nm⁻²
1	4.01	—	3.87	2.33
3	6.01	6.00	3.56	2.14
6	9.23	8.99	3.51	2.11
10	12.72	12.62	3.41	2.05
14	15.51	15.63	3.26	1.97
18	17.65	17.58	3.04	1.83
22	20.03	20.03	2.98	1.80

Table 2
SURFACE COVERAGES OF PHASES WITH TERMINAL FUNCTIONAL GROUPS[20]

Functional group	Carbon (%)	Surface coverage	
		μmol/m²	nm⁻²
$-NH_3$	4.63	2.35	1.41
$-N(CH_3)_2$	6.89	3.11	1.87
-CN	6.43	3.32	2.00
-Cl	5.49	3.49	2.10
-H	5.48	3.36	2.03
-C=C	7.32	3.27	1.97
-cyclohexyl	10.66	3.17	1.91
-phenyl	10.81	3.20	1.93

of small reactive silanes (e.g., trimethylchlorosilane) than from bulky, less reactive silanes (e.g., dimethyloctadecylethoxysilane). One of the highest (monomeric) ligand densities reported[19] for silica was 4.78 μmol/m², using trimethyl(dimethylamino)silane. Coverages ~ 3.9 μmol/m² and less are more typical for phases prepared from dimethylmonochlorosilanes (see Tables 1 and 2).

Most bonded phases are currently prepared by the action of reactive organosilicon reagents on silica. Chloro- and alkoxysilanes are, perhaps, the most common silanizing agents; however, hydroxy-, amino-, and dimethylaminosilanes are also used. A wide variety of silane reagents are commercially available that are suitable for bonded phase synthesis, both for routine and novel phase synthesis. Silanes can also be synthesized from a "parent silane molecule" that contains a reactive substituent (chloro, alkoxy, etc.) and from the alkene form of the ligand of interest. For example, dimethylbutylchlorosilane can be prepared from dimethylchlorosilane and butene. Berendsen and de Galan[20] have described and applied this method of synthesis, termed the hydrosilyation reaction, to the preparation of dimethylalkylchlorosilanes with various alkyl chain lengths. Briefly, this reaction involves the anti-Markovnikov addition of a hydrosilane to a terminal alkene (Equation 4):

$$R\text{–}(CH_2)_m\text{–}CH{=}CH_2 + (CH_3)_2\,ClSiH \rightarrow R\text{–}(CH_2)_{m+2}\,Si(CH_3)_2\,Cl \qquad (4)$$

The reaction proceeds rapidly in the presence of a catalyst (hexachloroplatinic acid hexahydrate, $H_2PtCl_6 \cdot 6H_2O$) to give nearly quantitative yields (~ 80%). Substituted alkenes may also be used with this reaction with the stipulation that functional groups that react with the chlorosilane must be shielded from reaction by protecting groups which are removed after hydrosilylation. The silane product is purified by vacuum distillation. For polar silanes, distillation may be impractical because of high boiling points. Recently, the need for a final purification step has been questioned. Shih-Hsien et al.[21] prepared octyl- and octadecyldimethylchlorosilanes by hydrosilylation. Phases were synthesized from the crude silane product without separation from reactants or byproducts. In part of the study, activated carbon was added prior to phase synthesis to remove the hexachloroplatinic acid catalyst (the activated carbon was removed by filtration). Phases produced by this *in situ* synthesis procedure were found to be reproducible and comparable to phases prepared from pure silanes. When the catalyst is not removed by activated carbon, significant levels of platinum are present in the sorbent. No chromatographic difference, however, was observed between the phases with high or low platinum levels, at least for the solutes used. This *in situ* silanization procedure was suggested as applicable to polar, ionic, or other silanes with high boiling points. Also applicable would be exotic silanes that, due to expense or difficulty in synthesis, make conservation desirable.

In a study by Unger et al.,[22] monomeric alkyl phases ranging from C_1 to C_{18} were prepared by reaction of anhydrous silica with monochloro- and monoalkoxysilane reagents. The reactions were carried out without using a solvent by allowing the silane to condense onto the silica while under reduced pressure. The temperature was set at 20°C less than the boiling point of the silane, and the reaction was carried out for 8 hr. Surface coverage values were highest for the trimethylsilyl phase (C_1, 4.5 μmol/m^2) and lowest for the triphenylsilyl phase (1.5 μmol/m^2). A second type of bonded phase synthesis was described that involved the cohydrolysis and cocondensation of organic silanes with tetraethoxysilane (TES) and poly(ethoxysiloxane) (PES). This type of reaction, termed bulk modification, results in silica with the organic moieties uniformly distributed throughout the substrate. A practical limit of about 30 mol % of organic silane in TES is imposed by structural requirements of the substrate. At higher contents, swelling occurs in the presence of organic solvents and such substrates tend to be compressible.

Surface modification reactions were also carried out using mono-, di-, and trifunctional silanes. Careful evaluation of the phases indicated that for the di- and trifunctional silanes the stoichiometric factor (F) on average was between 1 and 2, i.e., one or two groups per silane molecule react with the silica surface. No evidence was found to suggest that three linkages per silane molecule were formed. In another study by Roumeliotis and Unger,[23] the effects of alkyl chain length and silane reagent functionality were examined as related to bonded phase surface coverage (α_{exp}, μmol/m^2). Mono-, di-, and trichlorooctadecylsilanes were used under identical reaction conditions to synthesize monomeric C_{18} phases. Surface coverage values for the resulting phases were 2.35, 2.40, and 2.35 μmol/m^2, respectively, essentially no difference. Residual silanol groups were also measured for these phases using an isotope exchange method with tritium-labeled water (HTO).[24] The silanol surface concentration values were considerably different: monochloro phase, 3.73 μmol/m^2; dichloro phase, 6.74 μmol/m^2; and the trichloro phase, 11.70 μmol/m^2. Thus, unreacted chloro groups on the silane hydrolyze after surface modification to give rise to newly formed silane silanols. These groups are accessible for interaction with solutes as are the silica silanols and can seriously affect chromatographic properties. Roumeliotis and Unger[23] also prepared a series of phases of alkyl chain lengths ranging from C_1 to C_{16}, using dimethylchlorosilanes. In general, phase surface coverage values decreased with increasing chain length. This trend has also been observed in other work[20,25-27] (e.g., see Table 1). Other physical properties of the alkyl phases were assessed, including changes in surface area and pore volume that occurred as a result of the surface modification. Measured values were then compared to theoretical values, which were calculated assuming a cylindrical pore model. Although the agreement between measured and theoretical surface areas was poor, pore volume comparisons were very good. The authors[23] concluded that the bonded phase thickness ranged from 0.3 nm for the trimethylsilyl phase to about 2.3 nm for the hexadecyldimethylsilyl phase. The reduction in pore volume for the latter phase was about 40%.

Berendsen and co-workers[18-20] also have studied the physical and chemical nature of dimethylalkyl bonded phases. Three classes of phases were prepared from dimethylalkylchlorosilanes: (1) octyl phases with surface coverages ranging from 0.8 to 3.5 μmol/m²; (2) propyl phases with various polar functional groups at the terminal position; and (3) C_1 to C_{22} dimethylalkyl phases with "maximum" surface coverages of 3.0 to 3.9 μmol/m², respectively. Silane reagents were synthesized through the hydrosilylation reaction.[28] Berendsen and co-workers[18-20] examined the effect of endcapping reactions on carbon loading for the "maximally loaded" phases. In each case, no significant difference in carbon loading values was observed after treatment of the alkyl phases with trimethylchlorosilane (see Table 1). From this result and from chromatographic tests, the authors concluded that endcapping is unnecessary for fully covered silicas operated in the reversed-phase mode. They are careful to point out that the effect of endcapping was not studied in the normal-phase mode.

Surface coverage values for polar-substituted dimethylpropylsilyl phases are listed in Table 2. With the exception of NH_2 phase, surface coverages are nearly independent of the polar substituent. This suggests that steric hindrance effects of bulky substituents are of reduced importance when removed a distance of only three to four carbons from the silica surface. Steric hindrance effects were also examined with regard to pore size. Surface coverage values were observed to increase as a function of pore diameter for phases prepared under identical conditions on different substrates. The explanation for this phenomenon is illustrated in Figure 3. For short alkyl ligands such as RP-3, surface coverage is limited by methyl groups attached to the silane silicon atom. As alkyl chain length increases, however, the curvature of the silica surface becomes important and surface coverage is limited by steric hindrance of the terminal ends of the alkyl chains. In the words of the author "the decrease in coverage is not determined by the available BET-surface area of the bare silica support, but by the area at the point where the bonded chains end." Thus, surface coverage values are smaller on narrow-pore substrates than on wide-pore substrates. The thermal stability of several alkyl bonded phases was examined by thermogravimetry combined with mass spectrometry (TG-MS). With this technique, the bonded phase sample is heated and mass spectra are recorded as a function of time. Two experiments were carried out. In the first, a C_{10} phase was heated in vacuum until mass spectra could be seen. Phase decomposition began at 310°C and was very pronounced at 420°C. The second experiment was carried out in air. The presence of CO_2 in the mass spectra was evident at only 205°C, indicating that the chains begin to burn off at this temperature! In light of this information, it seems prudent that temperatures well below 200°C be used in any drying procedures for bonded phase materials.

Berendsen and de Galan have also investigated possible geometrical models that might account for surface coverage values obtained for trimethylsilyl (TMS) and phenyldimethylsilyl (PDS) phases. Space requirements for TMS and PDS ligands were calculated based on various Si–O–Si bond angles, and these values were then compared to experimental values. It was concluded that the most likely Si–O–Si bond angle was between 120 and 140°. Free rotation about the Si–O–Si bond is unlikely (or impossible) due to geometric or energetic constraints. At the estimated bond angle, the phenyl ligand of the PDS phase points away from the silica surface. This geometry is also predicted for *n*-alkyl bonded phases.

Monomeric alkyl phases of various lengths have also been prepared by Hemetsberger et al.[27,29,30] In the first paper, alkyl and alkylaryl phases were synthesized using trichlorosilanes under anhydous conditions. Pyridine was added to the reaction mixture to tie up the HCl liberated in the silanization. The addition of pyridine is thought to increase phase loading. Retention (k') was found to correlate linearly with alkyl chain length for most solutes

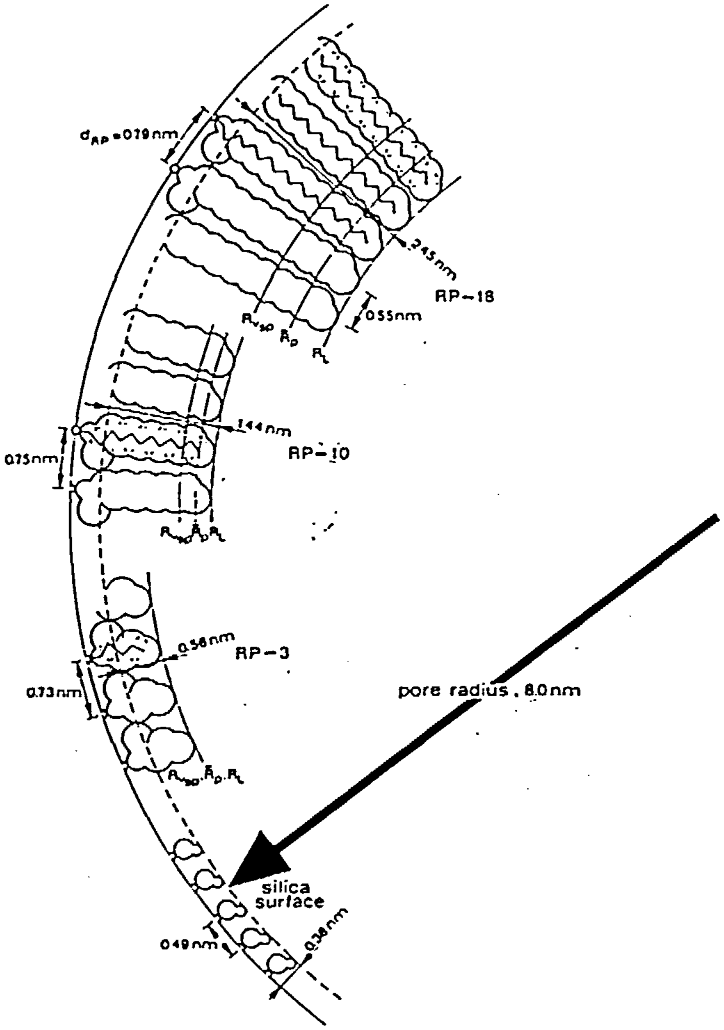


FIGURE 3. Pore model for bonded phases illustrating the steric hindrance effect originating from pore curvature and phase length. (Reprinted from Berendsen, G. E., Pikaart, K. A., and de Galan, L., *J. Liq. Chromatogr.*, 3, 1437, 1980. With permission.)

chromatographed. A similar relationship was observed for alkyl phases prepared from methyldichlorosilanes;[29] however, this phase was deemed chromatographically superior to the trichlorosilyl phases because of the reduction in silanols. A wider variety of alkyl phases were later synthesized[30] in an effort to study the effect of bonded phase structure on retention and selectivity.

The effect of silica pretreatment procedures, choice of reaction solvent, reaction temperature, reaction time, and endcapping procedures were investigated by Evans et al.[31-33] Based on the work of Bather and Gray,[34] it was suggested that no form of heat pretreatment be applied to the silica. Molecular water is evolved from the silica in the temperature range up to 120°C, between 120 and 450°C water is lost from silanol condensation, and above 500°C only free hydroxyls remain on the surface. Phases produced using silica treated at 800°C had low retention and degraded over time. Using octadecyltrichlorosilane as the silanizing reagent, phases were prepared using 14 different reaction solvents. The solvents ranged in

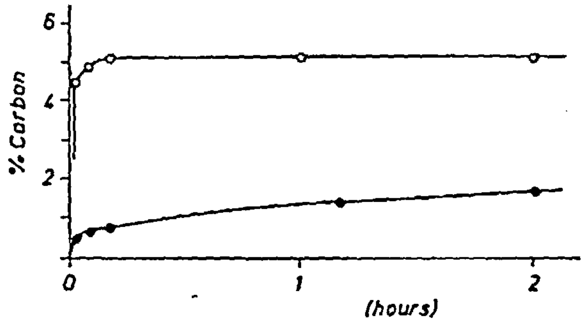


FIGURE 4. Dependence of bonded phase loading (percent carbon) on reaction time. Reaction solvents: (○) toluene; (●) methanol. (Reproduced from Evans, M. B., Dale, A. D., and Little, C. J., *Chromatographia*, 13, 5, 1980. With permission.)

polarity from acetic acid to carbon tetrachloride. Carbon loadings for the phases were remarkably constant with the exception of the phase prepared using methanol; that phase had about 25% reduction in phase loading. Chromatographic properties varied considerably among the columns. Phases prepared using acetonitrile, nitromethane, and pyridine had higher than normal back pressure and gave little or no resolution of the test mixtures. This behavior was attributed to the formation of a polymeric film over the surface of the silica which was thought to restrict solute access to the pores. Phases with the most desirable chromatographic properties resulted from the use of nonpolar, water-immiscible solvents. Dense solvents were also found to give better results than "light" solvents. From these observations, the authors recommended the use of carbon tetrachloride (CCl_4) as the diluent in bonded phase syntheses. The rate of silanization was studied in toluene and methanol (see Figure 4). At room temperature, maximum phase loading was achieved in about 15 min using toluene as the reaction solvent. However, in methanol, maximum loading could not be achieved even after a 24-hr reaction. The conversion of the trichlorosilane to trimethoxysilane apparently lowers the reactivity of the reagent. Finally, endcapping procedures were investigated for phases prepared from alkyltrichlorosilane. The chromatographic effects of endcapping were pronounced (see Figure 5). Retention of aromatic solutes increased significantly on columns after endcapping. The "best" phases resulted from reaction of octadecyltrichlorosilane with silica for 2 hr, followed by filtering and rinsing with methanol (methanolysis of unreacted chloro groups), and room temperature reaction with trimethylchlorosilane for 2 hr (endcapping). In contrast, Sander and Wise[35] observed only minor changes in the retention of three-, four-, and five-ring PAH between capped and uncapped polymeric C_{18} phases. Due to the variations in solutes and chromatographic conditions between these studied, it is difficult to assess the significance of these differences observed for endcapping (see Section II.C).

Rassi and Gonnet[36] studied the effects of thermal pretreatment of silica on phase coverage and chromatographic performance for trimethylchlorosilane phases. Silica was heated in 100° increments over the range 300 to 800°C. Surface area and pore volume measurements were made at each temperature, and the substrates were bonded with trimethylchlorosilane. Two stages in thermal treatment were distinguished. Below 600°C, surface area and pore volume measurements did not change with heating. Above 600°C, area and volume measurements decreased with increasing temperature. Silanol surface concentration decreased over the entire temperature range. The largest phase coverage values were obtained for the untreated silica; however, the largest percentage of silanols was modified for the silica treated at 400°C (see Table 3). The fewest silanols were present on the phase pretreated at 600°C. It was concluded that for nonpolar or slightly polar solutes, retention and selectivity were

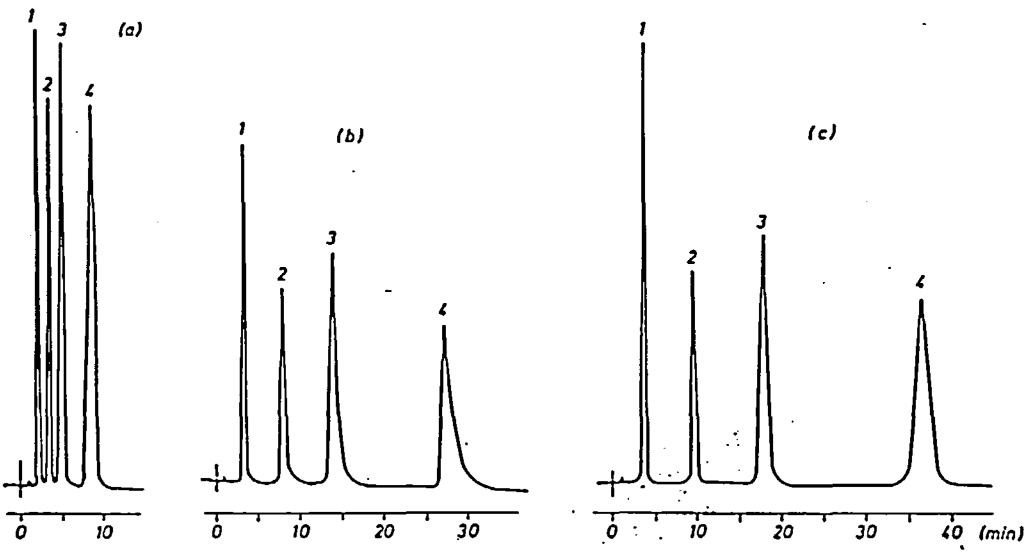


FIGURE 5. Effect of endcapping on retention for capped and uncapped phases: (a) uncapped bonded phase, (b) capped bonded phase, (c) bonded, methanolyzed, and capped phase. Solutes: (1) benzene, (2) naphthalene, (3) diphenyl, (4) anthracene. (Reproduced from Evans, M. B., Dale, A. D., and Little, C. J., *Chromatographia*, 13, 5, 1980. With permission.)

Table 3
SURFACE COVERAGE OF THERMALLY PRETREATED SILANIZED SUPPORTS[36]

	Untreated	400°C	500°C	600°C	700°C	800°C
Unbonded silica						
Chemisorbed H_2O (%)	3.6	2.5	2.2	1.8	1.7	1.2
S_{BET} m^2/g	400	432	403	399	368	200
μmol/m² OH	9.9	6.3	5.9	4.9	4.9	6.6
Bonded silica						
μmol/m² $(CH_3)_3$–	3.23	2.63	1.96	1.73	1.60	2.33
Reacted OH (%)	33	42	33	35	33	35

most closely related to the surface concentration of bonded ligands. For polar solutes these properties are strongly influenced (negatively) by the concentration of silanol groups. With such compounds, better results are obtained by using phases with fewer silanols.

Gobet and Kováts[37] also investigated the effect of thermal and hydrothermal pretreatment on phase coverage and reproducibility. In an effort to improve reproducibility in chemisorption experiments carried out on different silicas, pretreatment procedures were examined to make more uniform silica surfaces. The procedure finally settled on consists of three parts: (1) thermal treatment of the silica at 1173°C for 120 hr, (2) hydrothermal treatment (boiling in water) for 70 hr, and (3) freeze-drying of the wet silica. Reaction of silica so treated with trimethyl-(dimethylamino)-silane gave consistently uniform surface coverages of 4.75 μmol/m². Hydrothermal treatment for shorter periods resulted in lower surface coverages, indicating incomplete rehydration. The treatment procedure was found to cause small decreases in specific surface areas and corresponding increases in substrate density. In a subsequent study[19] the preparation of mixed stationary phases was described using similarly pretreated silica. Trimethyl-(dimethylamino)-silane and decyldimethyl-(dimethylamino)-silane were mixed to produce phases of predictable composition. The phases were characterized by alkaline hydrolysis[38] and gas chromatography of the butyl derivatives.

Boksányi et al.[39] studied reaction kinetics and other variables for bonded phase synthesis utilizing hydroxysilanes (also referred to as organosilanols) as the silanizing reagent. The reactivity of organosilanols is intermediate to chloro- and alkoxysilanes. An advantage of this reagent is that the byproduct of its reaction with silica is water (HCl formed during the reaction of alkoxychlorosilanes with silica is thought to attack the etheric carbon oxygen bonds and cause decomposition). The dependence of phase coverage on reaction temperature was studied, and it was found that surface concentrations increased with temperature to a constant value over the interval 200 to 340°C. Above this temperature surface concentrations decreased sharply. Surface coverage values increased gradually with time below 200°C; however, within the range 200 to 340°C, the reaction proceeded more rapidly and surface coverages reached a constant value within 5 hr. Successive treatment of the bonded phases increased surface concentrations to about 4.0 μmol/m^2 for trimethylsilyl phases and to about 3.5 μmol/m^2 for alkyldimethylsilyl phases with longer alkyl chains. Wickramanayake and Aue[40] presented evidence showing that phase thickness of monomeric phases depends, at least in part, on pore size, with the "thickest" phases occurring for wide-pore substrates.

While most investigations of silanization procedures are carried out on porous silica, some work has been performed on fume silica (e.g., Cabosil).[19,37] Recently, Dawidowicz and Rayss[41,42] studied the properties of controlled pore glasses as related to surface modification reactions for gas and liquid chromatography. Controlled pore glass (CPG) differs from precipitated silica both in chemical composition and in the way it is prepared. Silica is prepared by precipitation from a silica sol solution, followed by dehydration by heating.[24] Other types of silica are created by spray drying silica sols. CPG is manufactured from borosilicate glass by heating and leaching processes. During the heating process, two distinct glass phases form. After cooling, the phase rich in alkaliborate can be selectivity leached using a sodium hydroxide solution to create a pore structure. The process yields a material with a much narrower pore size distribution than is found in conventional silicas, but with a relatively high surface concentration of boron atoms. Dawidowicz and Rayss found that the boron atom concentration could be controlled and increased by heating the controlled pore glass (after the leaching process) at 700°C for extended periods of time. Boron concentration increases proportionally with heating time. The effect of boron atom concentration on surface modification reactions was examined using dimethyloctadecylchlorosilane (ODS) and dimethylaminodimethyloctadecylsilane (AODS) (see Table 4). Surface coverage values increased with the boron atom concentration. The exception, Glass 100, is explained by the formation of "borate crystallites" on the CPG surface. Also noteworthy is the observation that higher surface coverages were usually obtained with AODS than with ODS. A maximum surface coverage value of 5.03 μmol/m^2 was obtained for Glass 70 (thermal treatment at 700°C for 70 hr) using ODS. This value is significantly larger than ligand surface concentrations previously reported for monomeric C_{18} phases. The chromatographic behavior of the C_{18} CPG phases in Table 4 were studied for the retention of alkylbenzene homologs.[42] Plots of log(k'/S) vs. n_c were found to be linear in all cases. The slope of these lines, corresponding to methylene unit selectivity, remained constant for surface coverages within the range 2.3 to 4.0 μmol/m^2, but increased above this range. From these data it was concluded that the elution mechanisms were similar for the lower coverage phases, but altered for coverages exceeding 4 μmol/m^2.

The on-column synthesis of monomeric bonded phases for packed microcapillary columns (PMC) has been described by several research groups.[43-50] PMCs are produced by drawing glass tubing packed with silica particles at high temperatures. Because of the extreme conditions involved, surface modification reactions must be carried out after the column is prepared. The on-column reaction of trichlorosilanes has been described; however, certain

Table 4
PROPERTIES OF CONTROLLED POROSITY GLASSES[41]

Support	Area (m^2/g)	Density of C_{18} coverage ODS	Density of C_{18} coverage AODS
Glass 0	70.9	1.87	2.58
Glass 5	66.2	2.44	3.84
Glass 10	62.9	3.27	3.78
Glass 25	46.5	3.32	3.86
Glass 70	20.9	5.03	4.87
Glass 100	10.8	3.26	4.05

problems have been noted concerning polymerization. With polyfunctional silanes water must not be present if polymerization is to be prevented. Clogging of narrow bore columns with on-column silanization using trichlorosilanes has been reported. Tanaka et al.[43] chose octadecyldimethyl-*N*,*N*-diethylaminosilane because its physical properties make purification manageable, and because high phase coverage values have been reported for alkyldimethyl-(dimethylamino)silanes. In practice, phases were synthesized by pumping a toluene solution of the silane onto the column. After filling the column, the flow was interrupted and the reaction was allowed to proceed at 110°C for 1 hr. This procedure was repeated three or four times to ensure maximum surface coverage. The chromatographic properties of the phases prepared in this way were found to be similar to phases prepared by a more convention batch process (although the former phases possessed more silanols and were slightly more polar). Ligand concentrations for the on-column phases were estimated to be ~ 3 $\mu mol/m^2$.

A variety of novel alkyl phases have been synthesized in the past to examine changes in selectivity and retention as a function of bonded ligand shape. Lochmüller et al.[51] prepared cyclic and *n*-alkyl phases with similar overall carbon loadings and studied differences in solute retention originating from ligand shape. Three phases were synthesized using methyldichlorosilanes: *n*-heptyl (2.4 $\mu mol/m^2$), cyclohexyl (2.9 $\mu mol/m^2$), and bicycloheptyl (2.2 $\mu mol/m^2$). These phases were chosen as a progression from linear to puckered planar to highly angular conformations. Reaction conditions were typical, i.e., the silica was refluxed in toluene containing an excess of the silane and pyridine (to react with HCl byproduct) for a period of 24 hr. After careful washing to remove unreacted silane, the bonded silica was endcapped using trimethylchlorosilane. Retention was normalized for the differences in carbon loading for the phases (which were small). Linear and cyclic hydrocarbons were chromatographed on the three phases, and in all cases the *n*-heptyl phase was the most retentive and selective towards the selected solutes. It was concluded that flexible bonded phase ligands, such as *n*-alkyl phases, promote a stronger solute-BP interactions than rigid ligands such as the bicycloheptyl phase.

Rehak and Smolkova[52] also studied differences related to alkyl ligand shape, i.e., the influence that branching of the alkyl chain may have on retention. A list of the phases synthesized is given in Table 5. Solute retention on branched and unbranched bonded phases was found to depend strongly on solute structure and mobile phase composition. With strong mobile phases, retention was always greatest on unbranched phases. In weaker mobile phase environments, some solutes were retained longer on branched chain phases than on the *n*-alkyl phases. It was further noted that retention of branched chain solutes on branched stationary phases is enhanced compared to *n*-alkyl solutes.

Tanaka and co-workers[53] examined phase structure effects for a series of nine alkyl, aryl,

Table 5
BRANCHED AND UNBRANCHED BONDED PHASES

Phase	No. of C atoms	Carbon (%)	Coverage μmol/m²
2,4,4-Trimethylpentyl	8	9.29	3.40
4-Butyloctyl	12	11.74	2.94
1-Ethyladamantyl	12	12.49	3.15
n-Butyl	4		3.21
n-Hexyl	6		3.30
n-Octyl	8	10.88	4.09
n-Dodecyl	12	12.43	2.90
n-Octadecyl	18	16.32	2.90
Methyl	3	3.74	3.31

From reference 52.

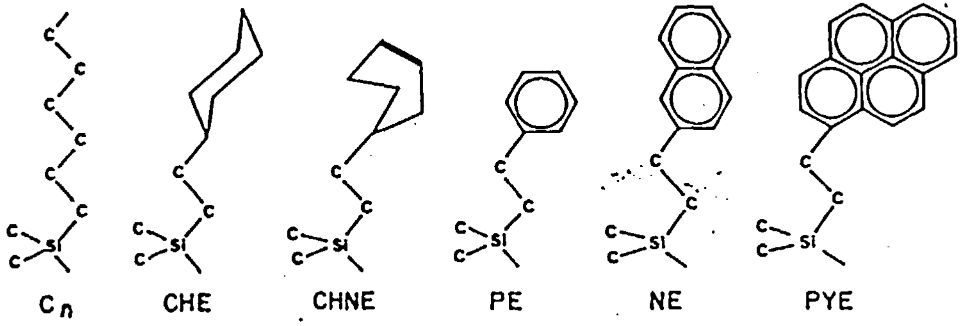


FIGURE 6. Novel alkyl, aryl, and alicyclic bonded phase structures. (Reproduced from Tanaka, N., Tokuda, Y., Iwaguchi, K., and Araki, M., *J. Chromatogr.*, 239, 761, 1982. With permission.)

aralkyl, and alicyclic ligands (see Figure 6). Each of the phases was prepared under conditions intended to yield maximum coverage. Surface concentration values for all of the phases were at least 3.4 μmol/m², and the phases were subjected to endcapping treatment with trimethylchlorosilane. The chromatographic behavior of the bonded phases was probed with a variety of solutes which were classified into four groups: *n*-alkanes, planar PAH, nonplanar PAH, and alicyclic compounds. Selectivity plots of these solutes for various combinations of columns revealed some interesting trends. Planar solutes (PAH) were preferentially retained by planar stationary phases containing large aromatic rings, and they were rejected with nonplanar phases. In general, aromatic bonded phases showed preferential retention for aromatic solutes and lesser retention for saturated hydrocarbons than was observed with *n*-alkyl bonded phases. Dramatic differences were noted in the retention behavior of octadecyl and pyrene phases (see Figure 7). Using the same mobile phase conditions, a nearly complete reversal of elution order occurred on the pyrene phase as compared to the C_{18} phase. Larger changes in retention and selectivity were also observed with changes in mobile phase composition for the pyrene phase compared to the C_{18} phase. Because of the large differences in selectivity of these phases, aromatic phases were judged to offer an alternative to C_{18} phases complementary to their use.

Recently, the underlying factors affecting silane reactivity have been examined in independent research efforts. Hunnicutt and Harris[54] studied reaction kinetics of chloro- and bromo-substituted alkyl chlorosilanes using X-ray fluorescence to characterize the reaction

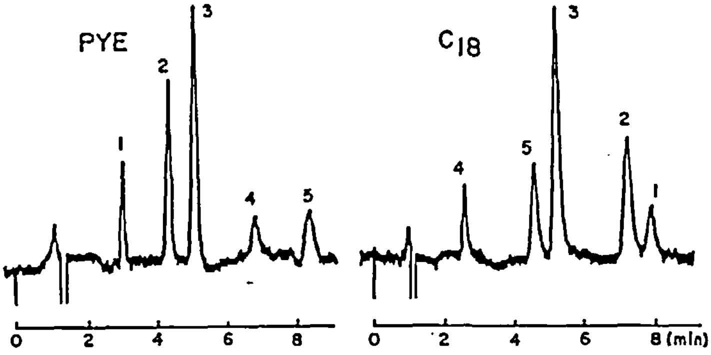


FIGURE 7. Reversed-phase separation on pyrene (PYE) and C_{18} bonded phases in 80% methanol/water. Solutes: (1) hexane, (2) butylbenzene, (3) diphenylmethane, (4) 1-nitronaphthalene, (5) triptycene. (Reproduced from Tanaka, N., Tokuda, Y., Iwaguchi, K., and Araki, M., *J. Chromatogr.*, 239, 761, 1982. With permission.)

products. Binary mixtures of chloro- and bromoalkyl silanes were used to modify Partisil silica and Cabosil fume silica. Various reaction parameters were examined. In one study, the mole ratio of Cl/Br silane was varied, and the resulting Cl/Br ratio in the phase was measured. Under the conditions of rapid silane addition and reaction catalysis, the smaller reagent was observed to react faster with the silica surface. This effect could not be attributed to a pore size effect, since equivalent results were obtained for both the nonporous fume and porous precipitated silicas. The authors make the point that for a diffusion-limited reaction (using competing reactants of different sizes), an unequal distribution of the species would result at the surface of the particle compared to deep within the pore network. This assumes the reaction occurs rapidly compared to diffusion processes and that a stoichiometric quantity of silane is used. To test this idea, Partisil was modified under these conditions and the resulting phases were characterized by X-ray photoelectron spectroscopy (XPS) for comparison with X-ray fluorescence spectroscopy (XRF). Because XPS is a surface analysis technique limited to a depth of a few nanometers, the resulting data reflect properties of the outside of the particle. Equivalent Cl/Br ratios were found using both techniques indicating that "size-dependent differences in reactivity and not diffusion rates are responsible (for the enhanced reactivity of small silanes)." In another experiment, silane size was not found to affect the Cl/Br ratio in the product, as long as the reaction was not catalyzed and the silane mixture was added slowly.

Lork and co-workers[55] examined the reactivity of monofunctional C_8 silane reagents under a variety of reaction conditions. C_8 silanes with the following functionalities were studied: chloro, hydroxy, methoxy, ethoxy, dimethylamino, trifluoroacetoxy, and bis-(siloxane) functional groups. Each of the silanes was prepared by reaction with octyldimethylchlorosilane (which itself was prepared by hydrosilylation of 1-octene). In many of the syntheses, 2,4-lutidine was added as a base and a catalyst to assist complete reaction. Although the presence of trace water in monomeric syntheses has in the past been considered unimportant, the authors disagree with this belief. Ligand density values were observed to drop dramatically with the content of water in the reaction mixture. This trend is explained by the formation of silane dimers in solution with low reactivity. Water is also believed to be initially adsorbed on the unbounded silica, further hindering modification by causing formation of low reactivity hydroxy silanes. The overall reactivity and temperature dependence of some of the silanes examined is illustrated in Figure 8. Ligand density increased for each of the silanes with increasing temperature. At most temperatures the dimethylaminosilane resulted in the highest ligand density. The overall order of reactivity (from highest to lowest reactivity) is

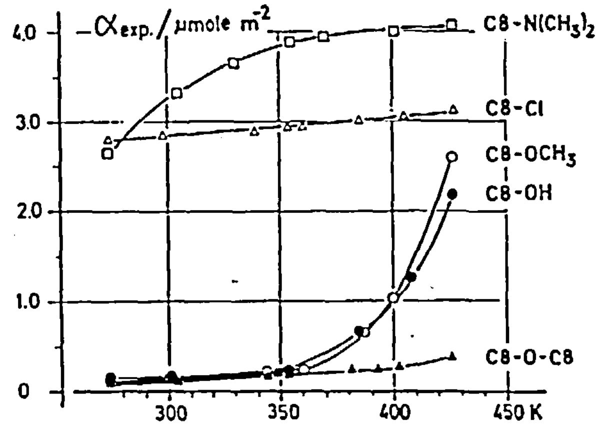


FIGURE 8. Dependence of bonded phase loading (μmol/m²) on reaction temperature for various dimethyloctylsilanes. (Reproduced from Lork, K. D., Unger, K. K., and Kinkel, J. N., *J. Chromatogr.*, 352, 199, 1986. With permission.)

C_8–N$(CH_3)_2$> C_8–O–$COCF_3$ > C_8–Cl » C_8–OH ≈ C_8–OC_2H_5 » C_8–O–C_8. The greatest ligand density using C_8–N$(CH_3)_2$ was about 4.1 μmol/m². Thermal pretreatment of the silica was found to have little effect on surface coverage values for phases prepared with the dimethylamino silane even at temperatures as high as 700°C. The authors concluded that phases of the highest density are best prepared using a dimethylaminosilane, elevated temperatures, and *N,N*-dimethylformamide as the reaction solvent. The base catalyst (2,4-lutidine) is not needed with dimethylamino silanes. A reaction mechanism is presented in which the silica silanol oxygen makes nucleophilic attack at the silane Si atom. The silanol hydrogen is abstracted by the dimethylamino portion of the molecule, which then leaves the reaction as HN$(CH_3)_2$ gas. Base catalyzed reactions are similarly pictured with the base aiding silanol proton abstraction.

B. Polymeric Alkyl Syntheses

Most of the attention in bonded phase research has focused on synthesis, characterization, and properties of monomeric alkyl phases. The study of polymeric syntheses, by comparison, has been largely ignored. Polymeric phases are synthesized by intentionally introducing a measured quantity of water into a synthesis involving polyfunctional silanes. Polymeric phases have been prepared in two distinct ways. Traditionally, polymeric phases have been prepared by adsorbing a known quantity of water onto dry silica.[56] This "wet" silica is then allowed to react with the polyfunctional silane ("water preequilibration synthesis"). At the silica surface, polymerization of the silane is initiated by the adsorbed water. Water hydrolyzes chloro- and alkoxysilanes to form silane silanols; these molecules then react with other silane molecules to form a polymer. Both linear addition and cross-linking reactions are possible, as shown in Figure 2. Only siloxane bonds (Si–O–Si) are involved in the polymerization and the alkyl side chains (e.g., octadecane) are incorporated as substituents on the polymer backbone. In the second method of preparing polymeric phases, water is added directly into the reaction slurry to initiate polymerization reaction ("water slurry synthesis"). This procedure will be discussed in more detail below.

A certain stigma is associated with polymeric phases that is probably due to early problems with polymeric phases on pellicular substrates. Problems have included low column efficiency due to limited mass transfer,[57-59] poor peak shape,[60,61] and difficulty in reproducing phase synthesis.[62] The use of di- and trifunctional silanes in phase preparation has largely

Table 6
EFFECT OF PERCENT WATER ADDED ON POLYMERIC PHASE LOADING[64]

Silane	Water (%)[a]	Phase loading (TGA) (%)[b]
$Cl_3SiC_8H_{17}$	0	11.6
	1.5	15.5
	3.0	18.5
$ClMe_2SiC_8H_{17}$	0	12.0
	1.5	15.0
	3.0	15.6
	7.0	18.5
$Cl_3SiC_{18}H_{37}$	0	10.5
	0.2	11.0
	0.5	12.0
	1.0	13.9
	1.5	16.0
	2.0	17.0
	5.0	25.6
	10.0	30.0

Percent water based on amount of silica used.
Percent loading is for total organic content (not %C).

been replaced by monofunctional reagents. The usual reasons cited for this choice is that monofunctional silanes necessarily result in a monomeric synthesis, and they do not contribute additional silane silanols to the phase. Only recently have the properties of intentionally polymerized bonded phases been reexamined.

The feasibility of using reactive cylic organosilicon reagents to prepare polymeric phases was studied by Aue and Wickramanayake.[63] Cyclic silane polymers with four to eight monomer units were reacted with silica to produce phases for gas and liquid chromatography. Phase coverages ranged from 3.5 to 6.8 μmol/m^2, these values based on the monomer units of the silane. In an interesting discussion on the nature of polymeric phases, it was suggested that the cyclic organosilane molecules open upon attachment to the silica, but it is not known how many points of attachment occur for each polymer unit. Based on experiments with silazane polymers (which can be characterized by nitrogen as well as carbon analyses), the authors appear to favor a model in which the cyclic silane molecules fragment and depolymerize upon bonding. Such a phase would consist primarily of monomer units rather than polymer chains. The cyclic polymeric phases were not critically compared to monomeric phases, but no disadvantages were reported. Additional studies on these phases are warranted.

Verzele and Mussche[64] prepared monomeric and polymeric C_8 and C_{18} phases on totally porous, narrow-pore silica. Silica was first dried and then heated in xylene at 80 to 90°C. Measured quantities of water were added to this slurry on a weight percentage basis, and the mixture equilibrated. Finally, a fourfold excess of the silane was added and the slurry refluxed for 4 hr. This synthesis procedure is somewhat different from earlier polymeric syntheses used with pellicular substrates[56] in that water was added to the reaction slurry rather than directly to the silica. The order of addition of reagents was not investigated in this work. A series of C_8 and C_{18} phases were prepared using different amounts of water (see Table 6). Phase loadings were measured by thermal gravimetric analysis (TGA) rather than elemental carbon analysis. As expected, phase loadings increased with the quantity of water added to the reaction mixture. Not expected were the phase loading increases observed

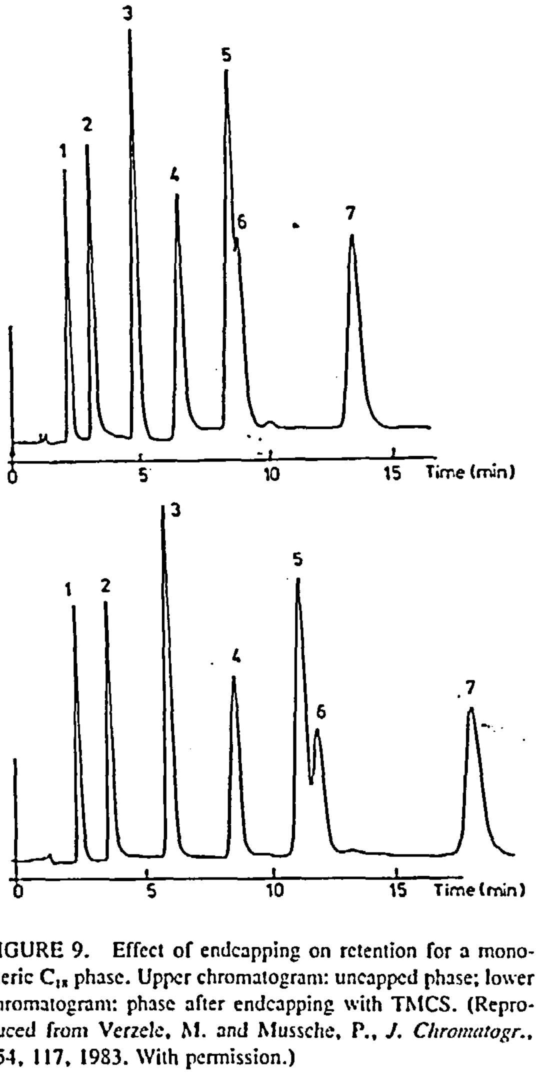


FIGURE 9. Effect of endcapping on retention for a monomeric C_{18} phase. Upper chromatogram: uncapped phase; lower chromatogram: phase after endcapping with TMCS. (Reproduced from Verzele, M. and Mussche, P., *J. Chromatogr.*, 254, 117, 1983. With permission.)

with the addition of water to octyldimethylchlorosilane (monomeric) syntheses. The authors hypothesized that hydrolysis of this silane permits the modification of "less reactive, better hidden" silanols on the silica and this accounts for the high phase loadings. Columns were prepared from each of the phases and it was noted that back pressure was higher than normal for the heavily polymerized phases. Physical examination of the 30% C_{18} polymeric phases showed it to be rubbery!

Because additional silanols are created in silane polymerization, the effect of endcapping polymeric phases was examined. The separation of polar and nonpolar solutes on capped and uncapped polymeric C_{18} phases is illustrated in Figure 9. Higher retention and slightly better resolution of the components were observed for the phase capped with trimethylchlorosilane. Better overall separation of the solutes was obtained with the polymeric C_{18} phase; however, it was concluded that there is actually little difference in the performance of monomeric and endcapped polymeric phases. The authors proposed that most phases prepared from di- or trifunctional silanes in the presence of trace quantities of water have properties similar to monomeric phases, as long as the surface coverage values do not exceed 3 to 3.5 μmol/m². Differences do exist, however, in the polarity of such phases.

Polymeric phase modification was further studied by Sander and Wise[35] using wide-pore

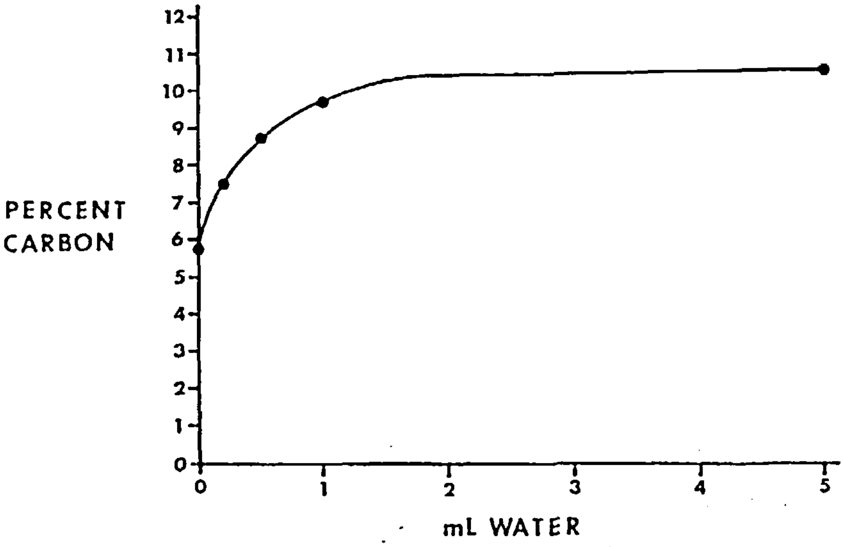


FIGURE 10. Effect of the amount of water on the resulting carbon loading for polymeric C_{18} phase syntheses. (From Sander, L. C. and Wise, S. A., *Anal. Chem.*, 56, 504, 1984. With permission.)

(~ 30 nm) silica substrates. Polymeric C_{18} phases were prepared by reaction of the silicas with octadecyltrichlorosilane in the presence of water. The reaction sequence was slightly different than with other studies. In a typical synthesis, dry silica was added to a hot solution of the silane in carbon tetrachloride. Polymerization was then immediately initiated by adding a measured quantity of water and refluxing for 4 hr. The influence of added water on carbon loading was examined for the given reaction conditions. A plot of percent carbon loading vs. milliliter water added is shown in Figure 10. Phase loading appears to reach a limiting value at high water concentrations. Reproducibility of the synthesis on a single batch of silica had surface coverage values that varied by less than 1% (relative standard deviation [RDS]). Phases prepared under identical conditions, but on different silica substrates, had slightly more variation, about 5% RSD. The greater differences observed with various silicas is attributed to uncertainty in the surface area values for the substrates.

The chromatographic properties of polymeric C_{18} phases were compared to monomeric C_{18} phases for the separation of PAH. Distinct differences in the retention behavior of polymeric and monomeric phases were observed. In general, better overall separation of complex PAH mixtures was possible on the polymeric phases than with monomeric C_{18} phases. The order of elution of certain PAH changed between monomeric and polymeric C_{18} columns. On the basis of the elution order of three selected PAH probes, an empirical classification test was devised that permitted phases to be classified as monomeric or polymeric in nature. This test proved valuable for the rapid screening of phases and allowed predictions to be made about column selectivity towards complex PAH mixtures. The test mixture employed is described in more detail in Section III.

A different type of C_{18} phase, distinct from monomeric and polymeric phases, was also prepared by Sander and Wise.[35] The phase was synthesized in a stepwise fashion by alternating silanization and hydrolysis reactions (see Figure 11). This process can be thought of as a controlled, sequential polymerization. Octadecyltrichlorosilane was reacted with silica under anhydrous conditions to give a phase with essentially monomeric properties. After thorough washing to remove unused silane, unreacted chloro groups on bonded ligands were converted to hydroxyls by refluxing the phase in a 50/50 tetrahydrofuran/water mixture for

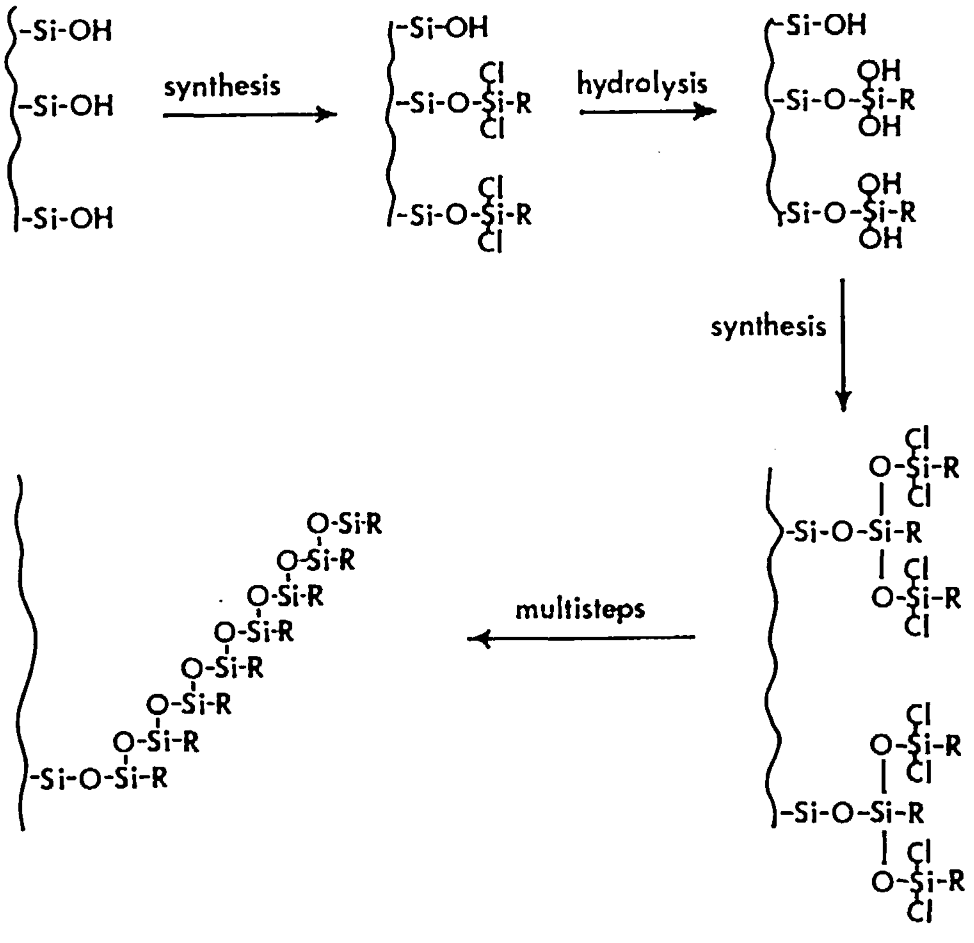


FIGURE 11. Oligomeric phase synthesis scheme. Anhydrous silanization and hydrolysis reactions are sequentially alternated so that the bonded phase is built up one unit at a time. The process can be considered a controlled, stepwise polymerization. (From Sander, L. C. and Wise, S. A., *Anal. Chem.*, 56, 504, 1984. With permission.)

1 hr. The phase was then dried at 150°C to remove physically adsorbed water. This reaction sequence was repeated a total of nine times. Samples were removed for carbon analysis after each silanization. Columns were prepared at steps 1, 3, 5, 7, and 9, and the columns were endcapped using hexamethyldisilazane (HMDS). A plot of percent carbon loading vs. reaction step number is shown in Figure 12. Although increases in percent carbon loading were measured for all reaction steps, the largest increases were observed during the first three steps. Measurable increases in carbon were also recorded after endcapping. The chromatographic properties of the oligomeric phases were intermediate to those of monomeric and polymeric C_{18} phases, with column selectivity becoming more "polymeric-like" with increasing step number. However, because the reaction is sterically limited, it is not feasible to prepare oligomeric phases with properties similar to polymeric phases. This suggests that for polymeric syntheses, the polymer forms in solution and is, subsequently, deposition onto the silica, rather than polymerization from the silica surface "outward".

The influence of pore diameter on column selectivity was investigated for monomeric and polymeric C_{18} phases prepared on a variety of substrates.[65,66] A total of 22 silicas with pore diameter ranging from 50 to 1000 Å were used in the study. Monomeric C_{18} phases prepared

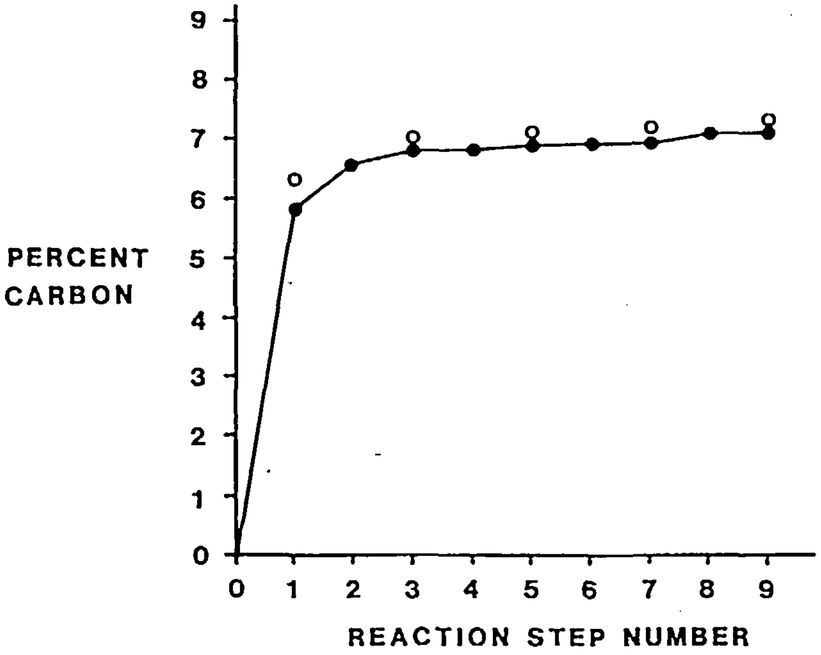


FIGURE 12. Carbon loading plotted as a function of reaction step number for the oligomeric phase synthesis (●). (○) Represents carbon loading after endcapping with hexamethyldisilazane. (From Sander, L. C. and Wise, S. A., *Anal. Chem.*, 56, 504, 1984. With permission.)

on various silicas differed primarily in terms of absolute retention. The differences are attributed to changes in specific surface area that occur as a function of pore size. The separation of 16 polycyclic aromatic hydrocarbons (Standard Reference Material, [SRM] 1647 from the National Bureau of Standards, Gaithersburg, Md.) is shown in Figure 13 for monomeric C_{18} phases prepared on experimental Zorbax® silicas. Retention decreases with increasing pore diameter (and with decreasing surface area), but the elution order and relative retention of the components remain constant. Polymeric C_{18} phases were prepared on the same substrates. Separation of SRM 1647 on these phases is shown in Figure 14. Surprisingly, absolute retention varies little among these phases; however, significant changes in column selectivity are apparent as a function of pore size. Baseline separation of all 16 components of the PAH mixture is achieved only with the polymeric phases on 300- and 150-Å substrates. Changes in the elution order of certain solutes are also apparent. Because these differences were not observed among the monomeric phases, the effect cannot be attributed directly to pore diameter, as with size exclusion. Instead, it is more likely that variations in pore size influence polymeric phase synthesis and result in bonded phases with different properties.

In the synthesis of polymeric phases, two species exist in solution: silane monomer and silane polymer molecules. Both species are reactive and can bond to the substrate surface. Unger[24] has stated that the diffusion and reaction kinetics of (monomeric) silane reagents may be affected by pore diameter. Because the silane polymer is larger than the monomer, size differentiation is possible within the pores. Such restricted access of the silane polymer to the pore network may account for the lower degree of polymeric phase behavior observed with narrow-pore substrates. A schematic illustration of this concept is presented in Figure 15. The irregular shapes denote silane polymer. For the narrow-pore case, polymer diffusion within the pore is restricted and monomeric modification is favored. For moderate and wide-pore substrates, the silane polymer has greater mobility within the pores and polymeric surface modification is increased.

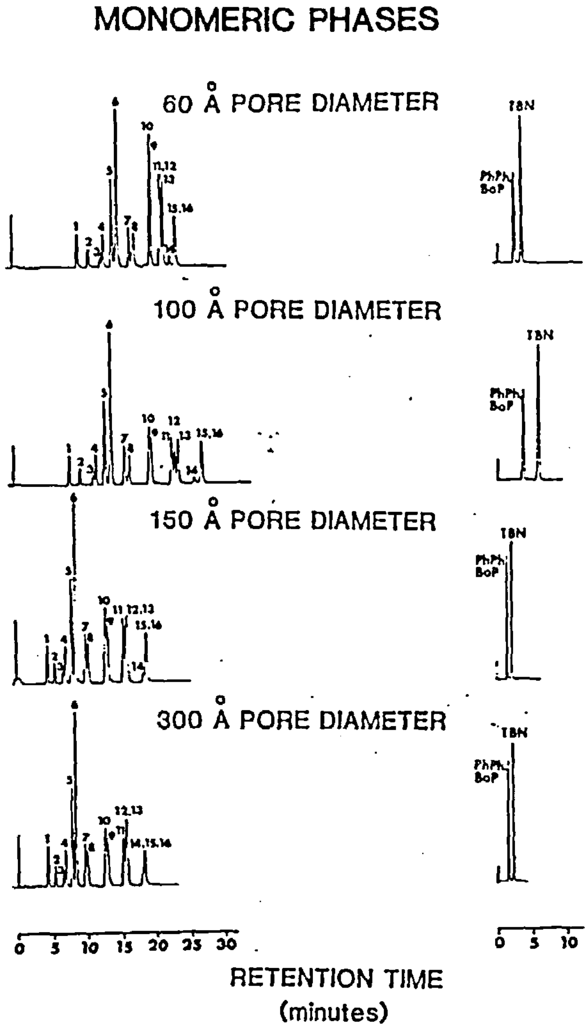


FIGURE 13. Separation of 16 polycyclic aromatic hydrocarbons (SRM 1647) on representative monomeric phases prepared on Zorbax substrates. The separation was performed by using gradient elution, 40 to 100% acetonitrile in water over 45 min at 2 mL/min. The three-component mixture was run isocratically at 85% acetonitrile/water. Component identification: (1) naphthalene, (2) acenaphthylene, (3) acenaphthene, (4) fluorene, (5) phenanthrene, (6) anthracene, (7) fluoranthene, (8) pyrene, (9) benz(a)anthrancene, (10) chrysene, (11) benzo(b)fluoranthene, (12) benzo(k)fluoranthene, (13) benzo(a)pyrene, (14) dibenz(a,h)anthracene, (15) benzo(ghi)perylene, (16) indeno(1,2,3-cd) pyrene. (PhPh) phenanthro(3,4-c)phenanthrene, (TBN) 1,2:3,4:5,6:7,8-tetrabenzonaphthalene, (BaP) benzo(a)-pyrene. (From Sander, L. C. and Wise, S. A., *J. Chromatogr.*, 316, 163, 1984. With permission.)

An entirely different class of polymeric phases has been prepared by Schomburg et al.[67-69] These phases consist of "mechanically coated" polymers that are immobilized on the substrate by cross-linking reactions. Because chemical modification of the silica (or other substrate) is not involved, silanol groups are not required for phase preparation. Silanols can be endcapped prior to polymer coating to improve surface deactivation. Figge and co-

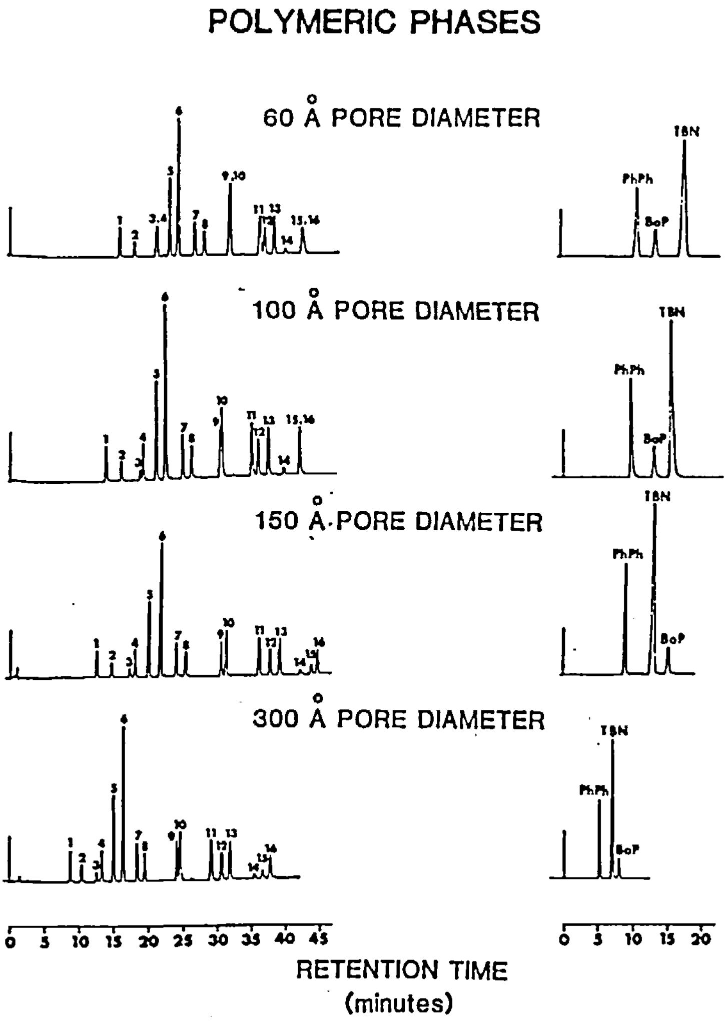


FIGURE 14. Separation of SRM 1647 on polymeric phases prepared on substrates with varying pore diameter. Chromatographic conditions and component identification are the same as in Figure 13. (From Sander, L. C. and Wise, S. A., *J. Chromatogr.*, 316, 163, 1984. With permission.)

workers[69] claim the phases prepared in this way are more homogeneous than conventional monomeric bonded phases. Because silanols are better shielded than with bonded phases, undesirable solute-stationary phase interactions are reduced (e.g., tailing of basic solutes). Also, since phase preparation does not depend on the chemistry of the underlying substrate, materials other than silica (such as alumina) can be modified. Several conclusions about immobilized polymer phases are reported.[69] Efficiency and sample capacity of polymer-coated phases can be comparable to conventional bonded phases. Phase thickness can be controlled, and better shielding of silanols results for phases thicker than 15 Å. Sample capacity is not dependent on phase thickness. The polarity of the phase can be modified by including polar functional groups in the polymer (e.g., cyano groups). Excellent phase stability was observed in both polar and nonpolar environments.

C. Endcapping Procedures

The term "endcapping" refers to a secondary silanization reaction intended to reduce

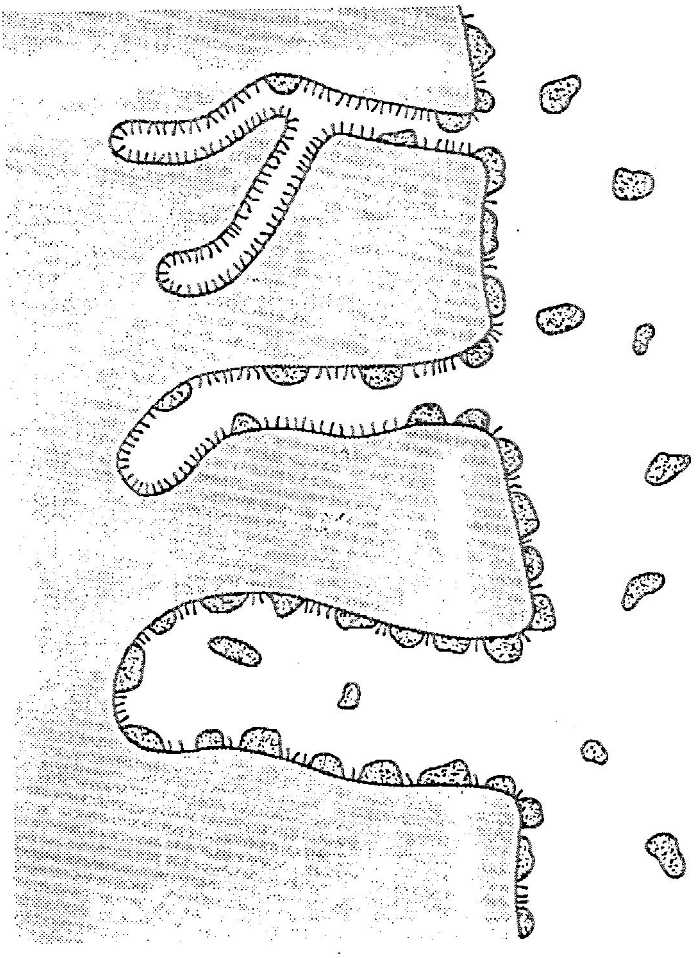


FIGURE 15. Schematic representation of a size-exclusion reaction mechanism that may limit polymeric coverage during phase synthesis. (From Sander, L. C. and Wise, S. A., *J. Chromatogr.*, 316, 163, 1984. With permission.)

residual silanols on a chemically modified surface. Trimethylchlorosilane or HMDS is commonly used for this purpose. Silanols inaccessible to modification by bulky silane reagents such as octadecylchlorosilane are more accessible to reaction with these small silanes. The intent of endcapping is to remove surface heterogeneity by modifying polar silica silanols with nonpolar TMS groups. Silane silanols are also modified during endcapping. Although monofunctional silanes react "completely" to give one silica bond linkage per molecule, di- and trifunctional silanes react only partially leaving chloro or alkoxy groups which later hydrolyze. These "silane silanols" are distinct from the silica silanols, yet they also contribute detrimentally to the retention of polar compounds. Roumeliotis and Unger[23] have shown that syntheses using trichlorosilanes can actually have more silanols after silanization than before. Endcapping is especially effective for phases prepared from polyfunctional silanes.

The need for endcapping densely loaded monomeric phases is controversial. Tanaka and co-workers[70] cautioned that the extent of endcapping cannot be assessed by the increase in carbon loading that results. This judgment was based on the imprecision of the carbon measurement and the small increases in carbon involved. Instead, Tanaka et al. recommended that endcapping effectiveness be measured chromatographically by measuring the normal mode retention of small, polar molecules. The use of hexane as the mobile phase, however, requires patience and careful equilibration for the results to be valid. Lower than actual k' values result for incompletely equilibrated phases, thus, indicating (false) silanol deactivation. Berendsen et al.[20] disagree about the utility of carbon measurements in measuring the extent of endcapping. For carbon analyses precise to 0.1% absolute, any increases in carbon due to endcapping should be apparent. They further claim that endcapping is unnecessary for fully covered silicas prepared from monochlorodimethylalkylsilanes operated in the reversed-phase mode. No significant differences in retention were observed with short chain length phases, before and after endcapping.

While the effectiveness of endcapping high coverage monomeric phases is open for debate, the need for endcapping phases prepared from di- or trifunctional silanes is clear. Evans et al.[31] compared capped and uncapped phases prepared from trifunctional silanes and found marked improvement of peak shape after endcapping. They further examined endcapping conditions and determined the greatest number of silanols were modified if the unreacted chloro groups from the silane were converted to methoxy groups prior to endcapping. Phases so treated had increased retention and more symmetrical peaks for the retention of PAH. Verzele and Dewaele[71] also proved the efficacy of endcapping certain commercial C_{18} phases by the use of naphthalene, nitronaphthalene, and acetylacetone test probes. Sander and Wise[35,72] found that measurable increases in carbon loading (~ 0.1 to 0.2% carbon absolute) resulted after endcapping polymeric and oligomeric C_{18} phases. A comparison of one polymeric C_{18} phase before and after endcapping revealed, however, only minor chromatographic differences. The authors conclude that endcapping is of lesser importance for nonpolar solutes such as PAH, compared to polar solutes.

Lochmüller and Marshall[73] studied the differences between trimethylchlorosilane (TMCS) and hexamethyldisilazane (HMDS) as endcapping reagents. Several trends were observed. Retention was usually less with HMDS capped phases compared to TMCS capped phases. HMDS phases were also usually more efficient than TMCS phases; however, better peak shape was found for the TMCS capped phases. The solute pair aniline/phenol behaved differently on phases capped with HMDS and TMCS. Peak tailing was greatest for phenol with HMDS phases, and for aniline with TMCS phases. Lochmüller and Marshall hypothesized that the reaction of HMDS produces basic species which are adsorbed to the silica surface. From photoacoustic and fluorescence spectra HMDS capped phases appear to be more heterogeneous than TMCS capped phases. The authors concluded that HMDS may be the more desirable endcapping reagent if basic solutes are to be separated, while TMCS may be more suitable for neutral or acidic solutes. Hunnicutt and Harris[54] compared the reactivity of TMCS with HMDS and concluded that the former reagent is more reactive than HMDS.

Mixtures of endcapping reagents were assessed by Nawrocki.[74] Hexamethylcyclotrisilazane (HMCTS) and HMDS were used separately and in various proportions for the deactivation of a gas chromatographic silica phase. HMCTS reacts by ring opening and fragmentation processes which give rise to mono- and difunctional silanes. The highest carbon loading values for the silanization reactions were obtained with a 2:1 HMCTS/HMDS mixture. Coverage after reaction with this mixture was approximately 4.4 μmol/m^2. Gas chromatographic properties of the phase indicated a high degree of surface deactivation.

Dewaele and co-workers[75] developed a chromatographic test to ascertain the effectiveness of endcapping with C_{18} bonded phases. Because normal-phase retention criteria are inconvenient to measure, a reversed-phase method was devised based on the relative retention of naphthalene and nitronaphthalene. The selectivity factor (α) for naphthalene/nitronaphthalene (α N/NN) was measured at various mobile phase concentrations, and it was found to increase and approach a constant value for k'>10 for naphthalene. On this basis, the mobile phase composition was set to give retention at least this great (mobile phase composition 60:40

methanol/water). A correlation of selectivity factors for capped and uncapped C_{18} phases showed that most capped C_{18} phases have α N/NN values of 1.4 to 1.6. C_{18} phases that are not endcapped have lower α N/NN values, typically 1.0 to 1.2. A similar comparison for capped and uncapped C_8 phases gave different results. Absolute differences in selectivity factors were smaller, and no general classification was possible based on this solute pair for the C_8 phases.

Tomellini and co-workers[76] studied the relative contribution to retention introduced by endcapping C_8 phase. Octyldimethylchlorosilane was used to prepare a series of C_8 phases with various phase loadings. These phases were then endcapped using TMCs. The retention of small, polar and nonpolar solutes was measured before and after endcapping. Retention increased nonlinearly with the mole fraction of TMS phase. A comparison was made to the retention expected from individual C_1 and C_8 phases, and the increase in retention could not be accounted for on this basis. The increase observed per mole of trimethylsilane was greatest for high C_8 coverage phases.

D. Aromatic and Charge Transfer Phases

Although the bulk of bonded phase research has centered on alkyl phases of various types, considerable study has been carried out on bonded phases prepared from aromatic ligands. Den and Kettrup[77] prepared a series of alkylphenyl-modified substrates using mono-, di-, and trifunctional silanes. Silanes of different alkyl length (zero to six carbons) were synthetized from phenyl-substitured alkenes using the hydrosilylation reaction. Relatively high surface coverage values were obtained (~ 3.4 to 3.7 μmol/m^2). Each of the phases was characterized by IR and solid-state ^{13}C-NMR, and correlations were made between peak positions and silane functionality. The retention behavior of the phenyl BP was tested in both normal- and reversed-phase modes. Using *n*-hexane as the mobile phase, aromatic solutes were only slightly retained. The k′ value for nitrobenzene was 0.77, indicating some residual silanol interaction. In acetonitrile/water mobile phase, retention behavior was found to parallel reversed-phase C_{18} columns. Plots of log k′ vs. n (n = number of alkyl carbon atoms in the alkylphenyl ligand) were linear for aromatic solutes. This trend has also been reported for C_{18} phases (see discussion in Section IV.B).

Okamoto and Yamada studied differences in phenyl phases prepared using phenyldimethylchlorosilane, diphenylmethylchlorosilane, triphenylchlorosilane, and benzyldimethylchlorosilane.[78,79] Phases were synthesized on six silica substrates with pore diameters ranging from 10.8 to 22.0 nm. Interestingly enough, for each substrate the surface concentration values increased in the order phenyl < triphenyl < diphenyl. This order was also reported by Jinno and Okamoto.[80] Apparently, the PDS ligand is less reactive or more sterically hindered than either the triphenyl or diphenyl ligands. Retention increased regularly as a function of ligand surface concentration. Phase selectivity toward PAH was similar among the phenyl phases; however, separation of the nitrochlorobenzene isomers was possible only with the benzylsilyl phase. Little et al.[81] found that, in general, solute retention and resolution decreased with increasing phase bulkiness. Thus, better separations of sulphonamides were obtained with phenylsilyl BP rather than triphenylsilyl BP. Smith[82] used a retention index scale based on alkylarylketones to compare retention properties of phenyl and C_{18} phases. Lower-than-expected retention of aromatic solutes was observed with the phenyl phase. Although retention indices for aromatic solutes changed considerably as a function of mobile phase composition, better resolution of alkylbenzene homologs was not obtained with the phenyl phase. The elution order and resolution of these solutes were similar to that observed for C_{18} phases.

Jinno and Okamoto[80,83-85] addressed the shape recognition ability of phenyl and benzyl phases using several probes. Based on the retention behavior of five PAH solutes it was concluded that better shape recognition, as is reflected by the length-to-breadth descriptor,[86] L/B, occurs with triphenyl phases than di- or monophenyl phases. Unfortunately, the L/B descriptor used by Jinno and Okamoto is only valid for isomers (it is not valid for the comparison of different molecular weight PAH as in these studies), so additional solutes need to be chromatographed for the argument to be convincing. Variations in column selectivity were noted for phases prepared on different pore diameter substrates. Jinno and Okamoto[85] hypothesized that TMS groups have restricted access within the pore network, depending on the pore size, and so pore size affects phase synthesis. This exclusion mechanism for syntheses, first proposed by Sander and Wise[65] for the synthesis of polymeric C_{18} phases, seems unlikely in the context of monomeric phenyl silanes. These reagents are small in comparison to pore diameters and little or no differentiation between phenylsilane and triphenylsilane seems possible (the exclusion limit of even small pore, 6.0 nm pore size silica is over 50,000 mw).

Van Damme and Verzele[87] described the normal-phase properties of a commercial polyphenyl bonded silica. Better separations of phenols and benzodiazepine antidepressants (as well as other classes of solutes) were achieved on the polyphenyl columns compared with silica or cyanopropyl columns. Peak symmetry with the polyphenyl phase was also improved over the other phases examined. Interestingly enough, polyphenyl silica was shown to exhibit greater polarity than bare silica gel. Polar compounds were retained to a greater extent on the polyphenyl silica column than with a silica column prepared from the base silica.

Other aromatic ligands besides phenyl have been bonded to silica, with notable results. Verzele and Van de Velde[88] prepared anthracene-modified silica and compared retention behavior with C_{18} and phenyl phases. The approach taken in the preparation of the anthracene phase merits special mention. Unlike most bonded phase syntheses which use unmodified silica, a commercial propylamino bonded phase was employed and the anthracene ligand was linked to this phase by the reaction of 9-chloromethylanthracene. This method of attaching ligands through amine linkages has considerable potential for the preparation and study of novel bonded phases which would otherwise be expensive or time consuming using corresponding silane reagents. A practical limitation of the utility of aromatic phases is the high UV background that results from their use. This high background apparently results from phase dissolution into the mobile phase. For gradient separations, artifact peaks elute near the completion of the gradient.[71] This effect has also been reported by Lochmüller et al.[89] for a pyrene phase. The efficiency of the anthracene column was compared to a C_{18} column based on the same silica. A loss in efficiency for the anthracene column (5000 vs. 9000 plates) was attributed, in part, to a higher mass transfer term for the anthracene phase. The primary source was, however, attributed to the column packing process. A comparison of selectivity toward PAH and polar solutes was made for the anthracene, C_{18}, and phenyl columns. The phenyl column had considerably lower retention than the other phases and different mobile phase conditions were required. Two-, three-, and four-ring PAH were separated on each of the phases, with differing results. Separation of the components improved in the order phenyl $<$ anthracene $<$ C_{18}, i.e., the best separations were obtained with the C_{18} column. Interestingly enough, a change in elution order occurred with chrysene and triphenylene. With the anthracene column, chrysene eluted before triphenylene, but for the C_{18} column chrysene eluted last. A similar change in elution order was found for the polar test mixture containing nitronaphthalene and naphthalene. For the latter solutes, the change in elution is explained by the formation of a donor-acceptor complex between nitronaphthalene and the anthracene phase, but before naphthalene with the C_{18} phase.

The chromatographic properties of larger bonded aromatic species and phases for charge transfer interactions have been investigated by Lochmüller et al.[89,89a] Dimethylmonochloropyrenylsilane was synthesized by reaction of 3-bromopyrene with *n*-butyl lithium and subsequent hydrosilylation of this product with dimethylmonochlorosilane.

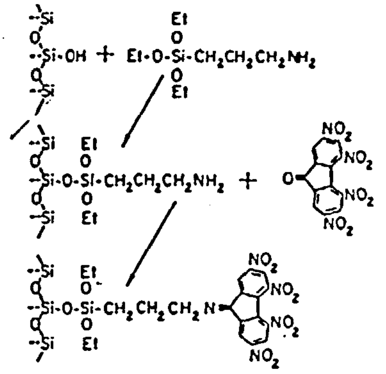


FIGURE 16. Two-step reaction synthesis scheme for 3-(2,4,5,7-tetranitrofluorenimino)-propyl bonded silica. The charge transfer moiety is bonded via condensation with propylamino silica. (Reproduced from Lochmüller, C. H. and Amoss, C. W., *J. Chromatogr.*, 108,:85, 1975. With permission.)

The pyrene bonded phase was endcapped using trimethylchlorosilane. Phase properties were studied in the normal-phase mode using hexane or acetonitrile-hexane mobile phases for the separation of PAH and nitro-PAH. PAH and nitro-PAH were clearly separated into two classes, with the nitro-PAH eluting last. This order of elution is the opposite of that observed on C_{18} phases operated in the reversed-phase mode. The bound pyrene ligand can be considered a π base (i.e., electron rich), and the nitro-PAH solutes, π acids (i.e., electron deficient). This charge transfer interaction accounts for the observed elution order and the resulting high selectivity toward nitro-PAH. Electron-deficient stationary phases were also prepared by Lochmüller and co-workers[90,91] by reaction of nitro-substituted fluorenone with propylamine bonded silica (see Figure 16). High selectivity toward alkyl-substituted PAH was observed for the tetranitro-substituted fluorenone phase. Retention was shown to be related to the degree of nitro substitution of the phase, i.e., retention decreased in the order tetranitro > trinitro > dinitro > nitro. The 3-nitrofluorenimine bonded phase did not give any measurable retention of PAH under the same conditions used to characterize the other phases.

Hemetsberger et al.[92] prepared a tetranitrofluorenone phase directly by reaction of TNF-chlorosilane with bare silica. To begin to understand the retention processes involved, k′ values for various PAH were correlated with donor-acceptor complex constants. These constants were measured for solution complexes of model ligand compounds with selected PAH, by a spectrophotometric method. Good correlation between the two values was observed, thus, supporting the belief that solute retention was the result of donor-acceptor complex formation. Hammers et al.[93] examined the dependence of donor-acceptor complex formation with various nitrofluorenone phases as a function of temperature and eluent composition.

Nondek and Ponec[94] prepared a different electron acceptor (EA) stationary phase based on the 2,4-dinitrobenzenesulphamidopropyl (DNSP) ligand. Other nitro-substituted ligands were prepared for comparison. The ligands were immobilized by attachment to a commercial amino propyl modified silica. Selectivity of the EA phases was found to increase with increasing electron affinity of the ligand (controlled by position and extent of nitration) as well as the nature of the "spacer" used to link the ligand with the silica. The properties of charge-transfer phases based on 3-(2,4-dinitroanilino)propyl (DNAP) ligands were further examined.[95,96] Retention was correlated with vertical ionization potentials of selected PAH solutes, thus, supporting a charge-transfer retention mechanism. Other charge-transfer phases have been prepared using tetrachlorophthalimidopropyl-modified silica[97] and picramidopropyl-modified silica.[98]

Fluorinated aromatic and alkyl bonded phases have been prepared and studied by a number of research groups. Properties similar to electron-deficient nitro-aromatic phases were expected and found for fluorinated phenyl phases. Haas et al.[99] synthesized phenyl, fluorinated phenyl, decyl, and fluorinated decyl phases and compared the retention behavior of each under normal- and reversed-phase conditions. Because perfluorinated alkyl phases have low stability, each of the fluorinated phases was prepared by attachment through a two- or three-carbon-long nonfluorinated "spacer". It is believed that the added flexibility of this spacer helps to increase stability. The fluorinated phenyl phases showed markedly improved selectivity toward PAH than did the normal phenyl phase. The authors explain this difference by complex formation between the electron-rich PAH and the electron-deficient fluorinated phenyl phase. This conclusion is supported by the fact that PAH separations were possible in a hexane mobile phase for the fluorinated phenyl phase, but not for the nonfluorinated phase. Solvophobic interactions are minimized or eliminated in hexane, so the primary retention mechanism must be attributed to donor-acceptor complexation. The fluorinated decyl did not show any improvement over the nonfluorinated phase for the separation of PAH. Lower retention was found for the fluorinated alkyl phase when used under the same conditions as the nonfluorinated phase. The fluorinated alkly ligands were viewed as a true bristle phase, because the chains are more rigid than nonfluorinated ligands. The authors concluded that fluorinated alkyl ligands are theoretically interesting, but not justifiable as a new phase type.

Berendsen et al.[100] also compared fluorinated and nonfluorinated decyl phases in the reversed-phase mode. In general, lower retention was observed for the fluorinated phase and small selectivity differences were noted. The most dramatic difference in the phases was apparent for fluorinated solutes. Even though the fluorinated phase was less retentive than the decyl phase (overall retention was similar to a C_3 phase), fluorinated solutes were selectively retained longer than with the decyl phase. This selectivity toward fluorine-containing compounds was observed even for solutes containing only a single fluorine atom. An application of the perfluoro stationary phase to the separation of fluorine-containing herbicides was presented. Investigations of fluorinated phenyl phases was continued by Ecknig et al.[101] and Felix and Bertrand.[102] Used in the normal-phase mode with hexane, separation of PAH by number of rings was demonstrated on the fluorinated phenyl phase. Ecknig et al.[101] found that alkyl-substituted PAH with different chain lengths had similar retention on fluorinated phenyl phases. Felix and Bertrand[102] observed changes in retention as a function of aromaticity. For PAH with the same number of rings, the elution order was generalized as noncondensed (e.g., biphenyl) < catacondensed (e.g., naphthalene). Other, undefined effects were thought to influence retention.

Colmsjö et al.[103] prepared an unusual sulfur heterocyclic stationary phase for the separation of aromatic sulfur heterocycles (PASH) from nonsulfur PAH. 2-Iodo-thiophene was converted to a Grignard reagent and reacted with allylbromide. The product was reacted with trichlorosilane (hydrosilylation) to give propylthiophenetrichlorosilane. Bonded phases were prepared using this silane and comparisons were made with commercial C_{18} phases. Complete separation of fluorene and the sulfur analog dibenzothiophene were obtained using the propylthiophene bonded phase. Dibenzothiophene eluted after fluorene with this column; however, with the commercial polymeric C_{18} phase, separation was incomplete and the elution order was reversed.

E. Novel Phases

The proliferation of phases in LC is not nearly as great as in gas chromatography; nevertheless, novel phases have been prepared that take advantage of unusual solute — stationary phase interactions. Felix and co-workers[104,105] synthesized a caffeine phase for the separation of polycyclic aromatic hydrocarbons. A physically coated caffeine phase was also used in preparative chromatography.[106] PAH separations using hexane/methylene chloride (95:5) were attributed to a charge transfer (donor acceptor) mechanism. Application to the separation of petroleum residues was presented using a normal-phase solvent gradient.

The separation of basic amines and other nitrogen containing compounds was carried out by Pharr et al.[107] by making use of chemical interactions between primary and secondary amines and carbonyls. Primary amines react with carbonyl compounds by condensation to give an azomethine linkage (Schiff base):

$$R\text{-}NH_2 + R'\text{-}(CO)\text{-}R'' \rightarrow R\text{-}N{=}CR'R'' \qquad (5)$$

Secondary amines also react with carbonyls as in Equation 6:

$$RR'\text{-}NH + R''\text{-}(CO)\text{-}R''' \rightarrow RR'\text{-}N\text{-}(C{=}R'')\,R''' \qquad (6)$$

Tertiary amines do not react with carbonyl groups. Each of these reactions is reversible and so can be used to advantage for chromatographic separations. Pharr and co-workers[107] decided to prepare an aromatic ketone bonded phase to evaluate the feasibility of this concept. 3-(*p*-Acetylphenoxy)propyldimethylchlorosilane was synthesized by hydrosilylation of 3-(*p*-acetylphnoxy)propene. The resulting bonded phase was characterized by carbon analysis (3.8 μmol/m^2) and fourier transform infrared spectroscopy (FTIR). Both techniques positively identified the presence of the bonded species. The mobile phase used to separate various amines was carefully chosen to permit chemical interaction with the bonded phase, but at the same time minimize adsorptive effects. The latter type of interaction was deemed undesirable because of lack of discrimination between primary and secondary amine groups. Hexane/isopropanol (95:5) was selected after some trial and error. A typical separation of chloroanilines is illustrated in Figure 17. Success was achieved in the separation of other difficult-to-separate compounds including nitroanilines, acetanilides, and amides. For para isomers of aniline the order of elution was $-Cl < -NO_2 < -OH < -COOH < -OCH_3$. This ordering is explained by the stability of the Schiff base intermediate, which has a positive charge. Electron-donating groups stabilize this intermediate resulting in increased solute retention. The retention of other substituted bases is explained on a related basis. The utility of the acetylphenoxypropyl phase was demonstrated for the separation of a polar shale oil fraction.

Mourey et al.[108] used a pyrrolidone bonded phase for the separation of shale oil fractions. The pyrrolidone phase was operated both in the normal- and reversed-phase modes. In the reversed-phase mode, retention of PAH was found to correlate primarily with the number of aromatic rings in the sample, at least for samples with one to six rings. The normal-phase mode was found to be most suitable for aromatic class separation for PAH with three rings or more. Little discrimination was observed among smaller PAH. Retention was attributed to electronic interactions rather than adsorption or reversed-phase "partitioning".

ω-Hydroxyalkyl silica phases were prepared by Matlin and Tinker[109] by reaction of Grignard and silane reagents with silica. In each case the terminal hydroxy group was protected by conversion to TMS ether. After modification of the silica, this protecting group was removed by aqueous hydrolysis. Slightly lower coverages were obtained by Grignard coupling than by reaction with trichlorosilane reagents. Chromatographic properties of the ω-hydroxy alkyl phases were examined for the retention of aromatic hydrocarbons, nitroanilines, and metal acetylacetonates. Contributions to retention from both the nonpolar alkyl portion and polar hydroxy portion of the bonded ligands were evident.

Crowther and Hartwick[109a] prepared multifunctional stationary phases that could be used

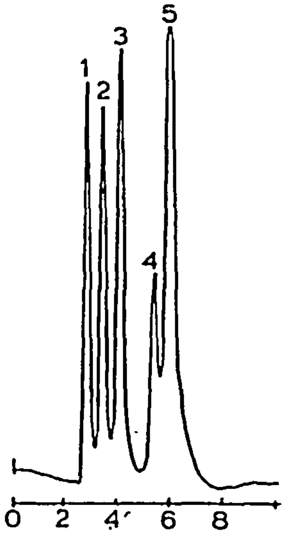


FIGURE 17. Separation of chloroanilines on 3-(*p*-acetylphenoxy)propyl bonded phase. Solutes: (1) 2,6-dichloroaniline, (2) *o*-chloroaniline, (3) 2,3-dichloroaniline, (4) 3,5-dichloroaniline, (5) dichloroaniline. (Reproduced from Pharr, D. Y., Uden, P. C., and Siggia, S., *J. Chromatogr. Sci.*, 23, 391, 1985. With permission.)

to emulate certain types of ion-pairing separations. Alkyl and ion exchange silanes were blended in various proportions to synthesize bonded phases with mixed ion-exchange and reversed-phase properties. Anion and cation exchange functional groups were both applicable to this technique. The overall retention of the solutes was similar for the three phases, which differed in the ratio of quaternary amine/C_8 ligands. Selectivity, however, varied considerably and is probably optimized for the given separation at 80% quaternary amine.

Another mixed stationary phase was produced by "diluting" diphenylphosphine ligands with octadecylsilyl groups.[110] The phase was synthesized primarily as a tool in understanding the nature of the chromatographic surface, but its chromatographic utility was also examined. Cross polarization and magic angle spinning (CP/MAS) NMR and elemental analysis were used to characterize the bonded silica. Surface concentration values for the diphenylphosphine ligands bonded to a narrow-pore silica (6.0 nm) were considerably lower than values usually found for alkyl ligands (~ 0.7 vs. 3.5 μmol/m^2 for C_{18} chains). This value increased when a larger pore (10 nm) substrate was used (~ 1.2 μmol/m^2). Heat treatment of the phase promoted the formation of multiple bonds to the silica surface. CP/MAS NMR was used to examine the diphenyphosphine phases before and after endcapping with trimethylchlorosilane and it was conclusively shown that trimethyl groups bond to the ligand silanols as well as the silica silanols. The mixed diphenyphosphine/C_{18} phase was found to exhibit both polar and nonpolar properties that could be used to advantage when operated in the normal- and reversed-phase modes. As an example, substituted nitroanilines were easily separated using an isooctane/ethanol/water (85:14.5:0.5) mobile phase and various substituted phenols were separated in acetonitrile/water (35:65).

The separation of hydroxybenzoic acids and nitrophenols has been demonstrated on a polyallylamine-coated pelicular substrate using a 5-m*M* sodium hydrogen carbonate mobile phase.[111] The retention of hydroxybenzoate esters decreased with phase loading, whereas nitrophenol retention increased with loading. At low concentrations, the polyallylamine is viewed as spreading out uniformly over the silica to give monolayer coverage. Amine groups pair with surface silanols, and the resulting phase is mostly nonpolar. At high phase concentrations, more amine groups are free to interact with polar solute. Thus, the relatively nonpolar benzoates are retained longer on a low loaded nonpolar phase than a more polar phase of higher loading.

Recently, Hagestam and Pinkerton[112-114] reported the synthesis of a novel nonpolar phase designed for the reversed-phase analysis of serum or plasma samples. Matrices containing proteins ordinarily must be denatured and extracted prior to analysis by LC. Attempts at analysis by direct injection of serum samples onto conventional reversed-phase columns usually result in loss of column efficiency and increased backpressure due to protein precipitation at the head of the column. This precipitation is due, in part, to adsorption and denaturation at the nonpolar surface of the bonded phase. The approach taken by Hagestam and Pinkerton makes combined use of size exclusion and hydrophobic retention. Three major proteins are present in blood plasma: globulin (30 to 60%, MW 90,000 to 160,000), albumin (52 to 66%, MW 65,600), and fibrinogen (6%, MW 400,000). The smallest of these proteins, albumin, is excluded from pores 80 Å in diameter and smaller. Thus, with small pore substrates, proteins are unable to enter the pore network and interact only with the external surface of the particles. Smaller analytes of interest (i.e., drugs, vitamins, etc.) are not excluded from the pore network. To minimize protein interaction with the sorbent, Hagestam and Pinkerton prepared a bonded phase sorbent with hydrophilic external properties and hydrophobic internal properties. Small pore silica was first modified with a glycerylpropyl bonded phase. To this phase was attached hydrophobic di- or tripeptide groups (glycine-L-phenylalanine or glycine-L-phenylalanine-L-phenylalanine). The final step in the synthesis was enzymatic cleavage with carboxypeptidase A (MW 35,000). Because this enzyme is excluded from the pore network, only peptides bound on the external surface of the particles were cleaved. The result is a modified substrate with dual hydrophilic and hydrophobic character, and this type of sorbent has been termed "internal surface reversed-phase packings" (ISRP). The utility of the novel phase was demonstrated for the direct isolation of phenytoin from plasma. It is interesting to note that phenytoin is bound to serum albumin. Release of the drug must occur on introduction into the ISRP column. This has wide implications for the separation of other protein-bound components in serum and plasma.

A second type of ISRP column was prepared using an amine phase rather than a glycerylpropyl phase.[114] The change in the primary phase was made to increase the yield of the subsequent silanization reactions (compared with the glycerylpropyl phase). Butoxy-L-phenylalanine (Boc-L-Phe) was linked to the amine phase with 1,1-ethyl-3-(3-diaminopropyl)carbodiimide (EDC). Externally bound Boc-L-Phe was cleaved with chymotrypsin and the phase was "capped" with 2,3-epoxy-1-propanol. The resulting phase was shown to be more retentive than previous ISRP phases, and its use for the separation of anticonvulsant drugs in serum was demonstrated.

F. Cyclodextrin Phases

Cyclodextrins are a class of compounds consisting of repeating glucose molecules bonded together in a cyclic structure. Three common cyclodextrin molecules have been illustrated by Armstrong and DeMond[115] and are shown in Figure 18. The molecules differ in the number of glucose units in the ring; γ-cyclodextrin (cyclooctaamylose), β-cyclodextrin (cycloheptaamylose), and α-cyclohexaamylose (cyclohexaamylose). Because of the cavity formed by the ring, cyclodextrin is sometimes viewed as a "molecular cup" (Figure 19). The interior of this cavity is primarily hydrophobic in nature, but hydroxyl groups do exist at the edge and exterior of the cavity. A variety of organic molecules are known to form inclusion complexes with cyclodextrins. Armstrong and DeMond noted that the unusual structure of cyclodextrins gave rise to several possibilities for chiral interactions. Each glucose unit has five chiral centers. Also, the 2-hydroxyls all point in one direction around the ring,

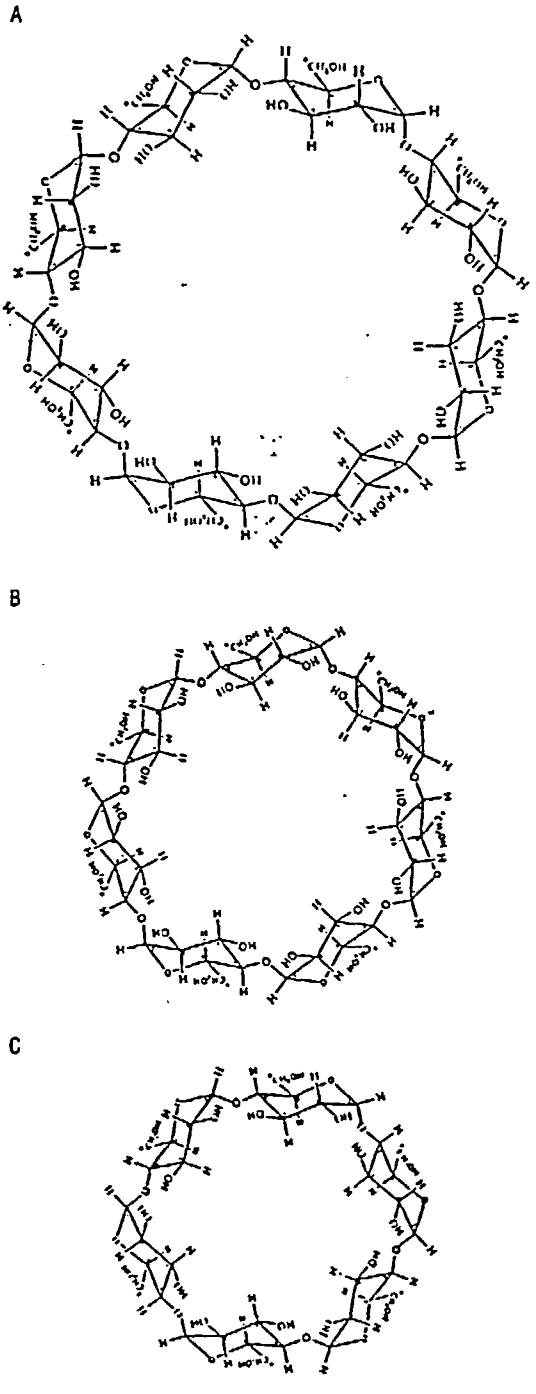


FIGURE 18. Schematic diagram illustrating the overall structure and relative size of three common cyclodextrin molecules. (A) γ-Cyclodextrin, (B) β-cyclodextrin, (C) α-cyclodextrin. (Reproduced from Armstrong, D. W. and DeMond, W., *J. Chromatogr. Sci.*, 22, 411, 1984. With permission.)

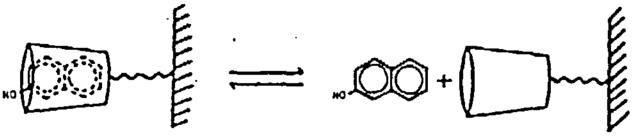


FIGURE 19. Schematic diagram of cyclodextrin bonded phase. Chiral interactions can occur at the edge of the cup, while hydrophobic interactions dominate within the cup. (Reproduced from Armstrong, D. W. and DeMond, W., *J. Chromatogr. Sci.*, 22, 411, 1984. With permission.)

and the 3-hydroxyls point in the opposite direction. Because of the combined chiral and hydrophobic nature of cyclodextrins, the potential of cyclodextrin-based bonded phases seems promising.[116] Cyclodextrins have been used as mobile phase additives, but only in about the last 5 years has real progress been achieved in designing and synthesizing cyclodextrin-based bonded stationary phases.

Tanaka and co-workers[117] prepared α- and β-cyclodextrin bonded phases on a polyacrylamide gel substrate. They reported the separation of ortho, meta, and para benzene isomers on these phases. Retention times were excessive — over 400 min for *o*-dinitrobenzene! The authors attributed the source of this unusually long retention to the low flow rates required by the gel substrate and suggested that silica-based cyclodextrin phases would reduce analysis times. The elution order for disubstituted benzene isomers was, in general o < m < p for the α-cyclodextrin phase. The β-cyclodextrin phase (with larger cavity) did not resolve benzene isomers as well as the α-cyclodextrin phase.

In a later study, Fujimura and co-workers[118] succeeded in immobilizing cyclodextrins on silica. Silica was first modified with diaminopropyl or aminopropyl groups by reaction with the appropriate trimethyoxysilane. The cyclodextrin was then tosylated with *p*-toluenesulfonyl chloride and linked via the tosyl species to the bonded amine phase. Although only small increases in carbon loading were measured as a result of the cyclodextrin reaction, the quantity of bonded phase was ample to separate various benzene and naphthalene isomers in 20/80% methanol/water. Separations were not possible on the amine phase prior to modification with the tosylated cyclodextrin. The same order of elution for disubstituted benzene isomers was observed as by Tanaka et al.,[117] i.e., ortho < meta < para. An exception to this trend was found for nitroaniline isomers, meta < ortho < para. In all cases the para isomers eluted last. This is thought to be due to a lower steric hindrance of para isomers to fit inside the cyclodextrin cavity. Polar functional groups are viewed as remaining outside of the cavity and, perhaps, interacting with "rim hydroxyls".

Silica-bonded cyclodextrin phases were acetylated with acetic anhydride by Tanaka et al.[119] Acetylated β-cyclodextrin was found to be superior to the unmodified phases for the separation of *o*-, *m*-, and *p*-isomers of toluidine and dinitrobenzene. The elution order was observed to change after acetylation from meta < ortho < para, to ortho < meta < para (for nitrobenzoic acid and nitroaniline isomers). Phase selectivity of α-cyclodextrin was not, however, markedly improved by acetylation. The effects of mobile phase composition and pH upon solute retention were examined in a later study.[120] While trends in retention as a function of mobile phase composition are similar to those observed in reversed-phase C_{18} systems, cyclodextrin phases are concluded to be superior for the separation of substituted aromatic isomers (e.g., cresol, iodoaniline, and nitrobenzene isomers).

The utility of cyclodextrin phases in chiral separations has been demonstrated by Armstrong and DeMond using a commercially available cyclodextrin column.[115,121] The separation of D and L isomers of β-naphthylamide derivatives of alanine and methionine is illustrated in Figure 20. Also shown is the separation of a trace quantity of dansyl D-leucine from dansyl L-leucine. Mobile phase conditions were 50/50% methanol/water. Water is necessary for

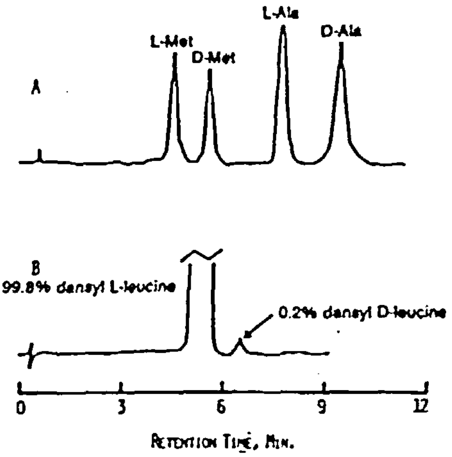


FIGURE 20. (A) Baseline separation of β-naphthylamide derivatives of alanine and methione enantiomers; (B) separation of enantiomers at low relative levels, illustrating the potential for determination of optical isomer purity. (Reproduced from Armstrong, D. W. and DeMond, W., *J. Chromatogr. Sci.*, 22, 411, 1984. With permission.)

inclusion complexation. Separations can be carried out in a normal-phase mode, but the retention mechanisms are thought to change and chiral solutes are usually not as well resolved as with hydroorganic solvent mixtures. Methanol and water mixtures are commonly used, because methanol does not associate strongly with the interior of the cyclodextrin cavity. Cyclodextrin phases can also be used to advantage for the separation of nonchiral molecules. Armstrong and DeMond[115] separated six barbiturates (barbital, butabarbital, sodium pentabarbital, phenobarbital, secobarbital, and amobarbital) in under 15 min using a β-cyclodextrin column. They noted that the selectivity of cyclodextrin phases changes with ring size. For example, the elution order of nitroaniline isomers with α-cyclodextrin phases is ortho < meta < para, but with β-cyclodextrin phases the order is usually meta < ortho < para. Changes in selectivity of the β-cyclodextrin phase are also noted with phase loading. With low ligand concentrations *p*-nitroaniline elutes before *o*-nitroaniline. For increased ligand concentrations retention of the para isomer increases faster than that for the ortho isomer, and *p*-nitroaniline elutes last.

G. Carbon Phases

Sorbents known as porous carbon packings are not chemically bonded, rather they consist of porous substrates with elemental carbon surfaces. Progress, to date, on porous carbon phases has been reviewed by Unger.[122] Carbon sorbents have been prepared in a variety of ways and from numerous starting materials including activated charcoal, hard cokes, polytetrafluoroethylene (PTFE), carbon black, and silica. Knox and Gilbert[122-124] developed a procedure for depositing a glassy carbon on silica by pyrolysis of a phenol/formaldehyde polymer coating. Colin et al.[125-129] prepared pyrocarbon phases on silica and on carbon black by pyrolosis of benzene vapor. Pyrocarbon phases have also been prepared by pyrolysis of dichloromethane.[130] Each of these processes shares two common steps: pyrolysis of organic compounds at high temperature and subsequent thermal treatment to improve the homogeneity of the surface.[122] Ideally, one would desire carbon packings to have physical properties similar to those of silica (i.e., strength, surface area, pore diameter, and particle size). Unfortunately, such ideal carbon sorbents have not yet been produced. The overall chromatographic properties of carbon-based phases are somewhat similar to C_{18} reversed-phase sorbents; however, important selectivity differences exist. For example, Unger[122] has reported that carbon adsorbents are much more selective towards PAH than are conventional alkyl modified silicas. Because solute retention occurs at a true surface rather than at a bonded layer (or multiple layer), retention mechanisms probably differ from those for bonded alkyl sorbents. These differences have motivated the study of carbon phases by several research groups.

Colin and co-workers[129] studied the chromatographic properties of pyrocarbon modified silica and pure carbon adsorbents. Emphasis was placed on changes in column performance as a function of temperature. The following trends were noted for increasing temperature: (1) little or no change in column selectivity, (2) decrease in absolute retention according to the relation in $k' \alpha 1/T$, (3) lower mobile phase viscosity resulting in improved column efficiency and lower column backpressure, and (4) slightly improved peak shape. The authors concluded that the major influence of temperature is on the mobile phase rather than the stationary phase. Very high retention was noted for certain PAH with hydroorganic mobile phases.

Zwier and Burke[131] combined the properties of bonded phases and carbon phases by synthesizing a C_8 alkyl phase on a porous carbon substrate. The carbon substrate was prepared by lithium amalgam reduction of polytetrafluoroethylene. A C_1 phase was produced on the carbon adsorbent by reaction with trichlorosilane; however, this phase was found to be susceptible to hydrolysis. More stable phases were attempted by a two-step synthesis. The carbon packing was first chlorinated with thionyl chloride and then reacted with octylmagnesiumbromide. The phases produced in this manner were found to be stable to hydrolysis. Peak tailing of polar compounds was reduced on the C_8 carbon phase, suggesting that adsorption at polar sites had been reduced. Retention of PAH was very large (sometimes infinite) on the carbon phase. The C_8 carbon phase had lower retention of PAH. The authors concluded that alkyl bonded carbon adsorbents may become an effective alternative to silica-based adsorbents if efficiency considerations can be overcome.

Dawidowicz and co-workers[130] prepared a pyrocarbon-coated silica in a similar manner to Colin et al.,[129] except dichloromethane was used in the pyrolysis instead of alcohols or benzene. The procedure described using dichloromethane does not have the drawbacks of previous methods, i.e., sooty particles were not formed as with benzene pyrolysis, nor were tarry residues formed as with pyrolysis of alcohols. Carbon loadings of pyrocarbon phases prepared on a 50-nm pore size, 10-μm particle-size silica ranged from about 2 to 10%. As expected, retention and selectivity increased with carbon loading, but unlike with pyrocarbon phases originating from benzene pyrolysis, selectivity decreased with increasing mobile phase strength.

A different approach based on the electrochemical reduction of poly(tetrafluoroethylene) (PTFE) with alkali-metal amalgams was used by Jansta et al.[132-134] Unlike the pyrolysis reactions involving benzene or dichloromethane, the reduction of PTFE was carried out at very low temperatures (~ 25 to 100°C) and, consequently, pyrolysis products were not formed at the carbon surface. The material isolated from the reduction was a free-flowing powder with the bulk of the particles in the size range 4 to 12 μm. Specific surface areas were very large — ~ 2500 m^2/g (BET) or 1550 m^2/g (small-angle X-ray scattering). Separation of *p*- and *m*-carborane was demonstrated for a PTFE-carbon column using heptane as the eluent.

III. CHARACTERIZATION TECHNIQUES

A. Chromatographic Evaluation

In the most general sense, all C_{18} columns have similar retention characteristics: nonpolar compounds are usually retained longer than polar compounds for reversed-phase separations.

Variations among C_{18} columns prepared by different manufacturers, however, can be great and separations that are easily achieved with one column may become difficult or impossible with other columns. These differences take the form of variations in column efficiency, absolute retention, and selectivity and may result from differences in phase polarity, silanol deactivation, phase coverage, carbon loading, the type of silane used in the synthesis, and even differences in the silica substrate.

A number of studies have made comparisons of the properties of commercially available C_{18} columns. Often this is carried out by chromatographing a series of solutes on the columns under consideration and contrasting the results. The importance of the choice of the solutes is clear: probes must be selected that will accurately reflect the overall and specific retention properties of interest. Unfortunately, a consensus has not yet been reached as to the composition of such test mixtures, and different probes are used from one study to the next. The feasibility of specifying column performance and evaluation by standard reference mixtures is currently under investigation in our laboratory.

1. Efficiency and Polarity/Silanol Activity

Most of the approaches taken in the chromatographic characterization of LC columns have utilized solute mixtures consisting of a broad range of dissimilar compounds. Nonpolar compounds are commonly used in the measurement of column efficiency, because they give rise to the largest theoretical plate values. Nonpolar compounds, especially homologous series, have been used to characterize hydrophobic retention and phase selectivity. Polar compounds are used to probe silanol activity. Little et al.[81] proposed the use of eight separate solute mixtures for the evaluation of reversed-phase sorbents. The mixtures were considered representative of a broad class of compounds including PAH, phenols, aromatic hetero-atom solutes, basic (nitrogen containing) solutes, ketones, sugars, and azo compounds. Column properties targeted for evaluation were alkyl chain length, basicity, acidity, steric effects, and phase polarity. Over 15 phases were compared using these mixtures and, in most instances, C_{18} phases were adequate for the separation. Sugars, however, were best separated on a propylethylenediamine phase and azo compounds on an octadecylsulphonic acid phase. The need for divergent test probes is clearly demonstrated.

Domagalska and Loscombe[135] used a series of polar and nonpolar solutes to evaluate column efficiency. Columns containing 3- and 5-μm-diameter particles were employed in the study, but the smaller particle columns did not always out perform the larger particle columns. The largest theoretical plate values were obtained for nonpolar solutes (toluene, naphthalene, anthracene). Somewhat smaller values were measured for polar solutes such as phenol and benzamide.

Significant changes in solute retention were observed by Engelhardt et al.[7] among C_{18} phases for the separation of aniline and related compounds (see Figure 21). The retention of basic solutes such as aniline was shown to increase and exhibit significant peak tailing on columns with accessible silanols. For example, C_{18} phases prepared with octadecyltrichlorosilane. In the latter case, peak tailing was excessive, but was observed to improve after endcapping the phase with HMDS. Little change was observed in the retention behavior of the ODS phase after endcapping, thus, suggesting that silanol groups for this phase were largely inaccessible to the solutes. Engelhardt and co-workers[7] further demonstrated the need for testing column performance over a wide range of mobile phase conditions. For the separation of nonpolar compounds, column selectivity was observed to be relatively constant among different C_{18} phases even when measured at different mobile phase compositions. The selectivity coefficient for *n*-methylaniline/benzene, however, was found to vary significantly between different columns at intermediate mobile phase conditions. At high and at low water concentrations, little difference in selectivity was observed among the various columns for these compounds.

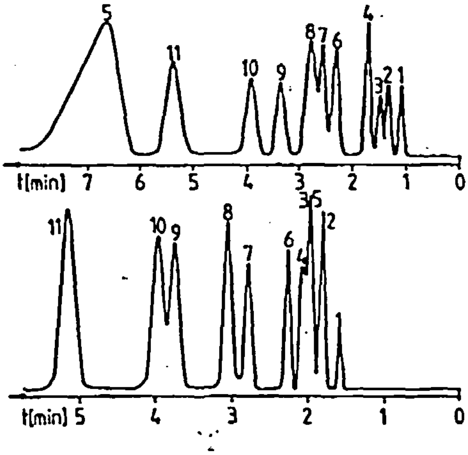


FIGURE 21. Comparison of retention and selectivity for two commercial C_{18} columns for the separation of polar and nonpolar solutes. Solutes: (1) D_2O, (2) benzamide, (3) phenol, (4) 2-phenylethanol, (5) aniline, (6) nitrobenzene, (7) methyl benzoate, (8) benzene, (9) ethyl benzoate, (1) toluene, (11) ethyl benzene. (Reproduced from Engelhardt, H., Dreyer, B., and Schmidt, H., *Chromatographia*, 16, 11, 1982. With permission.)

Rabel and co-workers[136] routinely use a mixture of aniline and phenol to evaluate phase polarity resulting from silanol groups. The solute pair is chromatographed isocratically at 60:40 methanol/water. Qualitatively, it is observed that aniline will elute before phenol on well-endcapped surfaces. Under these conditions, aniline elutes after phenol for phases that are not endcapped or that have accessible silanol groups. Rabel also reports that column efficiency varies considerably depending on mobile phase conditions and on the test solute employed. For example, benzene, naphthalene, and biphenyl were used in the measurement of column efficiency (80:20 methanol/water) for a certain 3-μm C_{18} column, and a value of 48,850 plates/meter was obtained. However, for the retention of methyl parabens at 40:60 acetonitrile/water, an efficiency of only 26,800 plates/meter was obtained.

A different set of probes has been used by Verzele and Dewaele[71,75] to measure silanol activity in various bonded phases. The ratio of the retention times of naphthalene and nitronaphthalene reveals the degree of activity or of deactivation by endcapping. Verzele and Dewaele suggested that columns that were acceptably deactivated should have selectivity coefficients for naphthalene/nitronaphthalene of at least 1.4 or higher. Lower values are observed for nonendcapped phases, ~ 1.1 to 1.2. A novel probe was used to evaluate the presence of trace metals in bonded phase sorbents. Verzele and Dewaele[71,75] observed that β-diketones are good indicators of trace metal activity. Acetylacetone was selected because of its availability. Most of the commercial C_{18} columns evaluated using this solute gave rise to distorted, badly tailing peaks. A demineralization technique using phosphoric acid was devised that removed a significant fraction of the offending metal species, as was evidenced by the improved peak shape for acetylacetone (see Figure 22). Such treatment does not appear to influence silanol activity. In a related study, the influence of trace metals on the chromatography of beer bitter acids was studied.[137] A series of polar and nonpolar compounds was chromatographed and theoretical plate values determined. As with other studies, the sharpest peaks were observed for nonpolar solutes. Particularly low plate counts were measured for cinnamic acid. Hop bitter acids and derived products were similarly affected. Verzele and Dewaele showed that the loss in efficiency for these compounds was related to the metal content of the silica. They described a procedure by which the concentration of trace metals in bonded phase sorbents could be reduced by boiling in methanolic

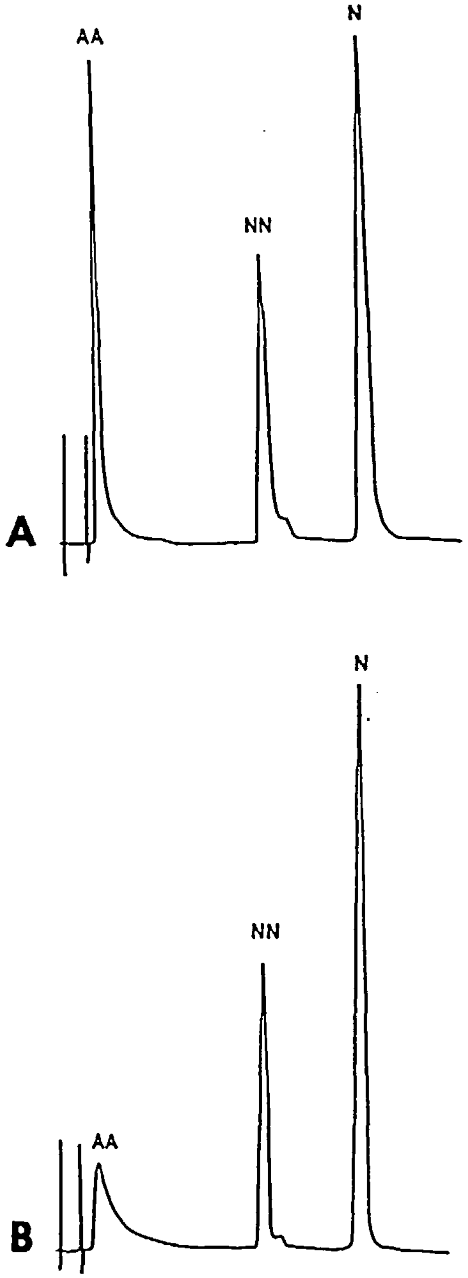


FIGURE 22. Comparison of column performance (B) before and (A) after demineralization. Acetylacetone tails badly before demineralization indicating the presence of metals in the sorbent. (Reproduced from Verzele, M. and Dewaele, C., *Chromatographia*, 18, 84, 1984. With permission.)

hydrochloric acid. Maximum effect is achieved by boiling at least three times with 6 *M* HCl. The quantity of bonded phase lost through hydrolysis was estimated to be about 5% (i.e., 18% carbon reduced to 17%).

Scott and Kucera[138] used a normal-phase procedure to characterize the extent of derivatization of several commercial C_{18} phases. In effect, the test that they devised was a measure

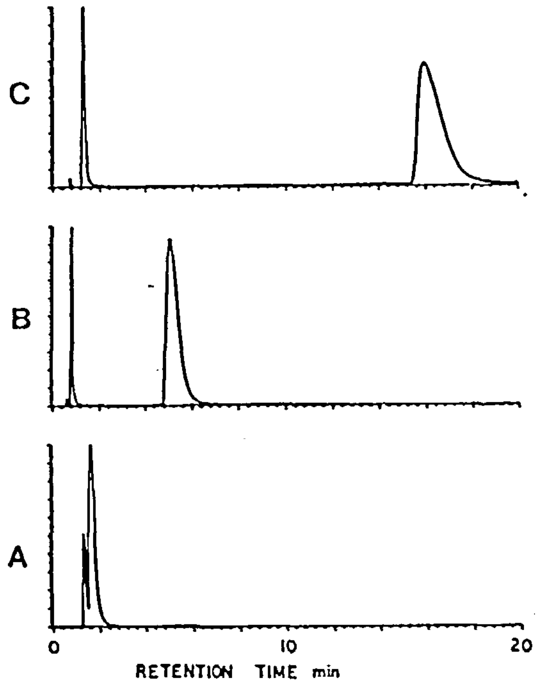


FIGURE 23. Normal-phase retention of benzene and nitrobenzene on three commercial C_{18} phases. Columns: (A) ODS (5% carbon); (B) ODS-2 (16.9% carbon); (C) RP-18 (19.8% carbon). (Reproduced from Scott, R. P. W. and Kucera, P., *J. Chromatogr.*, 142, 213, 1977. With permission.)

of the polarity of the bonded phase and, thus, was directly related to the number of accessible silanol groups at the modified surface. Each column was equilibrated by passing six column volumes of tetrahydrofuran, ethyl acetate, dichloroethane, and *n*-heptane through the column. Benzene and nitrobenzene were then chromatographed using the heptane mobile phase, and the relative retention of nitrobenzene/benzene was measured. Columns that were well deactivated retained nitrobenzene only slightly more than benzene, while for unloaded phases containing accessible silanols, retention of nitrobenzene was significant (see Figure 23). Karch et al.[139] also used a normal-phase procedure to characterize silanol accessibility. A series of solutes ranging from hexane to *o*-dinitrobenzene was chromatographed on reversed-phase columns using *n*-heptane as the eluent. Columns included C_1, C_4, C_{18} (two surface coverage values), and bare silica. Retention of the solutes increased with increasing solute polarity. Thus, hexane was unretained and *o*-dinitrobenzene was retained the longest on each of the columns. For a given solute, retention was lowest on the C_4 phase, suggesting that the C_4 ligands were the most efficient at shielding unmodified silanols from interaction with the probe molecules. Silanol accessibility was also evaluated by the methyl red adsorption criterion described by Shapiro and Kolthoff.[140] Karch et al. recommended the use of phases that have no methyl red adsorption and that have normal-phase k′ values for benzene and nitrobenzene of less than 0.1 and 0.5, respectively.

A quantitative measurement of silanols in 25 homemade reversed-phase materials was made by Welsch et al.[141] Silanol concentration was determined by reaction with methyllithium.[142] These values were then correlated with the normal-phase retention of polar compounds (nitrobenzene, benzonitrile, and benzyl alcohol) using *n*-heptane as the eluent. A plot of log k′ vs. silanol concentration is shown in Figure 24. The retention of nitrobenzene

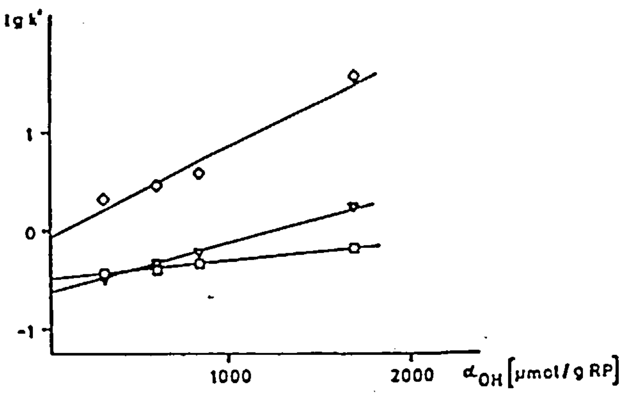


FIGURE 24. Dependence of normal-phase retention (ln k') on silanol concentration (µmol/g) using water-saturated heptane mobile phase. Solutes: (□) nitrobenzene, (∇) benzonitrile, (◇) benzyl alcohol. (Reproduced from Welsch, T., Frank, H., Zwanziger, H., Liebisch, S., and Engewald, W., *Chromatographia*, 19, 457, 1985. With permission.)

changes only slightly with variations in the residual silanol concentration. For this reason, the more polar benzonitrile or benzyl alcohol is preferable as an indicator of silanol concentration. Unfortunately (but perhaps not unexpectedly), the retention of these probes is also dependent on the nature of the alkyl ligand attached to the silica. Welsch and co-workers determined calibration curves for a series of bonded phases with chain lengths ranging from RP-1 to RP-18. The influence of silanol concentration on peak shape was also investigated under similar chromatographic conditions. Peak shape was found to be best for phases with either very high or very low silanol concentrations. Asymmetrical peaks were observed for intermediate silanol concentrations. Furthermore, this asymmetry was seen to increase with phase length. The explanation advanced is that peak tailing is related to the accessibility of the silanol groups. For phases with few silanol groups, there is little interaction with polar probe molecules. With active surfaces such as bare silica, silanols are fully accessible and interaction is complete (and uniform). Moderately accessible silanol groups give rise to partial interaction with polar solutes and it is thought that the heterogeneity of the surface gives rise to peak asymmetry. Welsch et al. were able to classify bonded phases into four distinct groups using multivariate statistical analysis and retention and asymmetry descriptors for aniline, butyrophenone, and benzyl alcohol.

Other polar probes have been used in a reversed-phase mode to characterize silanol activity. In general, the absolute retention of polar compounds decreases and the peak shape improves as phase polarity decreases. Such a reduction in polarity can be achieved by endcapping reactions. Dramatic results were achieved by Cooke and Olsen[5] by endcapping a commercial C_{18} column for the separation of PTH-amino acids (see Figure 25). PTH-histidine and PTH-arginine exhibited very broad peaks before endcapping, and narrow, well-separated peaks after endcapping. The absolute retention of the column for the third component (PTH-valine) was virtually unaffected by endcapping. Daldrup and Kardel[143] devised an evaluation mixture that emphasized column suitability for toxicological screening. The mixture consists of diphenhydramine, MPPH (5-[*p*-methylphenyl]-5-phenylhydantoin), and diazepam. The retention of MPPH was found to be relatively insensitive to phase differences among columns prepared by different manufacturers. For this reason, it was used as a standard from which relative retention of the other probes was based. Diphenhydramine is a basic drug sensitive to free silanol groups, and diazepam is a test probe used to indicate the surface concentration of bonded alkyl groups. The text mixture was used to classify 24 commercial reversed-phase columns into two groups: those with high retention for the basic drug diphenhydramine

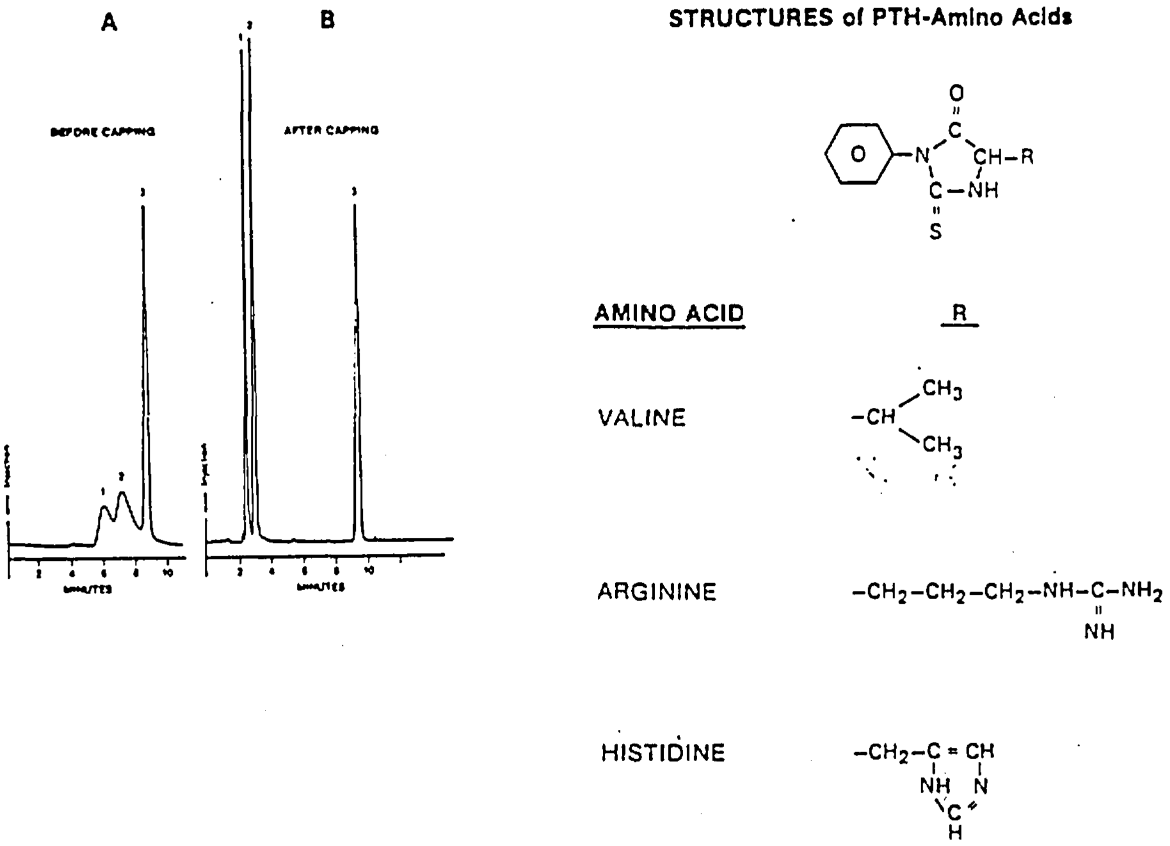


FIGURE 25. Effect of endcapping on the retention of PTH amino acids (A) before endcapping; (B) after endcapping. (Reproduced from Cooke, N.H.C. and Olsen, K., *J. Chromatogr. Sci.*, 18, 512, 1980. With permission.)

(diphenhydramine eluting after diazepam) and those with which diphenhydramine elutes before MPPH. The latter class of columns was considered the most suitable for the separation of basic and neutral drugs. Columns classified into the first group were thought to have a large number of unreacted and accessible silanol groups at the silica surface.

Sadek and Carr[144] used dimethyldiphenylcyclam, a cyclic tetraaza macrocycle, for the chromatographic evaluation of residual silanols. Cyclam was added to the mobile phase as a blocking agent for residual silanols. The change in retention of a probe sensitive to free silanols (5,14-dimethyl-7,12-diphenyl-1,4,8,11-tetraazacyclotetradecane (DMDPC)) was measured before and after the addition of cyclam. The change was taken as indicative of the concentration of free silanols for the phase. Comparison of the absolute retention of DMDPC with chrysene was also used as an empirical measure of relative silanol activity. The properties of 16 commercial columns were evaluated using this technique. Other methods for the determination of silanols have been based on isotopic exchange,[145] chemical reaction,[146] titrations,[147,148] and complexation with metal species.[149]

The chemical nature of the silica substrate has been shown to have a pronounced effect on the chromatography of basic and acidic solutes.[150] Engelhardt and Müller measured the pH of a suspension of silica in distilled water. pH values ranged from 3.9 to 9.9 for 1% suspensions. Columns were prepared from the silicas and the normal-phase retention behavior of acidic and basic solutes was measured. In general, basic solutes tailed severely on "acidic" silica columns, but gave more symmetrically shaped peaks on "basic" silica columns. The opposite was true for acidic solutes. The authors speculated that the acidic or basic nature of the substrate could also affect the properties of alkyl bonded phases prepared on them. In a later work they showed that this was, in fact, the case for C_{18} phases prepared on acidic (pH 6) and basic (pH 10) silica. Aniline and related compounds were retained longer and had more peak asymmetry on the acidic C_{18} phase than on the basic C_{18} phase. Interestingly, the acidic or basic nature of the silica was shown to change by washing the silica in an acidic or basic solution followed by rinsing to neutral pH with water. Because acidic solutes are best chromatographed on bonded phase columns prepared using acidic silica and basic solutes with basic silica, no one type of column is best for all classes of solutes.

Köhler and co-workers[151] used a diverse array of techniques (including diffuse reflectance Fourier-transform infrared spectroscopy, elemental and thermogravimetric analysis, ^{1}H and ^{29}Si cross-polarization magic-angle spinning nuclear magnetic resonance spectroscopy, and chromatographic evaluations) to characterize silica substrates before and after modification with silane reagents. The retention of *N,N*-diethylaniline relative to 1-phenylhexane was used as a measure of acidic silanols. Various silicas were modified with trimethylsilane and phase stability was measured (as indicated by the increase in accessible silanol groups) after passing measured quantities of water through the columns. Phase stability varied significantly among phases prepared on the different substrates. From NMR, IR, and TGA data it was concluded that isolated, acidic silanols are detrimental to phase stability and column performance for the retention of basic solutes. On the other hand, homogeneously distributed geminal (or associated) silanols give rise to phases that exhibit high hydrolytic stability and low adsorptivity towards basic compounds.

2. *Selectivity Differences*

Antle and Snyder[152] have classified column selectivity effects in two categories: solvophobic and chemical. Solvophobic selectivity effects originate from solvophobic-type interactions between the solute and the stationary phase. In contrast, chemical effects result from strong polar interactions between acidic or basic solutes and unmodified silanols. Because chemical effects are often associated with poor column performance, Antle and Snyder directed their attention to selectivity differences arising from solvophobic effects. They concluded that most solvophobic differences could be related to a column strength

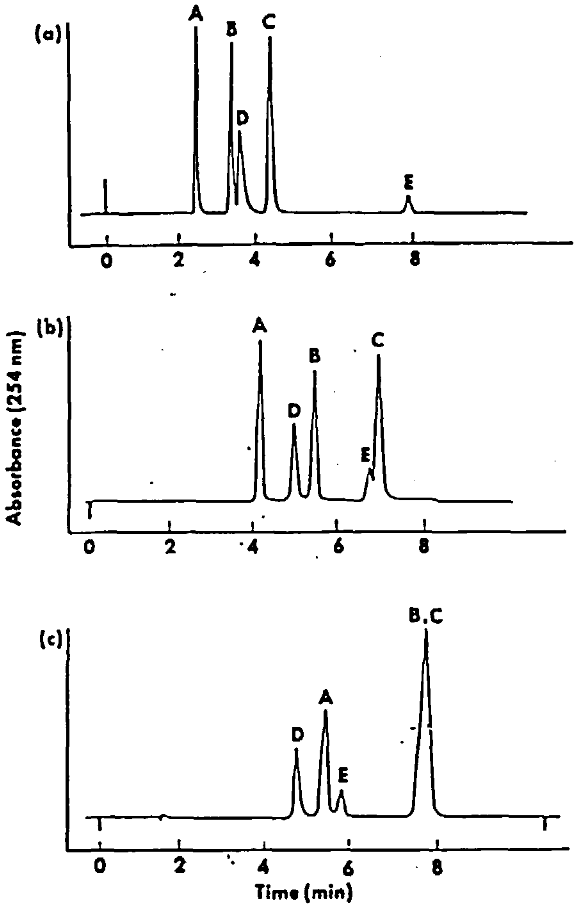


FIGURE 26. Changes in selectivity resulting from differences in column strength. Retention adjusted by varying mobile phase composition. (a) Zorbax ODS, 36:64 acetonitrile/water; (b) Zorbax phenyl, 32:68 acetonitrile/water; (c) Zorbax CN, 20:80 acetonitrile/water. (Reproduced from Antle, P. E. and Snyder, L. R., *Liq. Chromatogr. Mag.*, 2, 840, 1984. With permission.)

parameter (J) (related to the average retention of a sample on a given column). To measure this parameter, the retention of nonpolar compounds on various columns was related to retention on a standard column. Columns with lower-than-standard retention have small column strength values, while columns that strongly retain solutes have large column strengths. Column strength is dependent on stationary phase volume, surface area, and polarity. Thus, a reduction in phase volume or surface area, or an increase in phase polarity will result in a "low strength" column. Column strength variations originating from any of these causes may lead to gross differences in column selectivity (see Figure 26). The theoretical basis of these conclusions are treated in a later publication.[153] Jandera[154,155] used a different approach to characterize reversed-phase selectivity based on the retention of homologous series (alkylbenzenes). The utility of the method was demonstrated for the prediction of retention data.

Selectivity differences among commercial columns for the separation of polycyclic aromatic hydrocarbons (PAH) have been reported by a number of workers.[156-162] These differences have been related to the nature of the bonded phase, i.e., whether the phase was produced by monomeric or polymeric synthesis. Wise et al.[162-164] have shown that phase selectivity toward PAH varies continuously as a function of phase loading (ligand surface coverage) (see Figure 27). A three-component mixture was developed to assess the relative monomeric or polymeric nature of a phase, as related to PAH selectivity.[35] The elution order of the mixture consisting of benzo[a]pyrene (BaP), phenathro[3,4-c]phenanthrene, and 1,2:3,4:5,6:7,8-tetrabenzonaphthalene (TBN) was found to change with phase type. For monomeric phases the elution order in 85/15% acetonitrile/water is BaP, PhPh, TBN; however, for polymeric phases BaP is retained longer than either PhPh or TBN, and so elutes last. The synthesis of a third type of C_{18} bonded phase termed "oligomeric" was described that has properties intermediate to monomeric and polymeric phases (see discussion earlier). The oligomeric phase is essentially a polymeric phase that is synthesized in a controlled, stepwise fashion. The elution order of the three-component mixture for this phase is PhPh, BaP, TBN. Selectivity ratios for PhPh/BaP and TBN/BaP were determined for a series of monomeric, oligomeric, and polymeric phases with various surface coverage values. The selectivity ratios were observed to decrease for increasing phase coverage. In general, better separations of isomeric PAH were possible on heavily loaded polymeric C_{18} phases than on monomeric phases. Thus, depending on the elution order (or selectivity ratio of TBN/BaP) of the three-component mixture, new phases could be screened rapidly and column selectivity toward more complex PAH mixtures predicted. The separation of 16 PAH priority pollutants (SRM 1647) is shown in Figure 28 for representative monomeric, oligomeric, and polymeric C_{18} columns. Also shown in Figure 28 is the isocratic separation of BaP, PhPh, and TBN.

Numerous studies have endeavored to compare and contrast the general retention properties of commercially available LC columns. Goldberg studied the empirical retention properties of 31 commercially available bonded phase columns using polar and nonpolar solute probes. Under identical mobile phase conditions, capacity factors for anthracene varied from about 1.0 to 6.5 among C_{18} columns prepared by different manufacturers. Selectivity factors (k' anthracene/naphthalene) varied from one to three among the same columns. The retention of more polar probes such as diethyl phthalate revealed differences in the columns that were not apparent with the PAH solutes. Interestingly, the absolute retention of caffeine was similar on most of the columns examined (with a few notable exceptions). Selectivity factors for caffeine/theophylline were also similar among the columns, suggesting that the solutes are each retained similarly on the columns. Capacity factors for toluic acid varied considerably on the columns (0.8 to 7.5), but the selectivity factor k' toluic acid/k' benzoic acid was relatively constant.

In a two-part study, Colin et al.[165] studied analytical and theoretical[166] considerations in the differences that exist in reversed-phase columns. Included in the studies were pyrocarbon-modified silica gel (PMS) and pyrocarbon-modifed carbon black (PMCB) phases. In general, the carbon-modified phases were more selective in the separation of geometric isomers and homologous series than were bonded phases. This enhanced selectivity is attributed to adsorption effects on the pyrocarbon surface. On the other hand, the selectivity of chemically bonded phases (CBP) with polar eluents is thought to be due largely to the solubility of solute in the mobile phase and the two phase types are complementary. An important conclusion is that for CBP, selectivity varies little with changes in the water content of the mobile phase. More significant changes in selectivity result from changing the organic modifier.

Selectivity differences among several reversed-phase columns were examined in a work by DiCesare and Dong.[167] Capacity factors and selectivity ratios were measured for eight reversed-phase columns. Seven sets of test mixtures were contained in the study including aromatic compounds, phthalates; water-soluble bases, acids, and steroids. The separation of an ASTM proposed test mixture is shown in Figure 29 for three columns: P-E HS-5 C_{18} (5 μm), P-E Analytical C_{18} (10 μm), and μBondapak C_{18}. Peak identification for the proposed ASTM evaluation mixture is as follows: (1) benzyl alcohol, (2) benzaldehyde, (3) aceto-

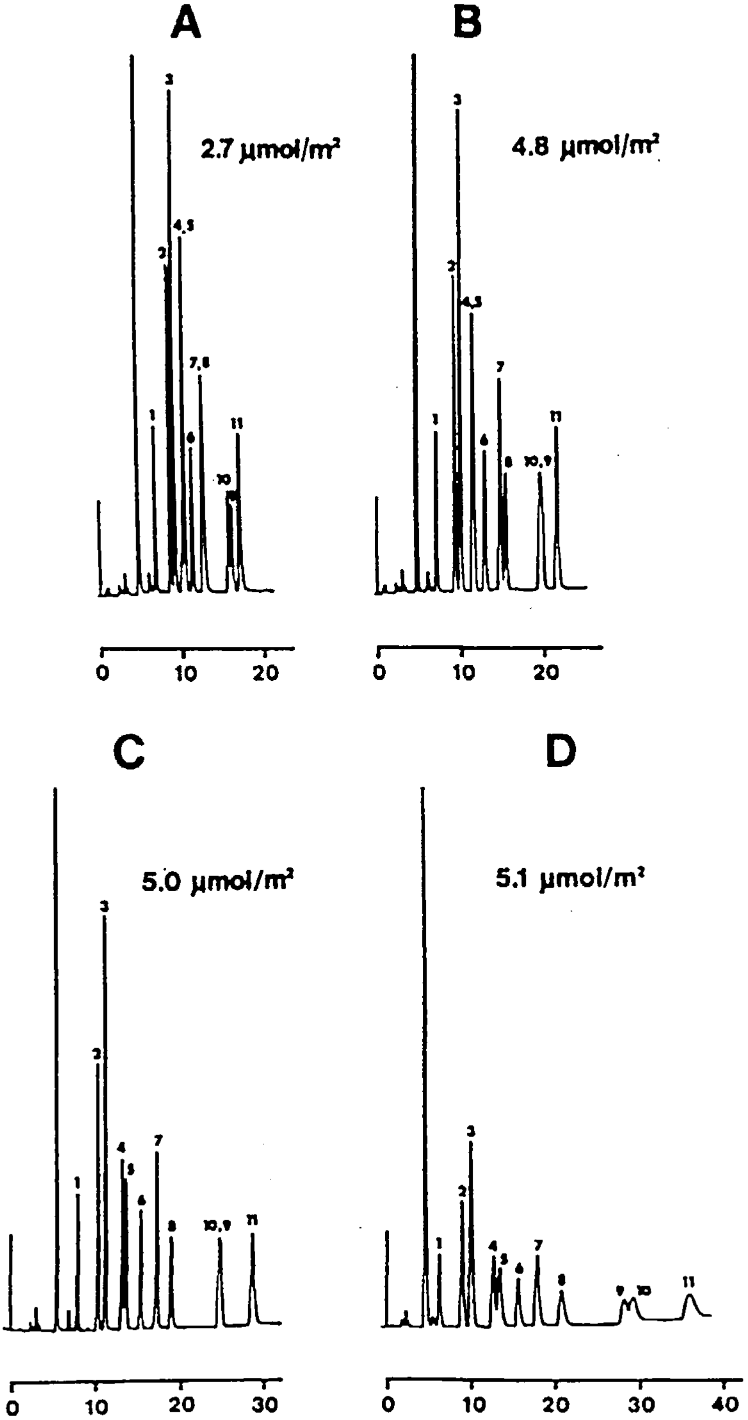


FIGURE 27. Reversed-phase separation of PAH isomers on four polymeric C_{18} columns (A to D) with increasing loading (see Figure 60 for peak identifications). (From Wise, S. A. and Sander, L. C., *J. High Res. Chromatogr. Chromatogr. Comm.*, 8, 248, 1985. With permission.)

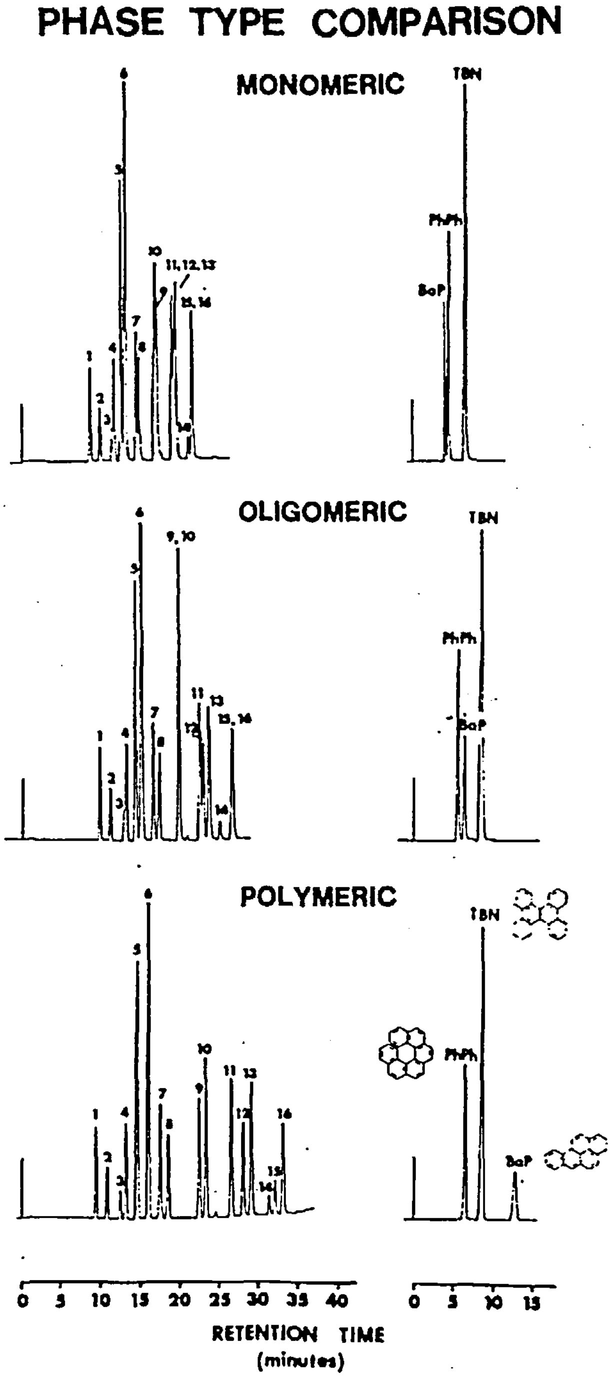


FIGURE 28. Separation of 16 polycyclic aromatic hydrocarbons (SRM 1647) on representative monomeric, oligomeric, and polymeric phases. Separation of the 16-component mixture was performed by using gradient elution, 40 to 100% acetonitrile in water over 30 min at 2 mL min. The three-component mixture was run isocratically at 85% acetonitrile/water. The elution order of benzo(a)pyrene (BaP), phenanthro(3,4-c)phenanthrene (PhPh), and 1,2:3,4:5,6:7,8-tetrabenzonaphthalene (TBN) is indicative of phase type. Solute identification is as in Figure 13. (From Sander, L. C. and Wise, S. A., *Anal. Chem.*, 56, 504, 1984. With permission.)

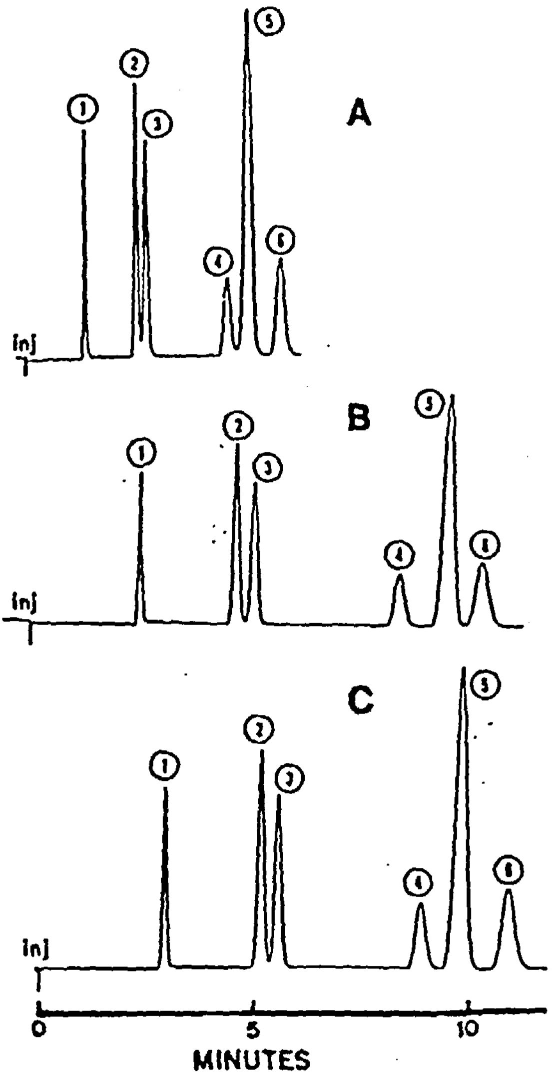


FIGURE 29. Comparison of the retention behavior of three commercial C_{18} columns: (A) P-E HS-5 C_{18} (μm); (B) P-E Analytical C_{18} (10 μm); (C) μBondapak C_{18}. Solutes: (1) benzyl alcohol, (2) benzaldehyde, (3) acetophenone, (4) methyl benzoate, (5) benzene, (6) benzaldehyde, (3) acetophenone, (4) methyl benzoate, (5) benzene, (6) dimethyl terephthalate. (Reproduced from Di Cesare, J. L. and Dong, M. W., *Chromatogr. Newsl.*, 10, 12, 1982. With permission.)

phenone, (4) methylbenzoate, (5) benzene, (6) dimethyl terephthalate. While absolute retention is seen to vary among these columns the selectivity is relatively unchanged.

Selectivity differences have also been examined for high and low loaded Partisil ODS columns.[168] The high loaded column, ODS-2, is described by the manufacturer as prepared using "polymeric" chemistry. Three test mixutures were employed consisting of low polarity (benzene, naphthalene, biphenyl, phenanthrene), medium polarity (acetophenone, propiophenone, methoxybenzene), and high polarity (phenol, aniline, nitrobenzene, ethyl benzoate) probes. As expected, less retention is observed for the ODS column than the more heavily loaded ODS-2 column for the nonpolar solutes. In general, larger selectivity factors were measured for low polarity solutes when separated on the ODS column. Solutes with moderate to high polarity were best separated on the ODS-2 column. The two columns are described as having complementary selectivity characteristics.

Evaluations of column selectivity have been carried out on commercially available columns for specific classes of compounds. Shaikh and Tomaszewski[169] examined eight commercial reversed-phase C_{18} columns for the separation of hydroxylated derivatives of 7,12-dimethylbenz[a]anthracene. The chromatographic properties of the columns studied differed considerably in terms of selectivity, absolute retention, and efficiency. For columns with similar substrate surface areas, retention was shown to correlate with percent carbon loading of the phase. Spherisorb ODS was judged the most suitable due to the low retention and complete separation of the solutes of interest.

Other commercial C_{18} columns have been evaluated[169a] for the separation of dansylamides (5-dimethylaminonaphthalene-1-sulphonyl [Dns] amides). Once again, the primary mode of solute retention was attributed to hydrophobic interactions; however, silanophilic interaction with basic amino groups was considered a likely source of the differences that were observed between the columns. Unlike certain other studies,[6] solute retention was not found to correlate highly with either percent carbon loading or carbon loading normalized to the packing density. The authors[169a] speculated that the differences were due to the polar and basic functionalities of the solutes (compared with purely nonpolar solutes in reference 6). The solvent strength of methanol/ and ethanol/water mobile phases was shown to be greater than acetonitrile/water mixtures for the dansylamides.

Gonnet et al.[170] examined the suitability of various commercial C_{18} columns for use in ion-pair and classical reversed-phase chromatography. Column selectivity toward nonpolar solutes (as evidenced by the selectivity coefficient for toluene/benzene) was observed to vary little among the columns tested, even though retention for the solutes varied significantly. More dramatic differences in column selectivity became apparent for the separation of polar compounds. Selectivity coefficients for caffeine/theophylline ranged from 1.4 to 4.5. Other differences were observed for the reversed-phase retention of acidic solutes (i.e., methyl substituted uric acid isomers) chromatographed under reversed-phase conditions with the addition of NaH_2PO_4 to control ionization. The reagents tetrabutylammonium phosphate (TBA) and sodium heptane sulfonate were used to further study column performance for the retention of acids and bases, respectively, in the ion pair mode. Differences among the various columns were more pronounced for ion pairing.

The retention behavior of pyrimidine bases was investigated on a variety of classes of bonded phases including C_{18}, phenyl, C_8, CN, and NH_2 columns.[171] Under mobile phase conditions of 20:80 acetonitrile/water, the longest retention of the bases occurred with the C_{18} column, and the shortest retention with the NH_2 column. The elution order of the pyrimidine bases on the latter column was nearly reversed from that on the C_{18} column. With few exceptions, the absolute retention of a given solute was observed to decrease in the order C_{18}, phenyl, C_8, CN, and NH_2. Differences in retention among the solutes is explained in terms of aromaticity and polarity of the base. Overall retention is, thus, due to a combination of hydrophobic, dipole-dipole, and solute-silanol interactions.

Melander and co-workers[172] compared the retention characteristics for phenyl, benzyl, phenethyl and octyl bonded phases using hydro-organic eluents and a variety of polar and nonpolar solutes. The retention characteristics of the aromatic phases were somewhat similar to the C_8 phase, but with certain distinct differences. However, little or no difference in retention characteristics were found among the three aromatic phases. The evaluation of differences between columns was facilitated by the use of a measurement termed "column modulus". This parameter is expressed as the ratio of k' values for the column under study and a reference column for the same chromatographic conditions. Retention of various PAH and steroids relative to toluene is shown in Figure 30 for the phenethyl and octyl phases.

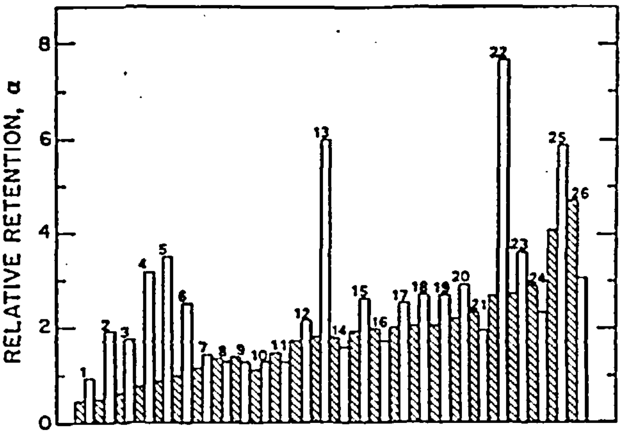


FIGURE 30. Comparison of the relative retention of aromatic hydrocarbons and steroids (relative to toluene) on octadecyl (hatched bars) and phenyl (open bars) columns. Solutes: (1) diethylstilbesterol, (2) Reichstein substance S, (3) corticosterone, (4) deoxycorticosterone, (5) Δ^4-androsten-17-dione, (6) testosterone, (7) naphthalene, (8) ethylbenzene, (9) *o*-xylene, (10) *p*-xylene, (11) *m*-xylene, (12) biphenyl, (13) progesterone, (14) cumene, (15) fluorene, (16) *n*-propylbenzene, (17) diphenylmethane, (18) acenaphthalene, (19) phenanthrene, (20) anthracene, (21) t-butylbenzene, (22) 5-α-pregnan-3,20-dione, (23) fluoranthene, (24) *n*-butylbenzene, (25) triphenylmethane, (26) *n*-amylbenzene. (Reproduced from Melander, W. R., Huang, J. X., Horváth, Cs., Stout, R. W., and DeStefano, J. J., *Chromatographia*, 20, 641, 1985. With permission.)

Notable differences are apparent for the retention of progesterone and 5α-pregnan-3,20-dione, as well as other steroids. Enhanced selectivity for certain PAH was also observed on the aromatic phase columns compared to the octyl column. This led the authors to suggest that the former class of phases might have utility in the separation of very large PAH that are difficult to elute in a reasonable time on conventional C_{18} columns. The differences in the retention behavior of arylsilica phases (compared to the octyl phase) were shown to result from solute — ligand interactions rather than interaction with accessible silanol groups. Zakaria and Brown[173] examined the retention behavior of purine and pyrimidine compounds on C_{18} and C_8 columns of varying surface coverage, polarity, and phase polymericity. Four columns were studied: Partisil ODS, ODS-2, ODS-3, and C_8 columns (Whatman, Clifton, N.J.). Carbon loading values for these columns were 5, 15, 10, and 9%, respectively. The ODS phases were referred to by the authors as "polymeric" in nature, because trichlorosilane reagents were used in their synthesis. Our experience has been that phases with monomeric-like properties can be synthesized using trifunctional silanes if care is taken to perform the reaction under anhydrous conditions. Of the three C_{18} columns listed, only Partisil ODS-2 is intentionally polymerized. ODS-3 is endcapped to reduce silanol accessibility, and ODS is synthetized to give a low phase loading. The C_8 phase was synthesized using dimethyloctadecylchlorosilane followed by endcapping with trimethylchlorosilane. Under aqueous mobile phase conditions (pH 2.95 using a phosphate buffer), nucleosides and bases were retained longer on the ODS-3 column than the C_8 column, even though the carbon loading values for the two columns were similar. Diphosphonucleotides, however, were retained longer on the C_8 column under the same conditions. The addition of small amounts of methanol to the mobile phase decreased retention for both classes of solutes. In a comparison of ODS-2 with ODS, virtually all solutes were retained longer on the column with the higher phase loading (ODS-2) for the phosphate buffer eluent. Surprisingly, at 60% methanol in the buffer eluent, all of the solutes were retained longer on ODS (with lower carbon and phase coverage than ODS-2). These unexpected trends in retention behavior are explained by changes in the stationary phase conformation that may occur with aqueous mobile phases. Zakaria and Brown[173] speculated that with high water mobile phase environments, the alkyl chains collapse against the silica and effectively shield silanol groups from interaction with polar solutes. Thus, in the first example above for the ODS-3 and C_8 columns, the collapse of C_{18} chains with ODS-3 gives rise to a more hydrophobic surface than with C_8 chains, and solutes that are retained primarily by hydrophobic associations will be retained the longest with ODS-3. By similar reasoning, more silanols are accessible with the C_8 column than the ODS-3 columns, and so the diphosphonucleotides are retained longer with the C_8 column, because this class of solutes is known to interact strongly with silanols. The effect of the addition of methanol to the aqueous eluent is to "open up" the collapsed C_{18} phase structure, thus, increasing the accessibility of silanol groups. It was also hypothesized that this change in stationary phase structure increases the surface area of the bonded phase available for solute interaction. For these reasons, the retention of both polar and nonpolar solutes is observed to increase with the addition of a small amount of methanol. It seems clear from the above discussion that the reversed-phase separation of purine and pyrimidine bases involves multiple retention mechanisms that are sensitive to changes in phase length, carbon loading, and the presence (or absence) of phase endcapping. Careful understanding of the effects that these parameters can have on retention is important for proper column selection and optimization of the separation conditions.

B. Spectroscopic Techniques

1. *Infrared and Photoacoustic Spectroscopy*

Chromatographic characterization of chemically bonded phases is valuable in predicting the bulk properties of LC columns, but such measurements yield little information about the immobilized ligand itself. Direct investigation of bonded phases is possible through spectral techniques. Infrared spectroscopy (IR) and the related technique of photoacoustic spectroscopy (PAS) are well suited to the study of bonded phases, both from the viewpoint of sensitivity and flexibility and from the type of chemical information the techniques provide.

Infrared spectroscopy has been used to investigate chemically modified surfaces with varying degrees of success. In early applications of the technique, bonded phase spectra were presented largely without conclusions. The primary goal of such studies was to prove the existence of the bonded ligands at the substrate surface. Berendsen and de Galan[18] presented IR spectra for a variety of phases and assigned absorption bands to specific ligand functionalities. Spectra taken before and after phase endcapping showed no evidence of change. Watanabe[174] used fourier transform infrared spectroscopy to study chiral bonded amino acid phases. Samples were mixed with KCl and spectra were recorded by diffuse reflectance. The amino acid phases were prepared through multiple reaction steps, and the progress of each step was monitored through the IR spectra.

Leyden and co-workers[175] used a combination of FTIR, ^{13}C-NMR, PAS, and UV spectroscopy to study an acetoacetamide stationary phase. FTIR spectra revealed the ligand to be hydrogen bonded, probably with residual silanols on the silica. Photoacoustic spectroscopy showed the ligand to be primarily in the keto form, but with small fractions of enol and enolate. Each of the techniques was considered complementary and suitable for a wide range of applications.

Unfortunately, the application of infrared spectroscopy to the study of conformational order in bonded phases is plagued with difficulties. The main problem arises from interferences from the silica substrate. Silica is a strong IR absorber and transitions arising from various alkyl conformations are weak. Furthermore, interferences from small particle scat-

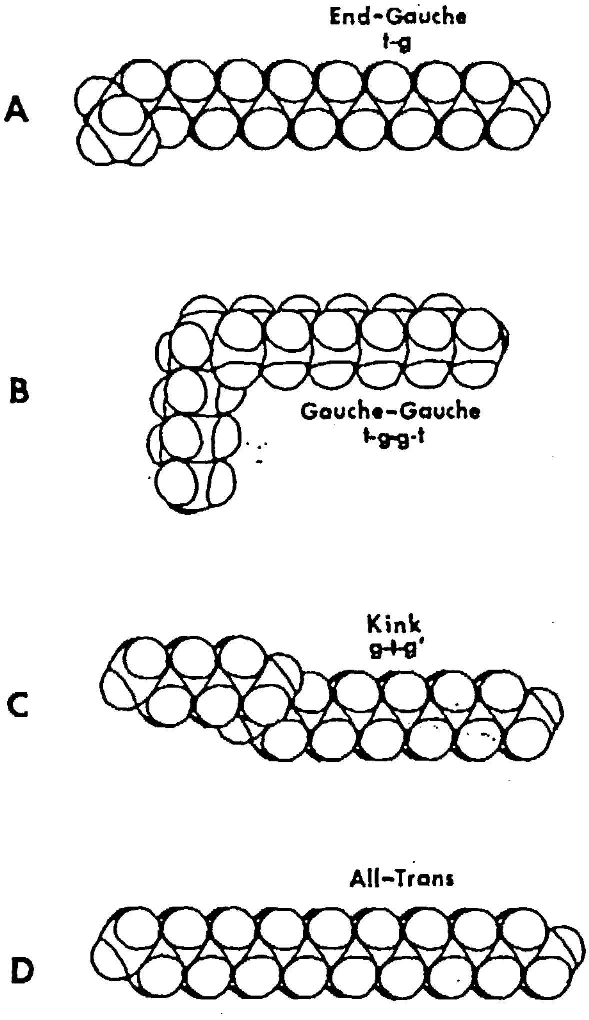


FIGURE 31. Alkyl chain conformations. The nonplanar conformations shown give rise to localized mode vibrations which are observed in the infrared spectrum: (D) all *trans*; (A) end-gauche, 1341 cm^{-1}; (B) gauche-gauche (or double-gauche), 1354 cm^{-1}; (C) kink, 1367 cm^{-1}. (From Sander, L. C., Callis, J. B., and Field, L. R., *Anal. Chem.*, 55, 1068, 1983. With permission.)

tering attenuate the signal. It is interesting to note that the particle diameter of chromatographic substrates is similar to the wavelength of infrared radiation (~ 5 vs. 3 to 12 μm). The problem can be summarized: signals of interest are weak and are superimposed on a strong, detailed background. To overcome these obstacles signal strength can be improved by signal averaging with fourier transform infrared spectroscopy, and the silica background problem can be largely eliminated by careful substraction and baseline linearization routines.

Sander et al.[176] performed such a study on dimethylalkyl phases of various lengths. High quality spectra were collected for C_1 to C_{22} bonded phases and compared to spectra for the corresponding chlorosilanes. Good overall agreement was found in all cases except the C_1 phase. For longer chain length phases, spectral assignments were made based on those of Snyder et al.[177-179] for *n*-alkanes. Assignments were made corresponding to various nonplanar alkyl chain conformations shown in Figure 31. The all-*trans* form is the most ordered state for an alkane. This conformation is usually associated with alkyl chains in an ordered,

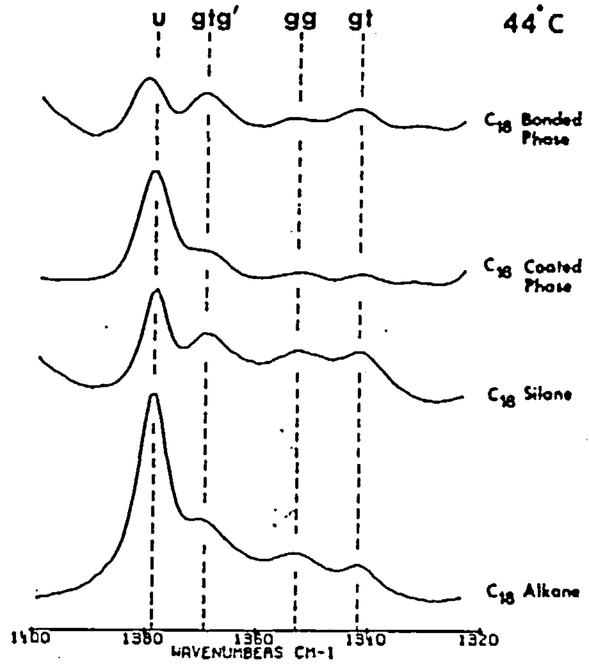


FIGURE 32. Infrared spectra of various C_{18} species showing conformational detail. Temperature: 44°C. Assignments are as follows: u, CH_3 symmetric deformation (umbrella mode); gtg', kink conformation; gg, gauche-gauche conformation; gt, end-gauche conformation. (From Sander, L. C., Callis, J. B., and Field, L. R., *Anal. Chem.*, 55, 1068, 1983. With permission.)

crystalline lattice. The end gauche (t-g) conformer has only slightly less order, but gives rise to a transition at 1341 cm^{-1}. The gauche-gauche (t-g-g-t, 1354 cm^{-1}) conformation results in a near 90° bend in the alkyl chain. Finally, the kink conformer, 1367 cm^{-1}, has parallel but laterally displaced segments. The transitions corresponding to conformations are weak, but can be observed in the corrected bonded phase spectra. Sander and co-workers collected spectra on four related alkyl systems: a pure alkane, the corresponding silane, a physically coated silica phase using thin layers of the alkane, and a monomeric bonded phase. Interesting differences were apparent in the spectra for the four samples (see Figure 32). The transition at 1380 cm^{-1} is assigned to a symmetric deformation of terminal methyl hydrogens (umbrella mode). This transition is more intense in the coated phase and neat alkane spectra simply because two terminal methyl groups are present per molecule. From the spectra it is clear that all samples have disordered chains with kinks and bends. At lower temperatures dramatic changes occur in the spectra as the alkane, silane, and coated phases "freeze" and become more ordered (see Figure 33). The bonded phase still retains considerable conformational disorder, however, even at low temperatures. Bonded phase spectra taken in the presence of deuterated methanol revealed that the alkyl chains are more ordered in this state than when dry. Even so, on average at least one gauche defect exists per chain.

Because photoacoustic spectroscopy does not rely on optical detection of absorption signals, it is particularly suited to the investigation of highly light scattering samples. Immobilized ligands with chromophores that absorb in the UV-vis spectral region can easily be studied using PAS without interference from the substrate. Solution spectra of solid-liquid slurries are also possible, so the potential exists for studying bonded phases in a mobile phase-like environment. One of the first research groups to employ photoacoustic

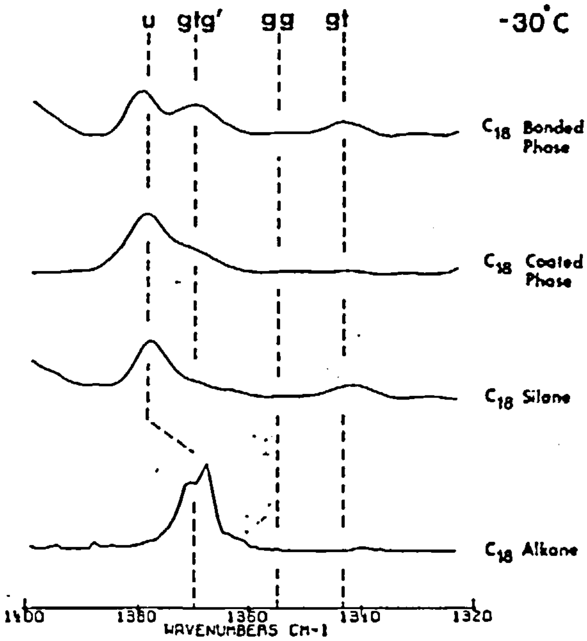


FIGURE 33. Infrared spectra of C_{18} species at −30°C. Reduction in band intensities compared to those in Figure 32 reflects ordering and chain extension at low temperatures. (From Sander, L. C., Callis, J. B., and Field, L. R., *Anal. Chem.*, 55, 1068, 1983. With permission.)

spectroscopy in the study of chromatographic phases was Low and Parodi.[180,181] They described the principles of operation and construction of a photoacoustic spectrometer, and they demonstrated its use for the characterization of bare and silylated silica. Silanol O-H stretching transitions decreased markedly after treatment of the silica with methanol (formation of $Si–O–CH_3$). Similar changes were observed upon reaction of the silica with trichlorosilane. In the latter case, a splitting of the O-H band was observed which was attributed to newly formed isolated surface silanols. The progress of silica dehydration was also examined by the technique.

Lochmüller and co-workers[182] studied aromatic chromatographic phases by PAS. Bonded phases incorporating charge transfer groups (2,4-dinitrophenyl [DNP] and 3-[2-quinazolineamino] functionalities) were prepared by linkage to previously bonded propylamine ligands. Phases were prepared in this way to space the charge transfer group away from the silica surface and allow better solution interaction. The 2-quinazoline system is sensitive to changes in pH and was included to probe the polarity of the mobile and stationary phase environments. Photoacoustic spectra taken for the dry DNP bonded phase were virtually indistinguishable from solution spectra for a reference DNP compound. However, although charge transfer acceptors are usually sensitive to the polarity of the environment, changes in the PAS spectra were not observed in the presence of polar and nonpolar solvents. Hypsochromic shifts in absorption maxima were observed with a decrease in solvent polarity for the reference DNP compound. The authors concluded that because of the loss in freedom of the immobilized ligand, association of DNP moieties may occur on the surface that mimic solid-state behavior. While changes in the spectra for the 2-quinazoline bonded phases were observed as a function of pH, the changes lagged the responses observed for solution of the 2-quinazoline reference compound. The authors further concluded that the solvation of DNP and 2-quinazoline chromophores is not complete, even for comparatively "good" solvents.

Photoacoustic spectra of bonded phases were later extended to the near infrared.[183] By utilizing this spectral region it is possible to characterize hydrocarbonaceous ligands that do not contain UV-vis chromophores. Absorption bands can be attributed to C-H stretch overtones, so the technique is widely applicable to most types of bonded phases. Although the information obtained from the near-IR spectral region is "less definitive" than the fundamental IR region, it is useful for determining the extent of a surface modification reaction or the presence of unsaturation in the phase ligands. Lochmüller and Wilder[183] characterized a series of alkyl and aryl bonded phases using near IR-PAS. They were able to make bond assignments corresponding to C-H stretches from saturated and unsaturated carbons. In general, the near IR-PAS bonded phase spectra were similar to neat IR solution spectra recorded for reference compounds. Because solution spectra were usually better resolved than bonded phase spectra, the authors believe this may parallel the inherently better resolution observed for solutions compared to solids. This, in turn, suggests a degree of solid-like behavior for the bonded phase ligands. The utility of near IR-PAS to assess the efficacy of endcapping reactions was further demonstrated.

The application of PAS to the characterization of bonded phases is not limited to qualitative analysis. Lochmüller and Wilder used near-IR PAS to assess carbon loading and phase coverage for aliphatic, aromatic, and aminoalkyl ligands. Correlations of the intensity of the PAS C-H stretch overtone was made with percent carbon values obtained by elemental analysis. Alkyl phases were prepared with different phase loadings by varying reaction time, and the correlation coefficient for this series was 0.999. The effect of the substrate on the PAS signal was examined by blending bare silica with bonded silica in various proportions. The correlation of PAS signal to percent carbon loading was again very high, 0.99. Phases with different ligand lengths (and, thus, different carbon loadings) also correlated well with PAS overtone absorptions. Similar experiments were carried out with aromatic and aminoalkyl ligands, and in all cases the validity of the method was upheld. Miller and coworkers[184] had similar success for the quantitation of octadecyl modified silicas.

2. *Fluorescence Spectroscopy*

In recent years several groups have used fluorescence spectroscopy to characterize chemically bonded surfaces and, in particular, to examine the polarity of the bonded phase microenvironment in various solvents.[185-191] Early studies by Lochmüller et al.[185,186] examined aminated silica (i.e., modified with aminopropyl reagents) that had been derivatized with a dansyl (5-dimethylamino-1-naphthalenesulfonyl) functionality to provide a highly fluorescent "tag" on the silica surface. The "tagged" silicas were exposed to solvent mixtures of varying polarities and the resulting emission spectra were compared with the spectra of a model compound, *n*-propyldansylamide, in the same solvents. The fluorescent emission spectra of the aminated silicas indicated a polar surface environment as a result of residual amine groups and unreacted silanol groups. Of more interest to conventional reversed-phase LC on alkyl bonded phases, they examined a series of "brush-type" (monomeric) alkyl dansyl silicas with low dansyl coverages and with varying alkyl chain length (C_1, C_8, C_{18}). These alkyl dansyl silicas were studied in common reversed-phase solvents (THF, methanol, acetonitrile, and water) and 50% aqueous mixtures of the same solvents. As expected, the surface polarity decreased with increasing alkyl chain length in each solvent.

Lochmüller et al.[186] also utilized fluorescence studies of dansyl-modified silica to study the microheterogeneity of the alkyl-modified silica surfaces. They observed that the fluorescence maxima of covalently bound dansylamide groups on silica surfaces, in both dry form and in acetonitrile, exhibit excitation wavelength dependence known as the "red edge" effect. Based on their results, they suggested a three-domain model of the modified silica surface,[26] i.e., fluorophores which are relatively inaccessible to solvent due to their location in the micropore or to being surrounded by paraffin-like agglomeration of alkyl chains, (2) fluorophores which are relatively accessible to solvent, and (3) fluorophores which are relatively accessible to solvent, but are hydrogen bonded to surface silanols. Their data suggest that modification of the surface with C_{18} groups does induce a significant degree of heterogeneity in the dry state.

Later, Lochmüller et al.[187,188] and other groups[189-191] utilized the fluorescence characteristics of pyrene, both adsorbed and chemically bound on the silica surface, to examine the alkyl-modified surfaces of reversed-phase stationary phases. Pyrene was selected as the luminescence probe for these studies based on its ability to exhibit short-range (3 to 5 Å) excimer formation. In dilute solution pyrene exhibits a structured fluorescence emission band with a 0-0 transition characteristic of the excited monomer at 27,000 cm^{-1}. As the concentration of pyrene is increased, the fluorescence intensity of the monomer decreases and a new, broad, and structureless fluorescence band, attributed to the presence of excited state dimers (excimers), appears at 21,000 cm^{-1}. Excimer formation has a critical interaction distance of 3 to 8 Å, but is thought to be closer to 3 Å.[192] Ware et al.[193,194] observed "excimer-like" emission of pyrene coated or adsorbed on silica; whereas Lochmüller and co-workers observed similar results for chemically bonded (3-[3-pyreneyl]propyl)dimethylchlorosilane (3PPS).

The observation by Lochmüller et al.[187] of "excimer-like" emission from the chemically bound 3PPS implies that the covalently bonded pyrene molecules must be within the critical interaction distance for excimer formation. The monomer and excimer fluorescence intensities for a series of 3PPS-modified silicas of varying surface concentrations were examined to determine the relative distribution of surface silanols. Based on this study, the average distance between a chemically bonded pyrene molecule and its nearest neighbor was determined to range from 12.3 to 34.9 Å. Using the calculation method of Unger and Roumeliotis[195] which assumes that all silanols are homogeneously distributed on the silica surface, the average distance between chemically accessible surface silanols on this particular silica (Partisil 10) was calculated to be 4.5 Å based on 8 μmol/m^2. The significant deviation of the observed range and the predicted values suggest that the chemically bound pyrene ligands are inhomogeneously distributed in high density regions where neighboring pyrene groups are less than 4.5 Å apart and low density regions where the average distance exceeds 8 Å. These results support the proposed model of Lochmüller and Wilder[26] for the formation of "liquid droplet"-like clusters of bonded alkyl ligands rather than the uniform, relatively ordered alkyl blanket that has been envisioned by others.[195]

More recently, Lochmüller and co-workers[188] investigated time-dependent luminescence of the bound pyrene molecules of varying surface concentration on silica to determine the distribution of the bound molecules and their organization in contact with different solvents. Based on precise space-filling models of 3PPS, the maximum distance between 3PPS bonding sites was estimated that would permit excimer formation assuming a fixed critical interaction distance of 4.0 Å. The structural conformations and corresponding maximum distances between 3PPS bonding sites for fully extended, collapsed, and pore representations for 3PPS molecules bound to silica are illustrated in Figure 34. Since the "pore" or interparticle excimer formation can be assumed to be negligible (since the Partisil 10 pore diameters are ~80 Å), the maximum possible distance between bonding sites for excimer formation occurs in the collapsed state (Figure 34B). Molecules with greater than 18.4 Å separation form the "isolated" monomer population. A quantitative estimate of the proximity and distribution of bound molecules suggests that there are three populations having nearest neighbors at distances greater than 18.4 Å (10%), between 18.4 and 11.2 Å (20%), and less than 11.2 Å (70%). Differences in the solvation of bound ligands can account for approximately a 20% change in the distribution of the various conformations in Figure 34. Solvation of the pyrene moiety in hexane results in extension of the ligand as in Figure 34A, whereas in hostile solvents (e.g., acetonitrile and methanol) the bonded ligands collapse upon each other as in Figure 34B.

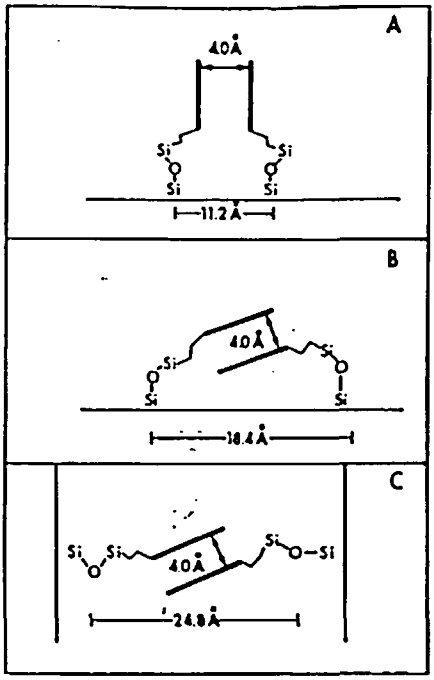


FIGURE 34. Schematic representation of structural conformations of the 3PPS bonded phase described in the text. (A) Fully extended; (B) collapsed; (C) pore or interparticle conformation. (Reprinted from Lochmüller, C. H., Colburn, A. S., Hunnicutt, M. L., and Harris, J. M., *J. Am. Chem. Soc.*, 106, 4077, 1984. With permission.)

Callis and co-workers[189] also used the formation of pyrene excimers to investigate the lateral diffusion of solutes in the C_{18}-modified silica surface. The rate constant for excimer formation was determined from both static and dynamic measurements and then used to estimate the diffusion coefficients for pyrene. From the diffusion coefficient they determined the "microviscosity" of the C_{18} surface to be about that of ethylene glycol (19cP). They interpreted these results as supporting the opinion that the solvated bonded alkyl phase is a dynamic (i.e., a partition mechanism) rather than a static, glasslike medium to which solutes are adsorbed.

Fluorescence spectrometry of pyrene adsorbed on the surface of C_{18}-modified silica surfaces has been reported recently by Ståhlberg and Almgren[190] and Carr and Harris[191] to investigate the polarity of the stationary phase environment and its dependence on the composition of the surrounding mobile phase. The emission spectrum of pyrene consists of five major vibronic bands usually labeled I to V in progressive order, i.e., 0-0 is I, etc. In the presence of polar solvents, the intensity of band I is enhanced at the expense of the other bands (e.g., band III). Thus, the ratio of the emission intensities of these two bands serves as a measure of the solvent polarity. For example, Ståhlberg and Almgren[190] found that the III/I ratio was 1.63 in hexane and 0.51 in water. They then adsorbed pyrene on RP-2 and RP-18 (LiChrosorb) particles suspended in water and found that these surfaces were comparable in polarity to 1-octanol. When the composition of methanol in the aqueous mobile phase was gradually increased from 0 to 30% (w/w), the surface polarity of both the RP-2 and RP-18 gradually decreased, suggesting that the adsorbed methanol molecules are probably hydrogen bonded to the free silanol groups and by turning the methyl groups from the surface, the polarity of the surface decreases. The results for the acetonitrile/water mixtures in contact with the alkyl-modifed surfaced indicated a more complicated behavior. For low concentrations of acetonitrile (0 to 14%), the behavior is identical to methanol, i.e., a decrease in polarity with increasing acetonitrile concentration. At concentrations of 14 to 28% the polarity increases, indicating that additional acetonitrile molecules enter between the alkyl chains and are more or less freely moving, which leads to the increase in surface polarity.

In contrast to the work of Ståhlberg and Almgren,[190] Carr and Harris[191] measured the III/I ratio over a wider range of mobile phase compositions using a microbore fluroescence cell packed with chromatographic silica and high solvent flow rates to yield stationary-to-mobile phase ratios comparable to chromatographic conditions. In addition, they compared the results on both a monomeric and a polymeric (Whatman ODS-2) C_{18} phase. The results for both phases indicate that intercalation of the organic modifier into the stationary phase produces an inverse relationship between polarity of C_{18} surface and that of the mobile phase over wide ranges of solvent composition (e.g., 20 to 60% acetonitrile in water). For dry silica surfaces (i.e., the solvent was evaporated after the pyrene was adsorbed onto the surface), the III/I ratio was 1.40 ± 0.02, which is characteristic of a very nonpolar environment approaching that of a hydrocarbon liquid, indicating relatively weak interactions with surface silanols under these conditions. The general behavior of the surface polarity for both the monomeric and polymeric C_{18} surfaces was similar. The greatest difference between the monomeric and the polymeric surfaces was the surface polarity at low concentration of organic modifier. The polarity of the monomeric phase at low concentration of acetonitrile or tetrahydrofuran exhibited a much higher surface polarity than the polymeric phase, whereas at high concentration they had the same polarity. These results may indicate that the monomeric phase is less effective in shielding the pyrene probe from surface silanols due to the more disperse organization of the alkyl ligand than the polymeric phase. At high concentration, the volume of the monomeric phase has been shown to increase with intercalation of solvent,[196] producing a surface environment similar to that of the polymeric C_{18} surface.

3. *Nuclear Magnetic Resonance Spectroscopy*

One of the more promising techniques to be applied to the study of chemically modified surfaces is nuclear magnetic resonance (NMR) spectroscopy. Until recently, progress in this field was slow, due to difficulties in obtaining high quality spectra for immobilized ligands. The restricted mobility of bonded molecules results in severe line broadening. This line broadening is caused by chemical shift anisotropy and carbon-proton dipolar interactions among the immobilized ligands. Fortunately, refinements in NMR technology now permit the measurement of solid-state spectra, and these new technques are also applicable to chemically modified silica substrates. A detailed discussion of NMR techniques is beyond the scope of this review, but Hays et al.[197] summarized the advantages and uses of solid-state techniques. Magic angle spinning (MAS) eliminates chemical shift anisotropy if the speed of rotation is fast enough. Cross polarization (CP) enhances sensitivity of solid-state measurements, and dipolar decoupling eliminates carbon-proton dipolar interactions. The resulting spectra for solids can have line widths comparable to solution spectra. The use of magic angle spinning with bonded phases does, however, remove much or all of the motional information about the bonded ligands, and so other techniques must be used to obtain such information.

One of the first studies employing ^{13}C solid-state NMR to characterize chemisorbed silanes was carried out by Chang and co-workers.[198] They prepared silylated derivatives of chrysatile asbestos using trimethyl, vinyl, and allyl silanes. NMR spectra of the samples proved the existence of surface-bound trimethyl and vinyl ligands. Bound methyl groups were observed in the spectra of all three phases; however, no evidence for the presence of allyl functional groups or oxidation products was observed. Subsequent experiments explained the source

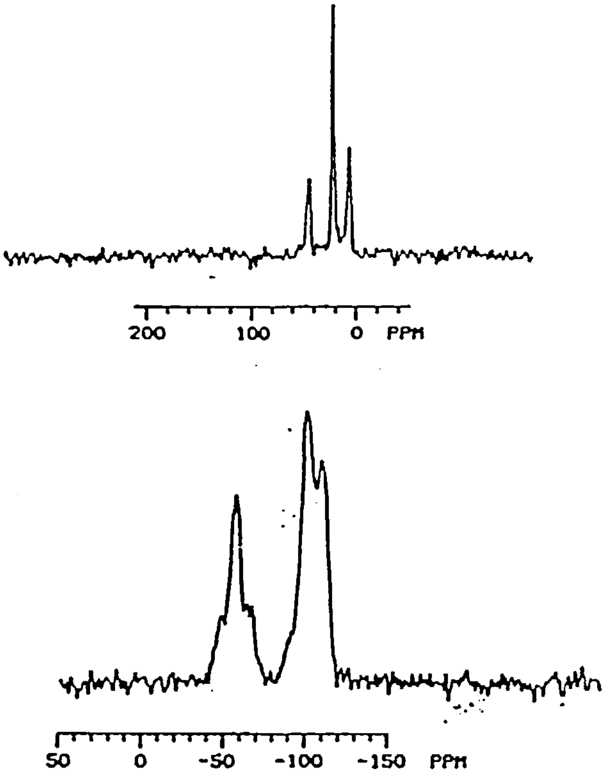


FIGURE 35. CP-MAS NMR spectra of silica bound (CH$_3$O)$_3$SiCH$_2$CH$_2$CH$_2$SH. (A) ^{13}C NMR spectra; (B) ^{29}Si spectra. (Reproduced from Maciel, G. E., Sindorf, D. W., and Bartuska, V. J., *J. Chromatogr.*, 205, 438, 1981. With permission.)

of the apparent discrepancy to be phase degradation through evolution of propylene during synthesis.

Maciel and Sindorf[199] have been instrumental in the application of solid-state NMR techniques to the study of silica and chemically modified silica. Bare silica was studied using ^{29}Si CP-MAS NMR, and signals were assigned to various features of the silica structure. Geminal silanols were identified at −90.6 ppm, isolated silanols at −99.8 ppm, and siloxane silanols at −109.3 ppm. The effects of dehydration and rehydration on silica were further studied in a later work.[200] Maciel and co-workers[201] demonstrated the application of ^{29}Si and ^{13}C cross-polarization magic angle spinning to trimethylsilyl, propylthiol, and nitrobenzylamide bonded phases. The ^{13}C and ^{29} Si spectra for the propylthiol phase is shown in Figure 35. ^{13}C peak assignments are as follows: 50.2 ppm = methyl °C, 27.9 ppm = α and β methylene °C, and 11.3 ppm = γ methylene °C (adjacent to the Si atom). In the lower ^{29}Si spectrum, the peak at −107.9 ppm is attributed to surface silicon atoms bound to four siloxane bridges; at −99.8 ppm to surface silicon atoms bound to one silanol and three siloxane bridges; and the shoulder at −89 ppm is assigned to silicon bound to two silanols and two siloxane bridges. The peak at −56.6 ppm is due to the silicon atom in the bonded ligand. Other spectra were presented for more complex phase structures.

Hays et al.[197] collected CP-MAS NMR spectra on 12 different aliphatic and aromatic stationary phases prepared from monofunctional silanes. Spectral line widths were narrow — in many cases less than 0.6 ppm — and resolution was great enough to permit discrimination of all seven types of carbon atoms in the dimethylhexyl phase. In general, chemical shifts for the immobilized ligands were similar to those for solution spectra of free molecules. This is an important observation, since consistent and predictable chemical shift values are required for interpretation of spectra from unknown phases. The authors concluded that solid-state NMR is a viable technique for the characterization of chemisorbed species that provides much more chemical information than can be obtained from infrared spectroscopy.

An even more extensive study of ^{29}Si and ^{13}C bonded phase spectra was carried out by Bayer et al.[202] Detailed assignments were made in the ^{29}Si and ^{13}C spectra recorded for homemade phases. The combination of the two spectral types proved invaluable in distinguishing phases prepared by mono-, di-, or trifunctional silanes. Monofunctional silane phases are easily identified by the presence of a signal between -3.4 and 0.5 ppm in the ^{13}C spectra, which is due to the two methyl groups adjacent to the silane silicon atom. Di- and trifunctional silane phases are most easily identified by reference to ^{29}Si spectra. The fact that trifunctional silanes bond with the silica to form mono- or bidentate ligands was verified by the presence of signals at -46 and -53.2 ppm, respectively. Perhaps most surprising is the observation that the largest signal is due to double bonded ligands! Difunctional methyl silanes produce other unique ^{29}Si signals by which identification can be made. Unfortunately, trimethylsilyl ligands from endcapping reactions cannot be distinguished from methyl groups in mono- or difunctional silanes in either ^{29}Si or ^{13}C spectra. Evidence of endcapping must rest with measurement of relative signal strengths. For trifunctional phases no such difficulty exists and proof of the existence of endcapping is absolute. In an evaluation of several commercial phases, Bayer and co-workers[202] concluded that most phases are prepared from trichlorosilanes, and the phases are subsequently endcapped. This conclusion is different from that reached by Fazio et al.[203] using a different characterization technique; however, a careful comparison shows that specific commercial phases in common to both studies were classified similarly (see Tables 7 and 8).

Sindorf and Maciel[204] also used ^{29}Si CP-MAS NMR to study the surface modification of silica with di- and trifunctional methysilanes. Under anhydrous conditions, single bonds to the silica were formed. Subsequent exposure to water resulted in hydrolysis of the unreacted chloro or alkoxy groups, and silanol condensation. This cross-linking reaction was pictured as "horizonal polymerization". Under conditions in which water was initially present at the silica surface, a higher percentage of multiple bonds was observed. These multiple bonds were also thought to originate from cross-linking as well as bidentate linkage to the silica surface.

Solid-state NMR techniques have been applied to the characterization of a wide variety of immobilized ligands. CP-MAS NMR ^{29}Si spectra were collected on silane polymers and silylated polysilicic acid by Engelhardt et al.[205] Comparison of the solid-state spectra with high resolution solution spectra revealed no special differences between the samples. Assignments and chemical shift information from solution spectra on model compounds were, thus, transferable to solid-state spectra. Besides normal alkyl phases, polar ligands including ethylenediamine,[206] (*N*-methyl)-propylamino, propylnitrile, propylthiol, benzyl chloride, acetyl, halogenated,[207] acetoacetamide,[175] and aminopropyl and *N*-β-(aminoethyl)-γ-aminopropyl ligands[208,209] have been studied by ^{13}C NMR. The information reported in most of these studies was chemical shift assignments for various carbons within the ligand molecules. Shinoda and Saito,[210] however, studied the motional behavior of immobilized amino groups. Line width increased in acidic environments. They attributed this change to the presence or absence of hydrogen bonding in the ligand. The nonprotonated ligand is thought to associate with residual silanols through hydrogen bonding. In the presence of an acid this hydrogen bonding is eliminated and ligand motion is less restricted giving rise to narrower signals.

Claessens et al.[211] correlated phase properties determined by ^{29}Si and ^{13}C NMR with chromatographic retention behavior. Quantitative measures of various NMR and chromatographic properties were not made. Instead, properties for several commercial phases were

Table 7
PROPERTIES OF SOME COMMERCIAL C_{18} BONDED PHASES

Brand	Technique	BET	Functionality	Endcapping	Carbon loading	Ref.
µBondapak C_{18}	Pyrol. GC[a]		Di			229
Adsorbosphere HS	HF[b]		Mono	No?	8.51[c]; 2.05[c](m)	203
Alltech	HF		Tri	Yes	8.91[c](m)	203
CP-Spher C-18	NMR	325		No	16%	211
Hypersil	Pyrol. GC		Tri	Probably		229
	NMR	170		Yes	9.5%	211
	Alkali[d]			Yes		227
LiChroprep RP-18	Alkali				0.59[c]	225
	Alkali			No		227
LiChrosorb RP-18	Pyrol. GC		Di			229
	HF		Di			203
	Alkali				0.60—0.80[c], 21.24%	225
	NMR		Di			202
	Alkali			No		227
	Carbon				19.0%	162
Micro-Pak MCH-10	Alkali			No		227
Nucleosil	Pyrol. GC		Tri	Lot depend.	13.3—14.9%	229
	HF		Tri	Yes	3.24[c](m)	203
	NMR		Tri			202
	Carbon				12.4%	162
ODS-SIL-X	Alkali				0.47—0.54[c]; 13.89%	225
Partisil	Pyrol. GC		Tri	Probably		229
	Alkali			Yes		227
Polygosil C18	Alkali				0.26[e]	225
Sep-Pak C_{18}	Alkali			Yes		227
Spherisorb	Pyrol. GC		Tri	Probably		229
	Alkali				0.22[e], 6.72%	225
	NMR		Tri			202
	Alkali			Yes		227
Techsil	HF		Tri	Yes	2.38[c](m)	203
Ultrasphere IP	HF		Mono	No?	4.15[c]; 2.01[c](m)	203
Versapack	HF		Mono	Yes	3.84[c]; 11.82[c](m)	203
Vydac 201-HS	HF		Mono	Yes?	6.71[c]; 4.62[c](m)	203
	Alkali				0.03[e]	225
Zorbax ODS	Pyrol. GC		Mono			229
	HF		Mono		6.19[c]	203
	Carbon				13.1%	162

[a] Pyrol. GC refers to pyrolysis GC.
[b] HF refers to digestion with hydrofluoric acid.
[c] Units = mol/g × 10^{-4} (ligand/silica); (m) = value for methyl groups only.
[d] Alkali refers to digestion with KOH.
[e] Units = µmol/mg (ligand/silica).

ordered among the phases, and the NMR and chromatographic rankings were compared. The ordering of phases by residual silanol concentration was the same by both techniques. Approximate agreement was achieved in the ordering of ligand coverage. This study shows that the usefulness of solid-state NMR as a technique to characterize chemically modified surfaces extends to include chromatographic retention behavior.

The use of cross polarization and magic angle spinning techniques has decided advantages over conventional NMR spectroscopy in elucidation of bonded ligand structure. Individual resonances from specific carbon atoms (or similar atoms) can be distinguished, permitting bonded phase identification. Other information about ligand bonding can also be discerned.

Table 8
PROPERTIES OF SOME COMMERCIAL C_8 BONDED PHASES

Brand	Technique	BET (m^2/g)	Functionality	Endcapping	Carbon loading	Ref.
Apex	HF	172	Mono		6.06^a	203
CP-Spher	NMR	325		Yes	11%	211
Hypersil MOS	HF	168	Mono		5.32^a	203
	Alkali			No		227
LiChroprep	Alkali			Yes		227
LiChrosorb RP-8	HF	280	Di		2.68^a—5.05^a	203
	NMR	250		No		211
	Alkali			No		227
Nucleosil	HF		Tri			203
	NMR		Tri			202
	NMR	300		No	11%	211
Partisil (C-8)	HF	347	Mono		4.85^a; 11.40^a(m)	203
Polygosil	NMR	500		No	8.5%	211
SIL-X5 (Perkin Elmer)	Alkali			Yes		227
Spherisorb	HF		Tri			203
Ultrasphere	HF		Mono		4.46^a; 1.45^a(m)	203
Zorbax C-8	HF	356	Mono		11.16; 2.34^a(m)	203

Note: Units are denoted as in Table 7.

Units = mol/g × 10^{-4} (ligand/silica); (m) = value for methyl groups only.

The use of CP-MAS NMR, however, removes information about chain mobility "in trade" for narrower line widths. A study of changes in line width using conventional FT-NMR should, in fact, provide insight to alkyl chain conformation and motion. Gilpin and Gangoda[212-214] reported convincing evidence for conformational changes in immobilized ligands using ^{13}C FT-NMR. A series of alkyl phases of various lengths were prepared using trichlorosilanes with ^{13}C enrichment at the terminal carbon. Two other phases were synthesized with ^{13}C enrichment at the C_1 position (closest to the silicon atom) and midway along the carbon chain. Spectra were collected in high water environments with varying percentages of dioxane (see Figure 36). The line width of the signal corresponding to the terminal methyl carbon can be seen to decrease with the addition of the organic modifier. This decrease in line width was interpreted as increased ligand mobility in the organic environment. A similar trend has been reported by McNally and Rogers.[215] Gilpin and Gangoda[213] noted two features of the signal: the broad base and the narrow top. These features were attributed to resonances originating from chains with two distinct degrees of mobility arising from polymerization at the surface and polymerization away from the surface. Line narrowing was not observed for a similar bonded phase prepared using a dimethylmonochlorosilane for which polymerization reactions are not possible (see Figure 37). Line width was also studied as a function of position along the alkyl chain. Very broad signals were observed for enrichment at the C_1 position, and slightly less broad signals for ^{13}C enrichment at the C_4 position. It was thus concluded that chain motion increases with distance from the silica surface. Signal width from terminal methyl groups was examined for various length alkyl chains and the width was found to be relatively constant for mid-length and longer alkyl chains.[213] The predominant motion of terminal methyl groups is rotation about the C-C bond, and, apparently, this is not influenced by chain length for sufficiently long alkyl chains. However, phase density was observed to affect signal width of terminally labeled alkyl ligands. Spin lattice relaxation times were observed to decrease substantially for only a 3% increase in carbon (phase loading). This change was interpreted to mean terminal methyl group rotation is hindered

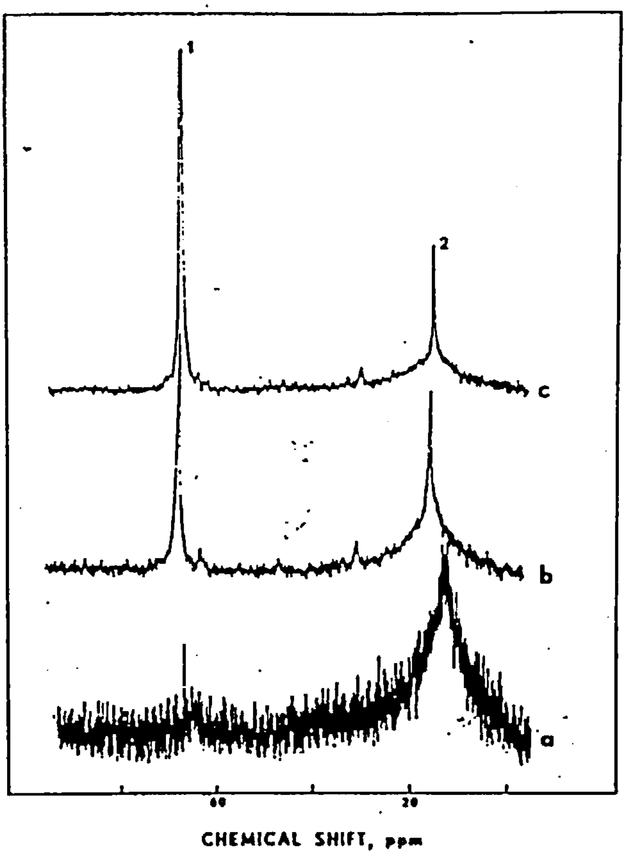


FIGURE 36. ^{13}C NMR spectra for a low coverage C_8 bonded phase prepared from octyltrichlorosilane with ^{13}C enrichment at the terminal methyl group. Changes in chain mobility are apparent as a function of the solvent environment. (a) 100% H_2O; (b) 2% dioxane, 98% water; (c) 10% dioxane, 90% water. Peak 1 = dioxane, peak 2 = labeled carbon. (Reproduced from Gilpin, R. K. and Gangoda, M. E., *J. Chromatogr. Sci.*, 21, 352, 1983. With permission.)

for sufficiently dense phases due to interaction between bound ligands. It suggests that a critical alkyl surface density for methyl group rotation may exist that could possibly affect chromatographic properties. Sindorf and Maciel[216] agree that alkyl motion increases with the distance from the silica surface; however, from cross-polarization relaxation measurements they concluded that motion does not become liquid-like even for carbons far removed from the surface. Instead, they found that increases in alkyl motion fall off after a distance of about eight to nine carbons.

The study of alkyl chain motion in bonded phases was approached in a similar fashion by Albert et al.[217] Spin lattice relaxation times (T_1) were measured for the ^{13}C signal assigned to carbons C_4 to C_{15} in a dimethyloctadecyl phase. Increases in T_1 values correspond to increases in chain mobility. By measuring the spin lattice relaxation of a group of similar carbons, an assessment of the average chain mobility was made. The effects of several interesting parameters on chain mobility were measured. T_1 values decreased for spectra recorded in the presence of increasing concentrations of water. Thus, the conclusions of Gilpin and Gangoda[212] are confirmed that alkyl chain mobility is reduced in the presence of water. Phases with different ligand densities were examined, and chain mobility was

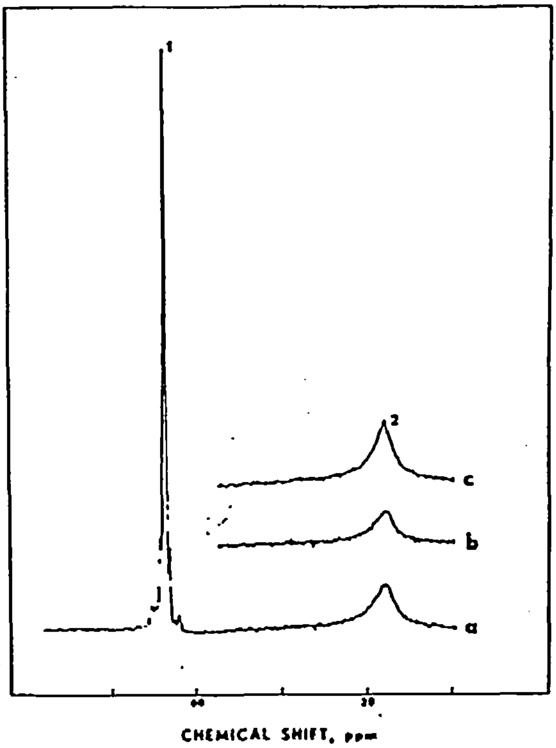


FIGURE 37. ^{13}C NMR spectra of a C_8 bonded phase prepared from octyldimethylchlorosilane. (a) 25:75 Dioxane/water; (b) 50:50 dioxane/water; (c) 100% dioxane. (Reproduced from Gilpin, R. K. and Gangoda, M. E., *J. Chromatogr. Sci.*, 21, 352, 1983. With permission.)

observed to decrease for increasing surface coverage. As the average distance between the bound ligands decreases, the alkyl chains interact more and are able to move less freely. Chain mobility was further observed to decrease after endcapping. The authors visualized a phase that folds back upon itself to associate with the nonpolar trimethylsilyl groups. This would result in a loss of chain motion. The results of initial NMR experiments dealing with solute-bonded phase association were reported; however, speculations were not made concerning the observed line broadening.

Kelusky and Fyfe[218] studied alkyl chain motion in alkoxysilanes immobilized on silica by ^2H NMR. Deuterated alkoxysilanes were prepared by reaction of deuterated alcohol with dichlorodimethylsilane. Phases of various lengths were prepared. The greatest motional line narrowing was observed for long chain length phases. Spectra were also recorded in the presence of hexane, benzene, and methanol. Some motional narrowing (compared to the solid-state case) was observed in the presence of hexane and benzene. However, motional line narrowing was apparently the greatest in the presence of methanol, indicating that methanol is able to solubilize a portion of the alkyl chain. An alternate explanation, not considered by the authors, is that the deuterated alkoxy chains are cleaved in the methanol environment. If true, the narrow line width observed would be the result of a solution spectrum. This possibility could be easily examined by analyzing the supernatant methanol solution (for the presence of the longer chain alcohol) by gas chromatography.

Sjotfeldt-Ellingsen and Resing[219] studied the motions of phenyl groups bound to silica by proton-decoupled cross-polarization ^{13}C NMR. By comparing changes in line width for various phenyl-modified silicas, a number of interesting conclusions were made. Phases of the type Si–O–Si$(OH)_n(C_6H_5)_{3-n}$ rotate about the O–Si bond. No rotation was observed about the phenyl-Si bond, and the angle between the phenyl plane and the O–Si–C plane was calculated to be about 65°. Furthermore, the O–Si bond (about which rotation occurs) was found to be approximately normal to the silica surface. Overall phenyl mobility was observed to increase with the length of the alkyl spacer used to attach the phenyl group to the silica. Thus, aromatic ^{13}C NMR signals for propylphenyl silica were narrower than for phenyl silica.

Miller and co-workers[220,221] used solid-state ^{29}Si NMR to examine the reactivity of geminal and free silanols at the silica surface. These species give rise to distinct signals in the ^{29}Si spectra of silica. The approach taken by Miller et al. was to measure the relative quantities of each type of silanol as a function of ligand density. Phases of varying surface coverage were prepared using octadecyldimethylchlorosilane. It was found that the intensity of the signal for geminal silanols at -91 ppm decreased to a greater extent than the signal for the free silanols (-100 ppm), suggesting tht geminal silanols are more reactive than free silanols, and geminal silanols are modified in preference to free silanols (Figure 38). Hydrogen-bonded silanols (vicinal silanols) are also expected to be more reactive than lone, isolated silanol groups, but this could not be demonstrated by NMR due to the fact that free and hydrogen-bonded silanols cannot be distinguished in the ^{29}Si spectra.

Claessens et al.[222] combined a number of techniques to study changes in LC packings that occurred with use. Octyl and octadecyl columns were used over a period of weeks with mobile phases containing buffers and/or ion pairing reagents, and column performance was observed to gradually degrade. Efficiencies for both columns remained fairly constant, but column retention and selectivity decreased. ^{13}C and ^{29}Si CP-MAS NMR, conventional HR-NMR, and FTIR were carried out on the bonded phases before and after use. Two changes in the phases were confirmed by ^{13}C and ^{29}Si CP-MAS NMR: hydrolysis of siloxane bonds to form new silanol groups and phase hydrolysis or cleavage. Cleavage of bonded ligands was most prominent for the C_8 phase, where in the worst case, loss of 50% of the phase occurred. The stability of the C_{18} phase was much greater, the primary deleterious effect being creation of new silanols by hydrolysis of siloxane bonds.

C. Chemical and Pyrolytic Cleavage

The nature of immobilized ligands precludes many direct approaches to the characterization of bonded phases. Because bonded phase are stable in most common solvents, they cannot be extracted by ordinary means. Many of the approaches to phase characterization seek to gain information on the immobilized ligand *in situ*, i.e., the properties of the modified substrate are assessed. Spectral techniques such as infrared and nuclear magnetic resonance spectroscopy are applicable to functional group analysis (for example, amine or nitrile moieties), but they are not suitable to quantitative determination of various ligands or mixture of ligands. Elemental carbon analysis gives the total carbon content of a phase, but no information about the types of ligands present. Other techniques have proven useful for determination of the supramolecular structure of alkyl phases and have aided in detailing microheterogeneity and bonded phase environment. However, one of the best approaches to the characterization of bonded phase composition appears to be cleavage of the phase followed by silylation with trimethylchlorosilane and analysis by gas chromatography.

A number of research groups have independently developed methods based on alkaline or acidic cleavage for determining bonded phase ligand content and the nature of the silane functionality (i.e., mono-, di-, or trifunctional silanes). The results of these procedures for a number of commercial alkyl phases are summarized in Tables 7 and 8. Verzele and co-workers[223,224] used potassium hydroxide in THF-water to cleave hydrophobic stationary phases. The addition of THF to 2 *M* KOH was found to be necessary to wet the phase

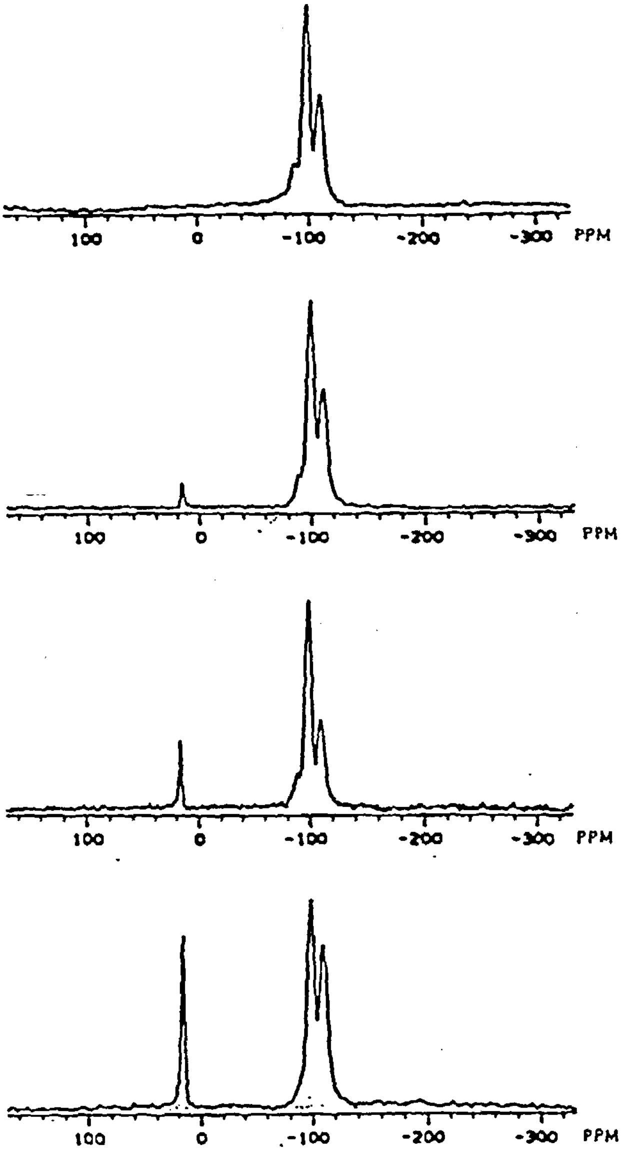


FIGURE 38. ^{29}Si solid-state NMR spectra of C_{18} phases with increasing phase loading. (Reproduced from Miller, M. L., Linton, R. W., Maciel, G. E., and Hawkins, B. L., *J. Chromatogr.*, 319, 9, 1985. With permission.)

(yields were low without this addition). The cleavage products were silylated with trimethylsilylimidazole (TSIM), although it was observed that some underivatized compounds containing free silanols could be chromatographed directly. The results are complex; however, peaks corresponding to the bonded ligands were identified by chemical ionization mass spectrometry. Quantitation was attempted for octadecyl phases, and the values compared to thermogravimetric analysis (TGA) results. Unfortunately, although trends in carbon loading matched TGA, the absolute values were only 20% of the thermogravimetric values.

A slightly different procedure that resulted in much simpler chromatograms for the cleavage products was used by Genieser and co-workers[225] to analyze C_{18} phases. Genieser et al. optimized reaction conditions for KOH to maximize ligand yield. Solid KOH was added to

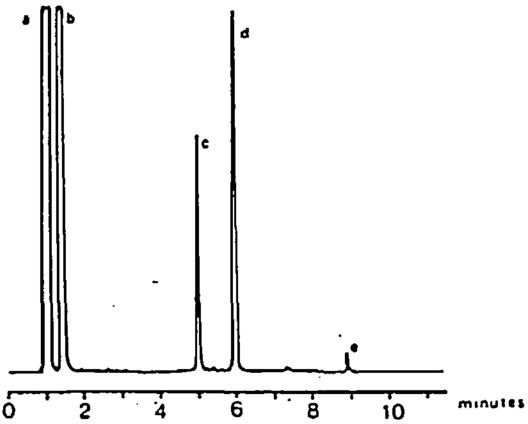


FIGURE 39. Gas chromatogram of the cleavage products from C_{18} modified silica, using fused KOH. Peaks: (a) hexane; (b) triethyleneglycol dimethyl ether; (c) *n*-heptadecane (internal standard); (d) *n*-octadecane; (e) octadecanol. (Reproduced from Genieser, H., Gabel, D., and Jastorff, B., *J. Chromatogr.*, 244, 368, 1982. With permission.)

the bonded silica and heated to 216°C in the presence of triethylene glycol dimethyl ether. The cleavage products were chromatographed directly without derivatization. A typical gas chromatogram of the products is shown in Figure 39. Peak c is *n*-heptadecane (internal standard), d is octadecane (96%), and e is octadecanol (4%). The presence or absence of endcapping could not be detected with this method, because no provisions were made to collect methane, although the method could be modified to do so. Phase loading values determined by this KOH fusion method were consistently lower than values from carbon analysis. This work was later extended to study a variety of commercial phase types with polar and ionic functional groups.[226,227] Headspace analysis was used to measure any evolved methane, which is indicative of endcapping reactions. Significant differences in the degree of substitution and type of parent molecule were observed among ion exchange silicas of the type SAX and SCX prepared by different manufacturers.

Erard and Kováts[38] developed a method for the determination of bonded phase content based on silica dissolution by hydrogen fluoride in diethyl ether. The resulting fluorosilanes were derivatized with butyllithium and quantified by gas chromatography. The method precision was about 2% and the values obtained did not differ significantly from those from elemental analysis. Fazio et al.[203] also developed a method for phase cleavage and analysis based on hydrofluoric acid digestion. The fluorosilane cleavage products were analyzed directly without derivatization by packed or capillary gas chromatography. Phases prepared from monofunctional silanes were quantitatively cleaved after reaction with methanolic HF for 2 to 3 hr. Maximum recovery for the phases prepared from di- or trifunctional silanes was only 40 to 60% based on elemental analysis (see Figure 40). A method for phase quantification was developed using empirical calibration curves based on elemental analysis. Method precision averaged 1.7 to 3.9%, and accuracy 2.2 to 4.0%. Discrimination between phases prepared from mono-, di-, and trifunctional silanes was possible with the method. A comparison of several commercial C_{18} phases revealed that most use monofunctional silanes in phase preparation. The presence and extent of endcapping reactions in commercial and homemade phases was easily assessed by the technique.[228] The value of this type of measurement was underscored by one commercial C_8 phase in which nearly 70% of the phase was shown to consist of trimethylsilane groups!

Hansson and Trojer[229] applied the technique of pyrolysis gas chromatography to bonded

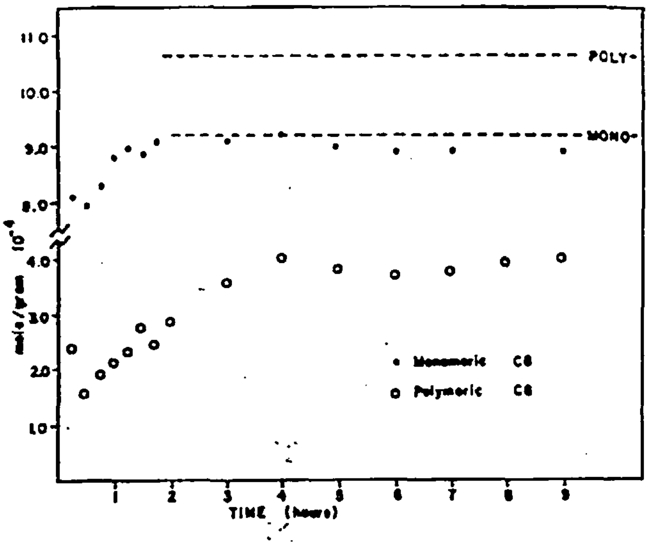


FIGURE 40. Phase density of monomeric and polymeric C_8 phases, as determined by gas chromatography of the HF cleavage products, plotted as a function of reaction time. Dashed lines represent phase densities determined by carbon analysis. (Reprinted from Fazio, S. D., Tomellini, S. A., Shih-Hsien, H., Crowther, J. B., Raglione, T. V., Floyd, T. R., and Hartwick, R. A., *Anal. Chem.*, 57, 1559, 1985. With permission.)

phase characterization. Using this technique, the modified silica was rapidly heated to 800°C and the pyrolysis products were analyzed by gas chromatography. For *n*-alkyl phases numerous peaks resulted from pyrolysis with the latest eluting peak corresponding to the alkyl ligand, and earlier eluting peaks to alkyl fragments. The type of silane used in the preparation of the phase was found to influence the resulting pyrogram. The peak height ratio of C_{18}/C_{17} was observed to decrease for increasing silane functionality (i.e., mono- > di- > trifunctional silanes, see Figure 41). This observation was used to evaluate the type of silane used in the preparation of various commercial phases. Similarly, the peak height ratio of C_1/C_4 peaks was found to increase after endcapping with TMCS of HMDS. Ratios of about 1 to 1.5 or greater were recorded for endcapped C_{18} phases. Phases not capped had C_1/C_4 ratios of about 0.5. In a later study[230] endcapping reactions were studied in more detail using a criterion based on the peak height ratio of C_2/C_1. It was shown that increases in the C_2/C_1 ratio occur after endcapping C_{18} phases prepared from di- and trichlorosilanes. No such increase was observed for a monochloro C_{18} phase. In fact, a slight decrease was noted and the authors feel it is inadvisable to attempt endcapping dimethyloctadecyl phases. This work was repeated by Mussche and Verzele[231] using a higher resolution pyrolysis-capillary GC technique and peak identification by mass spectrometry. Most of the conclusions reported by Hansson and Trojer[229] were verified. However, unlike Hansson and Trojer, Mussche and Verzele observed later eluting peaks than those attributed to the alkyl ligand. These peaks were identified as silane compounds probably formed, in part, from the substrate. This observation led the authors to question the validity of using thermal gravimetric analysis (TGA) to measure phase loading. Pyrolysis GC is viewed as an excellent technique for determining phase functionality and the presence of endcapping, but it is doubted if the technique could be used as a quantitative measure of phase loading.

The stability of silica-based alkyl phases has been examined by Wehrli and co-workers.[232] It is well known that bare silica is subject to attack and dissolution by mineral bases (e.g., NaOH). Wehrli et al. studied the kinetics of this reaction as a function of pH for a number of mineral and organic bases. The rate of dissolution of silica was found to be considerably

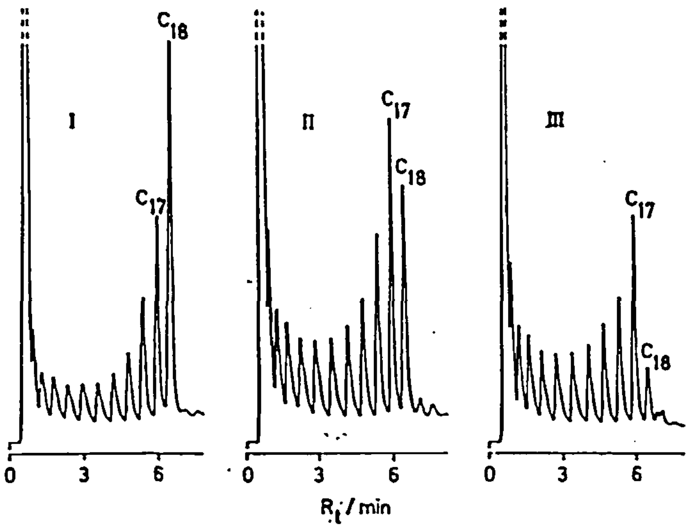


FIGURE 41. Pyrograms of C_{18} phases prepared using different silanes: (I) octadecyldimethylchlorosilane; (II) octadecylmethyldichlorosilane; (III) octadecyltrichlorosilane. (Reproduced from Hansson, L. and Trojer, L., *J. Chromatogr.*, 207, 1, 1981. With permission.)

larger for quaternary alkyl ammonium hydroxides than for primary, secondary, and ternary alkyl amines. The reactivity of NaOH was intermediate. This observation is potentially useful for the separation of compounds at high pH. Octadecyl and octyl phases were found to be more resistant to attack at high pH than bare silica, but above pH 11 with NaOH, column performance degraded in only 3 days. However, by using triethylamine above pH 11, columns were usable for several weeks.

D. Area and Pore Size Determinations

Two of the most important physical characteristics of silica substrates are specific surface area and pore diameter. Surface area values are required in the calculation of ligand density, and pore diameter has been shown to influence phase synthesis and column efficiency and selectivity. It is unfortunate that traditional methods for pore size and surface area determination are time consuming and require specialized equipment. As will be discussed later, alternatives to the traditional methods are becoming available that are practical without large investments of time and money.

The most common technique for the determination of surface area of powders is based on the adsorption of nitrogen (or other gas) at the boiling point of the gas. The uptake of gas is related to the surface area of the adsorbent by theory developed by Brunauer et al.,[233] and as such the measurement is often referred to as BET surface area. The characteristic shape of nitrogen adsorption isotherms is sigmoidal (see Figure 42). Three regions can be distinguished: the concave region at low pressures, a linear region for partial pressures $0.03 \leq P_0/P \leq 0.30$, and a convex region at high pressures. The onset of the linear region (point B) reflects coverage of the surface with a monolayer of the gas. Brunauer et al. derived an equation describing the isotherm:

$$\frac{P}{V(P_0 - P)} = \frac{1}{V_m C} + \frac{C - 1}{V_m C} \frac{P}{P_0} \qquad (7)$$

where P = gas pressure, P_0 = saturation gas pressure, V = volume gas, V_m = volume

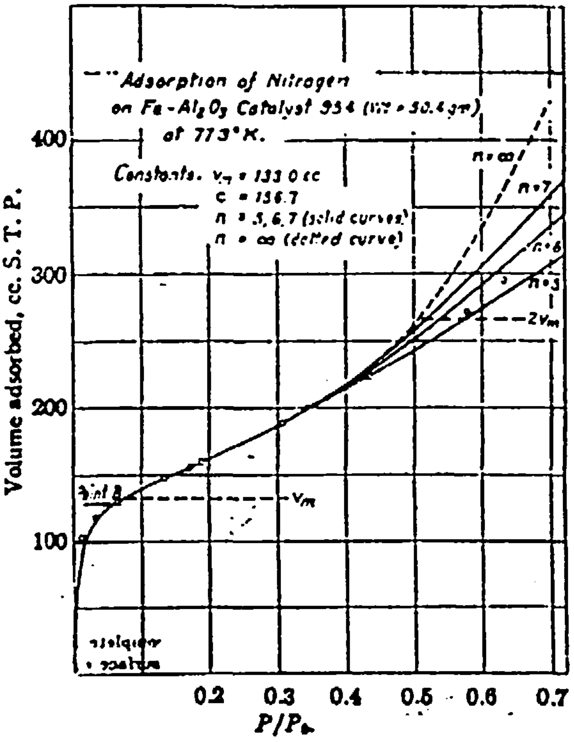


FIGURE 42. Typical adsorption isotherm for nitrogen. Point B represents monolayer coverage. (Reprinted from Brunauer, S., Emmett, P. H., and Teller, E., *J. Am. Chem. Soc.*, 60, 309, 1938. With permission.)

of monolayer of adsorbed gas, and C = a constant related to the heat of adsorption. A plot of $P/V(P_0 - P)$ vs. P/P_0 is linear over the range $0.03 \leq P/P_0 \leq 0.3$. From the slope and intercept of the plot, V_m can be calculated, and this, in turn, can be related to surface area. Measurement of nitrogen isotherms involves successive additions of measured quantities of nitrogen to the adsorbent cooled in liquid nitrogen. Calculation of area using Equation 7 above and isotherm data is sometimes referred to as multipoint BET. Because the term $1/V_mC$ is small in all cases, a good estimate of area can be obtained from a single P/P_0 value (within the linear isotherm interval) by setting the intercept to zero. This is sometimes referred to as single point BET surface area measurement.

A simplified apparatus for carrying out BET measurements has been described by Verzele et al.[234] Gobet and Kováts[235] developed a method for determining surface area based on chemisorption of trimethylsilyl or (3,3-dimethylbutyl)-dimethylsilyl groups. The space requirement of each type of ligand was determined using a reference silica of known surface area. Once calibrated, surface areas were determined by first modifying the substrate with the silane under controlled conditions. Phase loadings were then determined and surface area values calculated using these values and the appropriate space requirement values. Good correlation was obtained between chemisorption and nitrogen adsorption surface areas for fume and precipitated silicas. Larsen and Schou[236] also developed a method to determine surface area. ^{3}H-labeled alkydimethylchlorosilanes were used, and the bonded ligands were quantified by liquid scintillation counting. Determined in this way, lower surface area values could be measured with greater accuracy than was possible by elemental analysis of the bonded phases. The technique of using labeled silanes was also used to measure the extent of endcapping reactions.

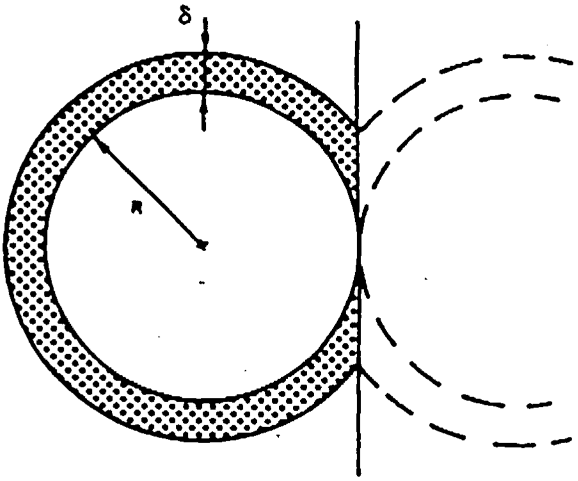


FIGURE 43. The "uniform globular model" for silica. Pores are formed by the spaces between the globules (radius R). The bonded-phase layer is represented as the thickness δ. (Reproduced from Gobet, J. and Kováts, E., *Adsorp. Sci. Technol.*, 1, 77, 1984. With permission.)

The validity of nitrogen adsorption techniques in the determination of specific surface area of silica is accepted; however, measurement of the surface area of chemically modified surfaces is more controversial. In general, the surface area values for silica substrates, determined by gas adsorption, decrease after modification with nonpolar ligands.[23] The source of this decrease is the central question: is the reduction in surface area representative of the true bulk properties of the sorbent, or is the decrease a consequence of a change in the nitrogen adsorption energy on the nonpolar surface?. This question has been addressed in the research of Gobet and Kováts.[37] Bonded phase surface areas were calculated based on two different substrate models, and these values were compared to surface area measurements based on nitrogen and argon adsorption experiments. From these comparisons, the "uniform globular model" (UGM) was selected to be more appropriate than the "naive or primitive model" of pore structure. Porous silica, regardless of type or origin, is viewed as an aggregate of nonporous silica spheres in the uniform globular model. Pores result from spaces between these spheres. For isolated nontouching spheres, surface area increases with sphere diameter, so chemical modification should increase surface area. However, in the UGM, the spheres do touch each other, and chemical modification results in a decrease in surface area at the points of contact (see Figure 43). These opposing effects are combined in the surface area equation for the uniform globular model:

$$S^*/S = CN(\rho - \rho^2)/2 + \rho^2 \tag{8}$$

where $\rho = (R + \delta)/R$, R = radius of sphere globule, δ = bonded phase thickness, CN = coordination number (number of contacting points per sphere), S^* = surface area after modification, and S = surface area before modification. With the primitive model, pore structure is viewed as a cylinder of radius r (see Figure 44). Modification reduces this radius by a distance δ:

$$S^*/S = (R - \delta)/R \tag{9}$$

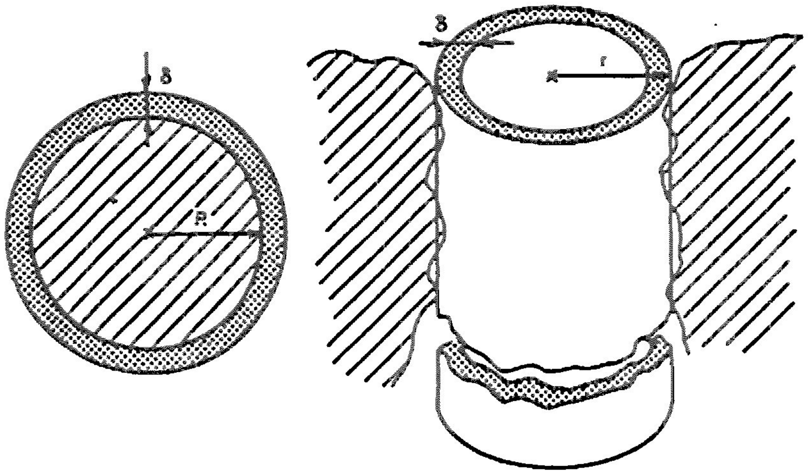


FIGURE 44. The "naive or primitive model". Pores are depicted as cylinders of radius r and bonded-phase thickness δ. (Reproduced from Gobet, J. and Kováts, E., *Adsorp. Sci. Technol.*, 1, 77, 1984. With permission.)

Gobet and Kováts[37] calculated surface area values for a variety of bonded phases using these models. Several assumptions were made. Phase thickness (δ) was estimated from van der Waals radii and bond lengths, and the surface was considered homogeneous (no accessible silica between ligands). The coordination number was estimated from the porosity using a previously established relation. Finally, the radius of the globules was determined from the BET specific surface area of the silica neglecting contact points. Better correlation between theoretical and experimental relative areas was observed for the uniform globular model, and on this basis the UGM was selected. Space requirements for the gas molecules were, in turn, adjusted to give better agreement between UGM and gas adsorption surface areas for the different phases examined. A value of ~ 20 Å^2 is recommended for low energy (nonpolar) surfaces compared to 16.2 Å^2 for high energy surfaces (e.g., silica). The error in the recommended technique is estimated at ± 10% or less.

A more empirical approach to the study of bonded phase surface areas was taken by Sander et al.[237] Three sets of bonded phases were examined: (1) C_{18} monomeric phases with surface coverages ranging from 0.27 to 2.7 μmol/m^2; (2) moderate coverage monomeric phases with different alkyl lengths C_1 to C_{18}; and (3) high surface coverage polymeric phases with chain lengths C_1 to C_{18} (~5.5 to 6.9 μmol/m^2). Apparent surface areas were determined by multipoint nitrogen adsorption, and corrections were made for the weight increase due to the bonded phase. Plots of apparent surface area vs. carbon loading are shown in Figure 45 for the various phase types. In every instance but one (C_4 monomeric phase), the measured surface area value was smaller than the unmodified substrate. When plotted on the same axis (Figure 45D), it is observed that the apparent surface area decreases as a function of overall carbon loading, regardless of the type of phase.

Nitrogen adsorption techniques are also used in the assessment of pore diameter and pore volume. At high relative nitrogen pressures, capillary condensation occurs within the pore network. The relative pressure at which this occurs depends on the size of the pores. Higher relative nitrogen pressures are required for larger pore substrates. Relative pressure and pore diameter are related through the Kelvin equation:

$$\ln(P/P_0) = -2\gamma\overline{V}/rRT \qquad (10)$$

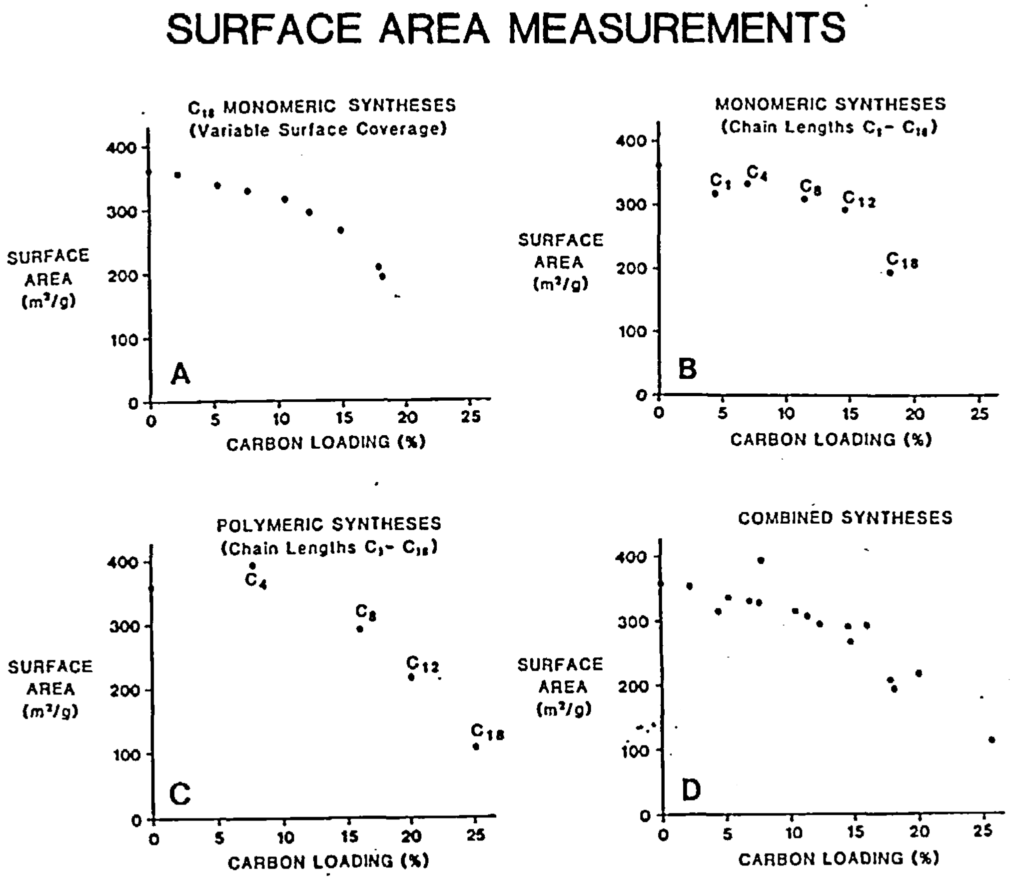


FIGURE 45. Apparent surface area values plotted as a function of overall carbon loading for monomeric and polymeric phases. (A) Monomeric C_{18} phases with different loadings; (B) monomeric phases of different alkyl lengths; (C) polymeric phases of different alkyl lengths; (D) combined plot of A to C. (From Sander, L. C., Glinka, C. J., and Wise, S. A., *Chemically Modified Surfaces: Silanes, Surfaces, and Interfaces*, Leyden, D. E., Ed., Gordon and Breach Science Publishers, New York, 1986, 431. With permission.)

where $\overline{V}$ = molar volume, P/P_0 = relative pressure, γ = surface tension, r = pore radius, R = gas law constant, and T = absolute temperature. Pore size distributions can be obtained in a different way by mercury porosimetry. For nonwetting liquids, a characteristic pressure is required to force the liquid into pores of given size. Measurement of the change in liquid volume as a function of applied pressure, thus, can be used to assess the pore size distribution.

A simple size exclusion chromatographic method for determining pore size distributions of silica has been reported by Werner and Halász.[238-241] A series of polystyrene standards of different molecular weight was chromatographed under conditions intended to eliminate interaction of the solute with the stationary phase. The resulting elution volumes were plotted as a function of log(MW) of the polystyrene standard or, alternately, as a function of the exclusion value (in Å) corresponding to that MW (see Figure 46). Differentiation of this curve gives a plot similar to the pore size distribution curves obtained by more classical methods (capillary condensation and mercury porosimetry). The measurement of pore size distribution by size exclusion chromatography has distinct advantages over porosimetry and gas condensation methods. The technique is inexpensive, rapid, and is carried out in a relevant mobile phase environment. By contrast, pososimetry requires expensive instrumentation and is tedious. Gas condensation is easier to carry out than porosimetry; however, the required equipment is also expensive and measurements are made at low temperature

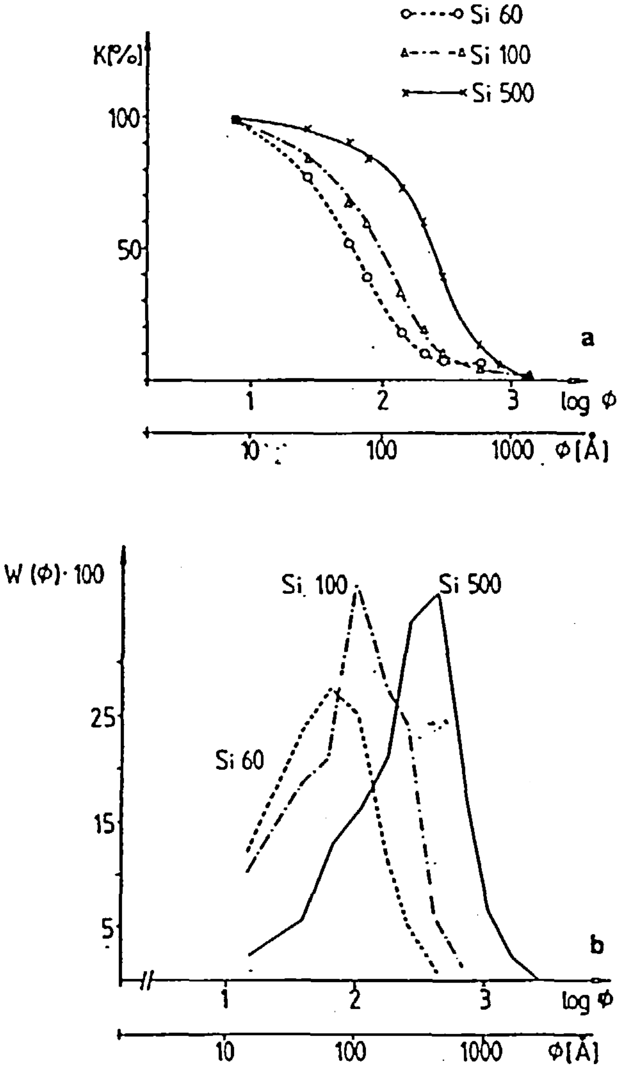


FIGURE 46. (a) Size exclusion calibration curve for 60-, 100-, and 500-Å pore diameter substrates; (b) pore size distribution curves as determined by size exclusion chromatography. (Reproduced from Werner, W. and Halász, I., *J. Chromatogr. Sci.*, 18, 277, 1980. With permission.)

on dry samples. Size exclusion is a viable alternative to these classical methods, but the information derived is not directly comparable with these methods. The size exclusion distribution plot is wider than those obtained from either of the classical techniques, but it is valuable for relative comparisons of pore size distribution, especially before and after surface modification. Nikolov and co-workers[241] showed that pore diameter is reduced by surface modification; "larger pores disappear and smaller pores are formed." The average layer thickness for C_{18} phases was 5 to 15 Å, which is considerably less than expected for fully extended octadecyl chains. In other experiments changes in pore structure resulting from changes in the C_{18} surface concentration seemed to indicate that ligand density varied rather than phase thickness.

Essentially equivalent results were obtained by Freeman and co-workers[242-244] using a size exclusion technique based on the theory of Giddings. Relations were derived that permitted measurement, not only of pore size, but of specific surface area as well. Pore diameter and surface area measurements were carried out on various samples by the size exclusion technique and were compared to values obtained by nitrogen adsorption (BET) and mercury intrusion. Good agreement between the techniques was found for a controlled pore glass substrate. Comparisons of surface area and pore diameter measurements for styrene-divinylbenzene copolymer gels, however, were in less agreement. BET surface area values for the gels were very low (or zero!). More reasonable values were found by the size exclusion technique. The discrepancy was attributed to changes in the gel pore structures upon drying and, thus, BET values on dry gels are suspect. SEC surface area measurements carried out in a mobile phase environment do not suffer from this limitation. The efficacy of the technique was further demonstrated by Kuga[245] for the determination of pore size distributions in Sephadex gels.

Warren and Bidlingmeyer[246] also used a size exclusion technique to characterize bonded silicas. Data were fit to a polynomial expression for smoothing. Polystyrene standards were supplemented with hydrocarbons to probe small pore structure. Significant differences were observed between nominal pore diameter values and the median values determined by size exclusion. This is expected since pore size changes upon surface modification. Similar pore size distribution plots were obtained for over 30 commercial LC columns; however, significantly narrower plots were observed for Zorbax-based sorbents.

E. Carbon Determination

Little has been published about the determination of carbon loading of chemically modified surfaces. Most techniques involve phase cleavage or pyrolysis; however, the methods vary in the way in which the carbon species so produced are quantified. One common technique is thermal gravimetric analysis (TGA). In its simplest form, the bonded silica is burned in an oxygen environment. The weight loss after burning is attributed to loss of the bonded phase. A conversion is made using the molecular weight of the ligand relating this weight loss to the carbon loading of the sorbent. Care must be taken to assure that the weight loss is due to loss of the bonded ligands and not other sources (such as loss of water). Cheng and McCown[147] reported a gravimetric technique for phase quantification based on the weight gain of silica after surface modification. A known quantity of silica was bonded and quantitatively transferred to sintered glass frit crucible. After drying to constant weight, the carbon loading (%C) was calculated from the weight increase from the modification. This technique was considered more accurate than other techniques; however, the uncertainty of the method was not reported. Because weight of the silica before bonding must be accurately known, this technique cannot be applied to commercial phases. Perhaps the most common method of phase quantification is elemental analysis. Elemental carbon can be measured directly by burning the sample in oxygen and quantifying the carbon dioxide evolved (usually by infrared spectrometry or titration). Diamondstone et al.[72] described such a method for determination of elemental carbon using apparatus originally intended for the measurement of carbon in steel. Relative precision of the technique was reported to be 0.8%. Several research groups have explored the possibility of phase quantification by phase cleavage and gas chromatographic analysis of the cleavage products (see Section III.C). While difficulties in quantitative phase conversion and reproducibility exist in practice, the approach is in principle workable. An alternate approach utilizing neutron activation analysis is under investigation in our laboratory.

F. Other Characterization Techniques

A few novel approaches have been described for the characterization of chemically modified surfaces. Hansen and Callis[247] studied phase transitions in alkyl phases by differential scanning calorimetry. C_{18} and C_{22} phases were prepared with surface coverage values of about 2.0 and 2.5 μmol/m². Unlike the work of Morel and Serpinet (see Section IV), no evidence for phase transitions was found within the temperature interval −20 to 150°C. The lack of phase transition was attributed to the moderate ligand concentrations of the phases employed. Phase transitions were observed in other samples prepared with physically adsorbed C_{18} and C_{22} *n*-alkanes. Experiments with adsorbed methanol on C_{18} phases seemed to indicate a layer of the solvent binds to the C_{18} chains, possibly hydrogen bonding to other methanol molecules in a cooperative fashion. Van Miltenburg and Hammers[248] observed second-order transitions in LiChrosorb RP-8 and RP-18 sorbents by adiabatic calorimetry. Transition enthalpy and entropy values for the bonded phases were about one fourth as large as for corresponding free alkanes. Rayss et al.[249] also studied thermal properties of adsorbed layers on chromatographic substrates. Differential thermal analysis was used to characterize *n*-octadecanol films on carbon-silica adsorbents. A method was devised by which the surface area ratio of bare silica to carbon-coated silica could be assessed from the thermograms of the coated substrates.

Hunnicutt and Harris[54] used the techniques of X-ray fluorescence (XRF) and X-ray photoelectron spectroscopy (XPS) to detemine the ligand density of chloro- and bromoalkyl modified surfaces. The application of XPS to the study of modified surfaces is noteworthy. Because XPS is a surface analysis technique (depth limit on the order of several nanometers), the information so obtained pertains to the outside of the silica particle. When compared to information obtained by XRF which is not depth limited, differences can be related to phase inhomogeneity between the outer particle surface and the inner pore network (see Section II.A).

Glinka et al.[250,251] determined pore accessibility in silica substrates by small angle neutron scattering (SANS). With this technique, low energy neutrons strike the sample and are elastically scattered by pores or other fine structures. Details within the size interval of about 1 to 100 nm can be probed, so the technique is well suited to the study of chromatographic substrates. Analysis of scattering patterns reveals information about the size and surface area of the scattering centers. Glinka and co-workers reported good correlation between surface area values determined by SANS and nitrogen adsorption techniques. Scattering results because of differences in scattering length densities (sld) of the solid (silica) and void phases (pores). Scattering length density can be thought of as an index of refraction for neutrons. When the difference in sld's for the two phases is large, scattering is strong. Likewise, if the difference is small or zero, little or no scattering results. Glinka et al. filled the pores of silica substrates with a mixture of H_2O/D_2O calculated to have the same sld as that for bulk silica. The reasoning is thus: if all of the pores are accessible to the solvent, no scattering should occur since the sld difference is zero. Any scattering that does occur is a result of incomplete masking or inaccessible (i.e., closed) pores. The reduction in scattering for the silicas examined was complete — less than 1% scattering remained indicating that essentially all pores are accessible. This work has been extended to include the study of bonded phase systems. The investigation of bonded phase structure is more complex, because scattering results from a three phase system — silica, bonded phase, and pores. The situation can be simplified by filling the pores of the modified substrate with a solution that has the same scattering length density as the silica. In such a case scattering can be attributed to the bonded phase structure (see Figure 47). Studies in progress show that scattering depends on both alkyl chain length and pore diameter. Proper interpretation of the data may eventually allow direct assessment of bonded phase thickness in a chromatographically relevant mobile phase environment.

Quasi-elastic neutron scattering experiments on bonded phases were carried out by Beaufils and co-workers.[252] This technique differs from SANS in that measurements are carried out at large angles and energy transfers of scattered neutrons are measured. Neutrons interact

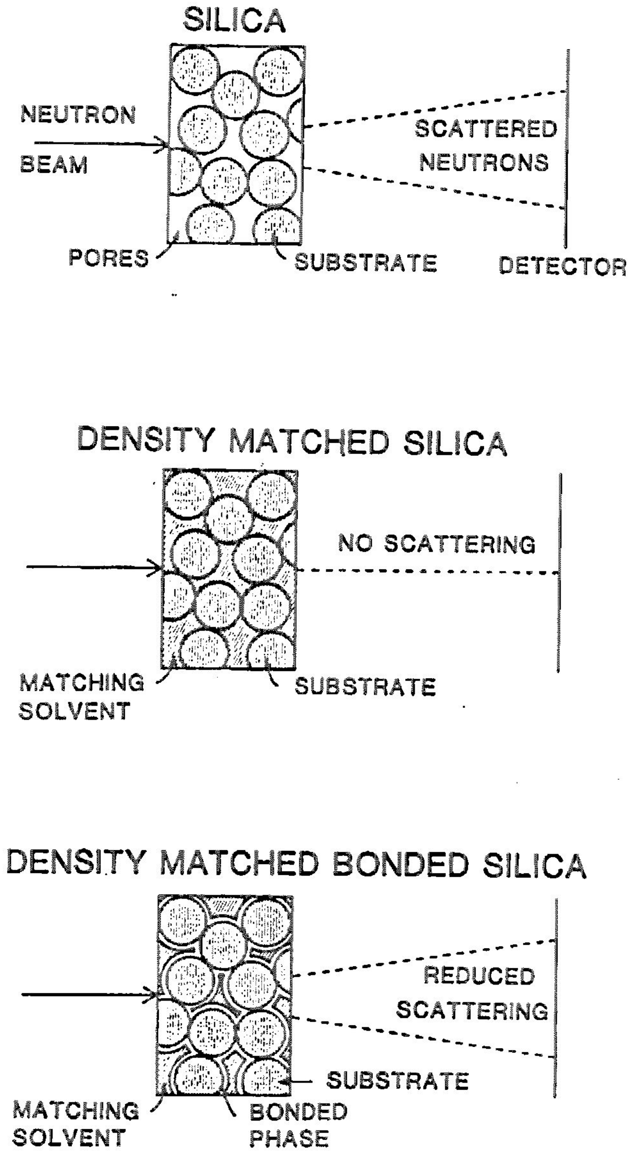


FIGURE 47. Schematic illustration of small-angle neutron scattering experiments. Upper figure: scattering from bare silica; middle figure: elimination of scattering by density matching; lower figure: scattering from bonded phase only by density matching. (From Sander, L. C. and Wise, S. A., unpublished research. With permission.)

during the scattering process to gain or lose energy. This energy transfer results in broadening of the elastic scattering peak, which can, in turn, be related to a characteristic time. Beaufils et al. used this technique to study alkyl chain motion in monomeric C_{18} phases. Chain motion is viewed as occurring in segmental jumps from one conformation to the next. The conformation changes involve five C-C bonds for dry C_{18} phases at 80°C. At lower temperatures segmental jumps involve three C-C bonds. The average time between two such jumps is 2×10^{-11} sec. Other experiments carried out in the presence of methanol show little evidence for strong associations of the methanol with the alkyl chains. The authors proposed a model in which the pore volume is divided into two parts: the volume occupied by the alkyl chains and the volume occupied by the methanol. The chains impose "shape" to the methanol volume, but do not strongly interact with the methanol molecules. Hydrogen bonding within the ordered methanol probably results in higher than normal viscosity for methanol molecules intercalated between the alkyl chains.

IV. THEORY

A. Hydrophobic Retention

In the past, several approaches have been used in the description of the origins of solute retention for alkyl bonded phases with varying degrees of success. The difficulty in modeling reversed-phase retention primarily originates from a lack of understanding about the physical nature of the alkyl modified surfaces. It is tempting to classify alkyl surfaces as either liquids or as solids. In so doing, retention at a thin liquid film could then be modeled by a partitioning mechanism, or retention at a solid surface could be attributed to adsorption. Monomeric bonded layers are neither true liquids nor solids, but, instead, have properties intermediate to each. Rotational and translational freedom is restricted in alkyl bonded phases compared to bulk liquids, yet molecular association (i.e., lattice formation) is not as complete as with solid paraffins. Phase density of monomeric alkyl phases used in liquid chromatography is about half that for bulk liquids or solids, although this value is sometimes substantially lower. For these reasons neither simple partitioning nor adsorption models of reversed-phase solute retention are satisfactory.

Hydrophobic and solvophobic retention models have been advanced by Horváth and Melander[253,254] and by Karger and co-workers.[255] These theories treat the stationary phase as a "passive acceptor" rather than an "active attractor" for the solute. Stated simply, the hydrophobic effect is the tendency of nonpolar molecules to minimize contact with water either by phase separation or by sorption of the molecules onto other nonpolar surfaces. Retention is driven by the reduction in exposed surface area of the ligand and solute by complex (ligand-solute) formation. This effect is opposed by attractive interactions (i.e., Van der Waals forces) between the solute and solvent molecules (see Figure 48). Unlike adsorption in normal-phase liquid chromatography, attractive forces between the solute and stationary phase for reversed-phase systems are thought to be small. Hydrophobic retention is, thus, attributed primarily to solvent effects.

The process can also be viewed thermodynamically. The positive free energy of solution for nonpolar solutes in water results, not because of positive enthalpies of solution ($\Delta H_s <$ 0 for hydrocarbons in water), but because of the large entropies of solution.[255] The formation of water "cavities" about the nonpolar molecules represents considerable ordering; to reduce this order, solute molecules clump together or are expelled from solution. For this reason, hydrophobic retention is often considered as entropy driven. Detailed thermodynamic investigations of hydrophobic solution processes have been reported by Gill and Wadsö[256] and Yaacobi and Ben-Naim.[257,258]

Each of the contributions to hydrophobic retention has been represented mathematically by Horváth et al.[253] The change in free energy associated with solute retention can be expressed as the difference in free energy required to bring the solute-ligand complex into solution from a hypothetical gas phase and the free energy required for solution of the solute and ligand individually. Horváth and Melander have derived the following relation combining all contributions:

$$\ln k' = \phi + (1/RT)[\Delta A(N\gamma + a) + NA_s\gamma(\chi^e - 1) + W - \Delta Z/\epsilon] + \ln(RT/P_0V) \tag{11}$$

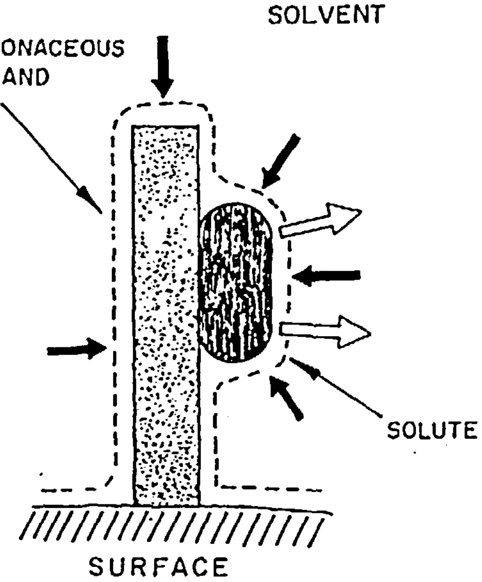


FIGURE 48. Schematic illustration of the hydrophobic association of a nonpolar solute with the nonpolar ligand of a chemically modified surface. Binding is due to the reduction in area exposed to the solvent resulting from the association. (Reproduced from Horváth, G. and Melander, W., *J. Chromatogr. Sci.*, 15, 393, 1977. With permission.)

where ϕ, W, a = solvent-dependent parameters (measurable); R = gas law constant; T = absolute temperature; $\Delta A = A_E + A_L - A_{EL}$ (change in exposed surface area of ligand and solute, the contact area); N = Avogadro's number; γ = surface tension of bulk solvent; A_s = surface area of solvent molecule; χ^c = correction factor for surface tension; ϵ = dielectric constant; ΔZ = electrostatic interaction term; and $\ln(RT/P_0V)$ = change in free volume term. In principle, each of the constants is measurable and, thus, the capacity factor can be evaluated. It is interesting to observe that the form of the equation corresponds at least qualitatively with observed trends in reversed-phase retention. The greatest effects on retention are contributed by surface tension (χ^c) and contact area (ΔA) terms. From Equation 11 it follows that a plot of ln k′ vs. eluent composition (e.g., % methanol/water, v/v) should be approximately linear, and this trend is commonly observed in practice. Linear relations between ln k′ and solute chain length (homologous series plots) can also be explained, at least in part, by Equation 11.

Stationary phase effects are not well accounted for by this model. Because the stationary phase takes a passive role in hydrophobic retention, selectivity differences sometimes observed between various types of nonpolar columns are not easily explained. One approach that retains the basic features of hydrophobic retention theory is based on solute accessibility at the bonded phase surface. If the contact area can be thought to vary because of stationary phase effects (rather than solute effects), then hydrophobic retention could be related to stationary phase properties as well as mobile phase properties. Sander and Wise[35,164] described such an effect in qualitative terms to explain selectivity differences observed between monomeric and polymeric C_{18} phases (see Section IV.E).

Karger and co-workers[255] related hydrophobic retention and selectivity to a topological index for the solute known as molecular connectivity. This parameter has been shown to be proportional to the solute cavity surface area, and it has the advantage of being easier to calculate than surface area. Karger et al. defined the molecular connectivity as:

$$\chi = \sum_{k=1}^{k} \frac{1}{(\delta_i\delta_j)^{1/2}} \qquad (12)$$

where δ = 1 to 4, i.e., the number of atoms attached to atoms i and j, respectively. Molecular connectivity was found to be a useful predictor in estimating hydrophobic selectivity. Methylene group selectivity was examined at various mobile phase compositions and at various temperatures. If the results are normalized to equivalent times, approximately equivalent selectivities were obtained under these conditions.

Recently, Katz et al.[259] developed basic theory to describe chromatographic retention based on the distribution of a solute between two phases. With bonded phases, however, the authors view the mobile phase and stationary phase as a ternary system in which the third phase consists of solvent associated with the bonded ligands. The utility of the retention theory is demonstrated by prediction of solute retention in reversed-phase LC.

B. Stationary Phase Chain Length

The physical nature and behavior of the alkyl bonded layer in reversed-phase LC has been a subject of considerable research and controversy. The effect of the chain length of the alkyl bonded phase has received considerable attention in studies of bonded phases often with contradictory results. Early investigations of the effect of the alkyl chain length on retention were performed by Majors and Hopper,[56] Gilpin and co-workers,[48] and Karch et al.;[139] all of these studies showed that increasing chain length provides increased retention and selectivity.

In a series of studies by Hemetsberger and co-workers,[27,29,30] the effect of chain length on retention and selectivity was considered. In their first study, they observed a linear relationship between k′ for small solutes and the amount of coverage (i.e., % carbon) that was independent of the chain length. Later, they compared phases of different chain length prepared with *n*-alkyltrichlorosilanes and alkylmethyldichlorosilanes. In all cases the alkylmethyl phases had greater retention and selectivity than the alkyl phases even though the carbon contents were comparable. These differences were attributed to the presence of less accessible silanol groups on the alkylmethyl phases. The effect of structure of the chemically bonded ligand was investigated by comparing monoalkyl and dialkyl phases. Retention was found to be greater for the dialkyl phases as compared to the monoalkyl phases at the same percentage of carbon.

In an attempt to characterize the physical nature of the bonded phases to determine whether it behaves as an adsorptive surface or resembles a liquid, Lochmüller and Wilder[26] studied the chromatographic capacity factor and selectivity as a function of the bonded phase chain length and the size of the solute and compared these data with analogous liquid-liquid partitioning systems. They compared liquid-liquid partition data and reversed-phase LC selectivity (ln α) for several relatively small solutes (methyl-substituted benzenes), as illustrated in Figure 49. The ln α values for alkane-water/methanol liquid-liquid partition systems were relatively constant over the range of carbon chain lengths examined (C_5 to C_{22}), whereas the ln α values for the bonded alkyl phases (C_6 to C_{22}) were smaller initially, but increased with chain length until at C_{12} the selectivities for the bonded phases and the liquid-liquid selectivities were essentially identical. They interpreted these findings to support a liquid-like behavior of the bonded phase and a partition mechanism, since an adsorption-dominated mechanism should produce a limiting value of ln α that is smaller than the liquid-liquid partition value. They proposed the formation of "liquid-droplet"-like clusters. Using larger-sized solutes (i.e., PAH), they again investigated selectivity as a function of bonded alkyl chain length (Figure 50). In contrast to the results with small solutes, ln α values did not approach a limiting value with increased length of the alkyl ligand. This result was interpreted as the inability of the bonded phase to completely solvate the relatively large solute molecules.

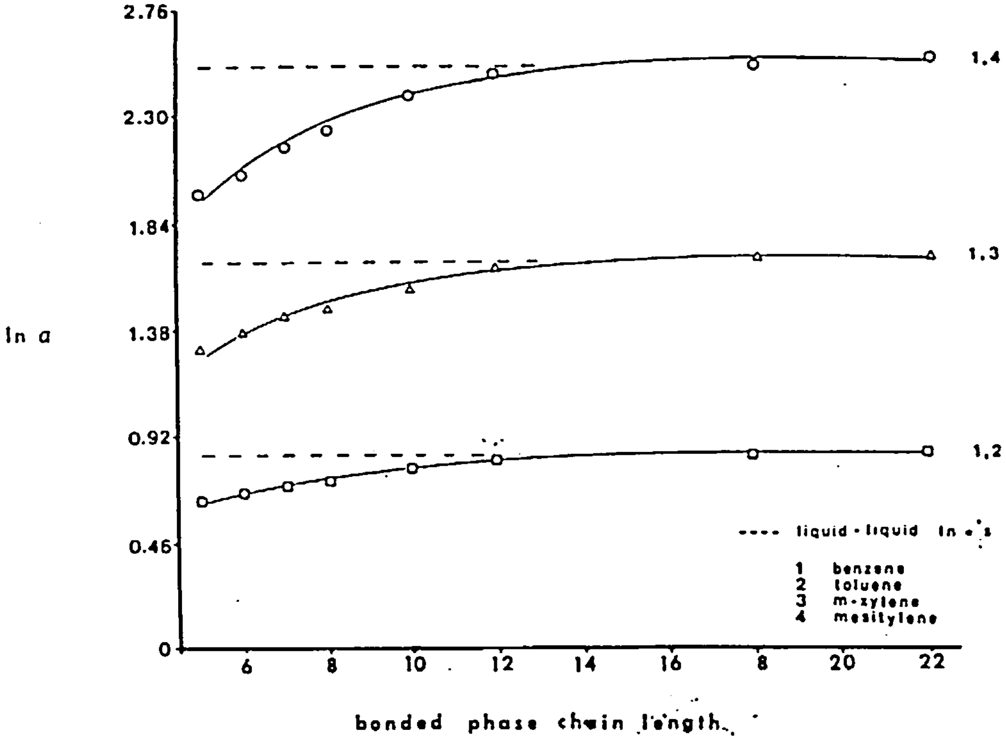


FIGURE 49. Plot of selectivity (ln α) against bonded carbon chain length. Dashed lines give ln α values for liquid-liquid systems. (Reproduced from Lochmüller, C. H. and Wilder, D. R., *J. Chromatogr. Sci.*, 17, 574, 1979. With permission.)

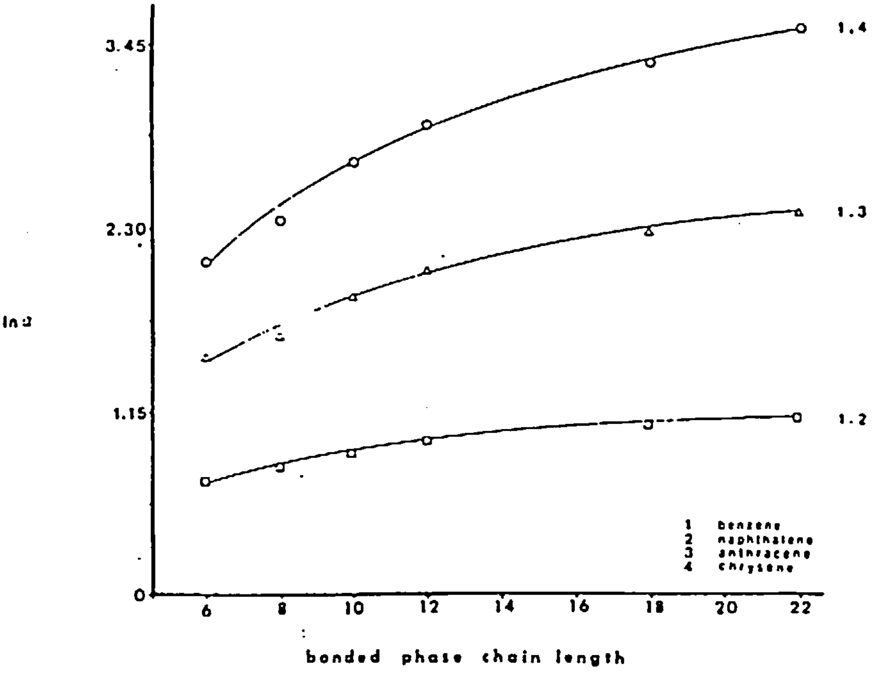


FIGURE 50. Plot of ln α vs. bonded carbon chain length for larger nonpolar solutes. (Reproduced from Lochmüller, C. H. and Wilder, D. R., *J. Chromatogr. Sci.*, 17, 574, 1979. With permission.)

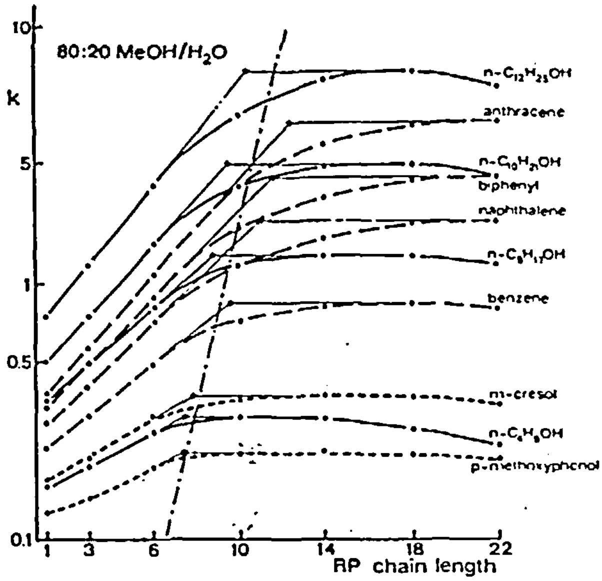
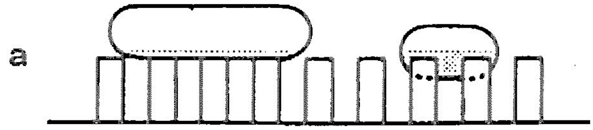


FIGURE 51. Capacity factor as a function of bonded phase length in methanol-water (80:20). Asterisks denote the critical chain length, determined as the intersection point of the extrapolated branches of the curves. (Reproduced from Berendsen, G. E. and de Galan, L., *J. Chromatogr.*, 196, 21, 1980. With permission.)

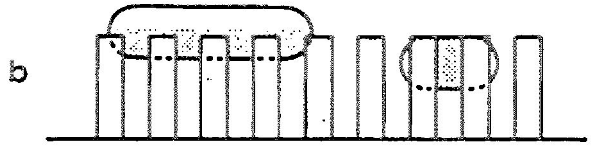
Lochmüller and Wilder[26] pointed out that the linear relationship between capacity factor and carbon coverage observed in studies of reversed-phase systems may result from the use of relatively large solutes.

Hennion et al.[260] investigated the effects of both C_{18} surface coverage and alkyl chain length on capacity ratio and selectivity for nonpolar (PAH) and polar (phenols) solutes. Capacity ratios increased exponentially with carbon content up to about 15% and then remained constant; selectivity also increased with increasing carbon content to the same loading. For bonded phases of constant surface coverage and alkyl chain lengths from C_4 to C_{18}, k′ increased exponentially with increasing number of carbon atoms for nonpolar solutes and linearly for polar solutes. Selectivity was found to increase with increasing number of carbons for both types of solutes.

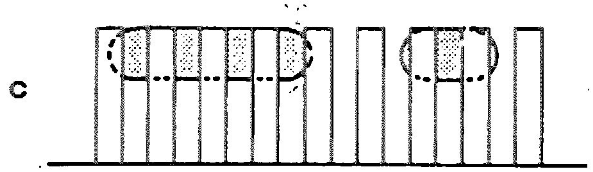
Berendsen and de Galan[262] investigated the influence of the chain length of *n*-alkyl bonded phases (C_1 to C_{22}) on retention in methanol-water mobile phases. In contrast to other investigations,[23,30,56,260] they did not observe a continuous increase in retention with increasing bonded chain length. Instead, it was found that the capacity factor increased exponentially up to a certain chain length after which the retention remained constant. The "critical chain length", where retention starts to become constant, was observed between C_6 and C_{10}, as shown in Figure 51, and was independent of the mobile phase composition. Extension of the chain length above C_7 did not increase the retention significantly, which indicates that for longer chains only the exterior part of the alkyl chains participates in the retention process. This conclusion contrasts the suggestion of Unger and co-workers[23,261] that the complete chain length is involved in the retention process. The conclusion of Unger et al.[261] was based on the observation that a plot of log k'/S_{BET} (where S_{BET} is the surface area of the underivatized silica) against the number of carbon atoms in the *n*-alkyl group for a number of PAH solutes (naphthalene to indeno[1,2,3-cd]pyrene) gives a straight line for each solute. Selectivity factors, on the other hand, did not change with varying chain length. The critical chain length of Berendsen and de Galan[262] was found to increase with increasing



Short chain length. No penetration of the larger sized molecule, less for the smaller sized one



Longer chain length. Larger sized molecule partially penetrated and the smaller sized one completely



Long chains. Both molecules are completely penetrated

FIGURE 52. Schematic illustration of partial penetration of solutes of different sizes in chemically bonded phases with (a) short chains, (b) intermediate chains, and (c) long chains. (Reproduced from Berendsen, G. E. and de Galan, L., *J. Chromatogr.*, 196, 21, 1980. With permission.)

solute capacity factor. Therefore, each solute requires its own minimum alkyl bonded chain length to maximize its retention, varying from a minimum of 6 for weakly retained solutes up to 14 for strongly retained solutes. For a series of *n*-alcohols and phenols they found an increment of critical chain length of 0.5 reversed-phase (RP) units per added methylene or methyl group. For PAH the addition of a phenyl ring increases the critical chain length by 1.3 RP units. The addition of a strongly polar hydroxyl group decreased the critical chain length by 2.2 RP units. Thus, the critical chain length is a function of the size of the nonpolar part of the solute which can be exposed to the bonded phase. Selectivity increases gradually up to the critical chain length and then remains constant. However, they found the influence of the mobile phase to far outweigh that of the stationary phase.

Berendsen and de Galan[262] postulated a reversed-phase retention mechanism based on the two major findings of this study: (1) the mobile phase provides the dominant influence on the retention and selectivity, whereas the stationary phase has a secondary role; and (2) the observance of a critical chain length indicates the interaction of the solute with only a part of the bonded chain. They visualized the influence of the RP chain length on selectivity, as illustrated in Figure 52, for two solutes of different nonpolar surface area. On short RP chains both solutes can penetrate the alkyl chains to only a limited extent (or they may reside on top of the chains). Since the larger solute possesses the larger absolute contact area, it will be retained longer (see Figure 52a). As the chain length increases the smaller molecule can penetrate more deeply and will reach its critical chain length sooner. The larger solute needs longer RP chains to become fully enclosed (i.e., a longer critical chain length). Since the contact area of the larger solute is much larger than for the smaller solute, its relative increase in retention will be greater. Consequently, the selectivity will generally increase with increasing chain length.

Results obtained by Tanaka et al.[25] suggest that retention is influenced by the participation of the solvent molecules in the stationary phase and the molecular structure (i.e., steric effects) of the solute. They studied the retention behavior of various hydrocarbons, including *n*-alkanes, PAH, and alicyclic compounds on C_1, C_8, and C_{18} dimethylsilyl phases. In general, a longer alkyl chain stationary phase provided longer retention. However, some interesting results were observed. Plots of log k' values for the various solutes on the C_{18} phase vs. log k' values obtained on C_1 and C_8 phases in 80% methanol and 80% acetonitrile are shown in Figure 53. The solid lines are drawn based on *n*-alkanes (compounds 5 to 8) and the dashed lines indicate the location of the PAH. A comparison of the behavior of two pairs of compounds, numbers 17 and 21, and 20 and 22, is of particular interest (see structures in Figure 53). Compounds 21 and 22 have the same number of carbon atoms and phenyl rings as 17 and 20, respectively. The only difference is that the phenyl rings are bonded directly in 21 and 22 which increases the planarity and rigidity of the molecules. The planar, rigid compounds (21 and 22) exhibited much longer retention on the C_{18} phase than the nonplanar solutes of similar structure (17 and 20); the difference in retention was much smaller on the C_8 and no difference in retention was observed on the C_1 phase. Thus, they concluded that the solute planarity or rigidity was able to visualize the difference in carbon chain length in the stationary phase using the same mobile phase. For 80% acetonitrile a similar tendency was observed; however, the effects of the chain length were much smaller in acetonitrile than in methanol. Wise and Sander[164] observed similar retention behavior differences for planar and nonplanar solutes on monomeric vs. polymeric C_{18} phases (see discussion later). From these retention behavior studies Tanaka and co-workers[25] also determined that C_1 and C_8 phases were relatively similar in nature. Increasing the content of organic modifier in the mobile phase resulted in much larger retention of the rigid planar aromatic molecules relative to other aromatic compounds on the C_{18} phase, whereas on the C_1 and C_8 phases this effect was smaller. These results suggest that with the increase in organic content of the mobile phase, the alkyl chains in the stationary phase are more extended and, thus, are able to better differentiate rigid planar solutes from nonrigid solutes.

Jinno[263] compared chromatographic retention on commercial C_2, C_8, and C_{18} phases and concluded that the interactions of the solutes and the different alkyl phases were similar in the mobile phase range investigated (40 to 70% acetonitrile in water). However, in a later study on similar commercial phases, Jinno and Kawasaki[264] concluded that some differences existed between the longer and shorter chain lengths for polar benzene derivatives. Both of these studies provided limited discussion of the findings.

C. Phase Transitions

Considerable effort has been expended in the study of the structure and dynamics of chemically modified surfaces. These studies have been motivated by a desire to understand the underlying mechanisms of solute retention and changes that occur as a function of temperature and mobile phase composition. Gilpin[265] has reviewed progress achieved in the study of bonded phase structure and dynamics. One approach that has been taken in the study of the supromolecular structure of modified surfaces involves the measurement of gas chromatographic properties of the phase under various conditions (termed "inverse gas chromatography"). Morel and Serpinet[266] presented evidence for the existence of phase transitions in C_{18} bonded phases using this gas chromatographic technique. Densely loaded octadecyl phases were prepared on a variety of substrates with pore diameters ranging from 15 to 1000 nm. Relatively high surface coverage values were achieved (~ 4.1 to 4.6 µmol/m²) by the bonding technique of Kováts et al.[267] The resulting sorbents were studied by gas chromatography over a temperature interval of −3 to 55°C. Solute retention plotted against reciprocal temperature was observed to be nonlinear over this interval, with an apparent phase transition near 22°C (see Figure 54). The presence of this transition was not dependent

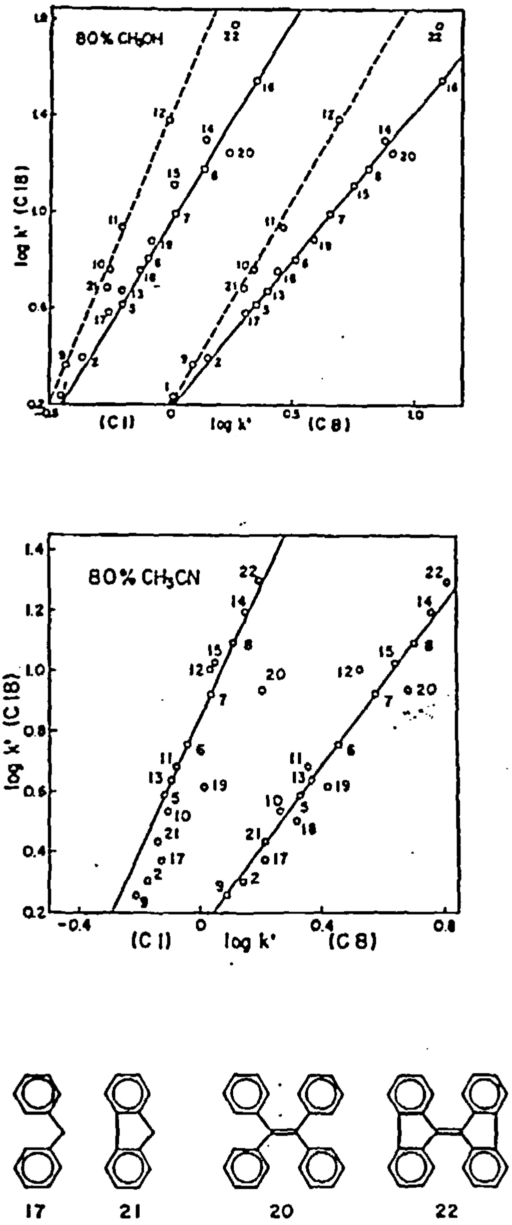


FIGURE 53. Upper figure: plots of log k′ values on a C_{18} phase vs. values for C_1 and C_8 phases in 80% methanol/water; middle figure: same as above, except 80% acetonitrile/water; lower figure: rigid and nonrigid solutes of similar overall shape. (Reproduced from Tanaka, N., Sakagami, K., and Araki, M., *J. Chromatogr.*, 199, 327, 1980. With permission.)

on the solute used. Retention volumes were normalized to the mass of bonded phase and compared to retention volumes in octadecane. In each case bonded phase retention was observed to be greater than for octadecane for temperatures above the transition. At lower temperatures greater retention was found in octadecane. The retention behavior was explained as follows. At temperatures below the transition point solutes were adsorbed onto the extremities of the alkyl chains. Retention is less than with octadecane because of limited

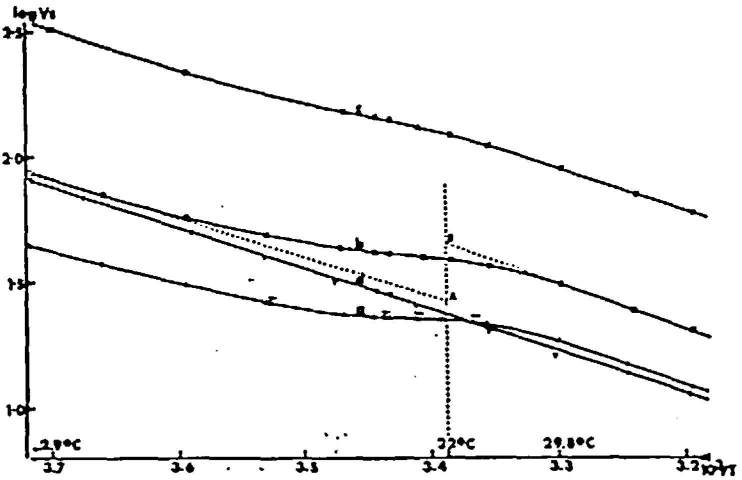


FIGURE 54. Variation in gas chromatographic retention (log V_g) of hexane as a function of temperature for C_{18} bonded Spherosils. (a) Spherosil XOB-015; (b) Spherosil XOB-030; (c) Spherosil XOA-200; (d) Spherosil XOB-015 (aberrant sample). (Reproduced from Morel, D. and Serpinet, J., *J. Chromatogr.*, 200, 95, 1980. With permission.)

association with the alkyl chains. Above the transition additional sites for solute interaction become available between the alkyl chains, and retention is greater than would otherwise be expected. The magnitude of the phase transition is reduced with decreasing pore size. Apparently, the curvature of the pore impedes chain association and the expanded (liquid-like) state is favored even at low temperatures. The authors further examined phase transitions for C_{18} bonded phases coated with free heptadecane. Transitions were observed at 22 and 51°C corresponding to the melting point of heptadecane and the ejection of free C_{17} chains from between the bonded C_{18} chains, respectively. One anomalous bonded silica did not exhibit phase transition behavior. Although speculations for the anomaly were not advanced, the authors did suggest that GC characterization might be valuable in the quality control of bonded phase synthesis.

More dramatic results were obtained with C_{22} ligands.[268] A transition temperature of 48°C was observed for the densely bonded (~ 4.3 μmol/m²) C_{22} phase. Over the interval 40 to 48°C, the gas chromatographic retention of heptane increased with increasing temperature. This suggests that a transition in the bonded phase occurs that gives the solute access to inner parts of the alkyl layer. Additional experiments were carried out to examine the influence a polar liquid might have on phase conformation. Small quantities of glycerine ethylene glycol, triethylene glycol, and glycine water were coated onto the C_{22} bonded phases and the gas chromatographic retention behavior was studied. Log retention volume vs. 1/T plots were characterized by marked increses in the transition temperature. This change in transition temperature was interpreted as an indication that the alkyl chains are forced by the polar liquids to associate with each other. In so doing, solute retention is reduced and the transition temperature increased. Extended to liquid chromatography, this suggests that the use of mobile phase environments containing a high percentage of water may lead to a transition in the phase and lower-than-expected retention. Experiments carried out with high density C_{22} phases in the liquid chromatographic mode have verified these predictions.[269] A phase transition was observed at 46.5°C in a methanol environment. This is slightly lower than for dry phases and suggests that the methanol binds to the alkyl chains and behaves partly as an organic liquid phase. The phase transition is viewed as a two-dimensional melting and "marks a change from adsorption dominated retention at the surface

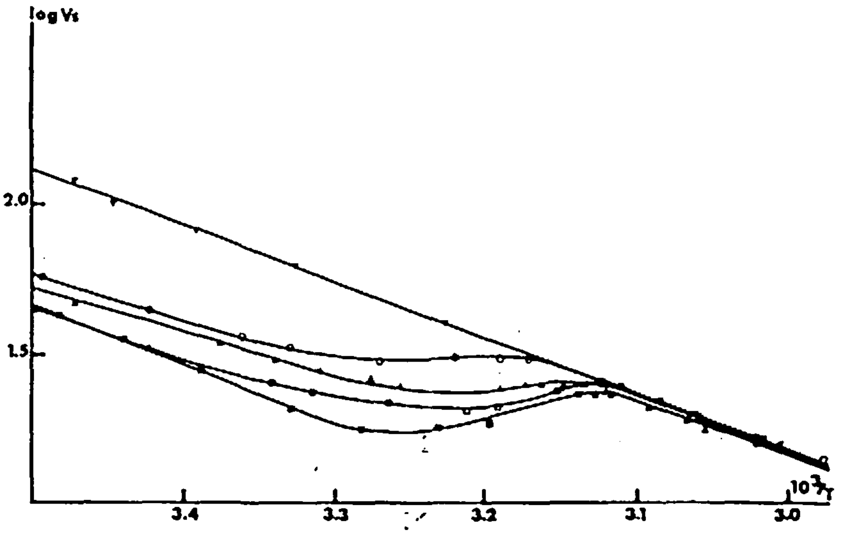


FIGURE 55. Variation in gas chromatographic retention (log V_s) of heptane as a function of bonded phase density. (■) 4.40, (□) 4.35, (△) 4.30, (○) 4.04, (▽) 2.90 μmol/m². (Reproduced from Claudy, P., Letoffé, J. M., Gaget, C., Morel, D., and Serpinet, J., *J. Chromatogr.*, 329, 331, 1985. With permission.)

of a solid monolayer to a solution dominated retention in a liquid expanded-like layer." In a later study,[270] transitions were also observed for a conventional lower density alkyl phases of the type more typically used in liquid chromatography. The transitions are thought to be a major cause of irreproducibility in liquid chromatography.

Claudy and co-workers[271] studied in detail phase transitions of modified surfaces as a function of surface coverage. A remarkable result was observed: retention of *n*-heptane was independent of surface coverage above the transition temperature (see Figure 55). Phase transitions were not observed for phase coverages less than about 2.5 μmol/m². Plots of log(V_s) vs. 1/T merged to a single curve above about 48°C, indicating that under these conditions the bonded ligands do not participate significantly in the retention process! Instead, retention appears to take place at the silica surface. Plots of log(V_s) vs. 1/T for bare silica supported this idea. Phase transitions were further studied using differential scanning calorimetry (DSC) and proton nuclear magnetic resonance spectroscopy (NMR). Unlike the work of Hansen and Callis,[47] phase transitions were readily observed in the DSC curves for the bonded phases. The difference was attributed to the use of narrow-pore, low-coverage phases by Hansen and Callis. Claudy et al. found that the temperature and magnitude of the phase transition increased with increasing surface coverage. NMR band width was measured as a function of temperature for densely loaded C_{18} and C_{22} phases (see Figure 56). Band width decreased with increasing temperature, indicating increased chain mobility. At 30 and 50°C a rapid narrowing of band width was observed for the C_{18} and C_{22} phases, suggesting that the mobility of the alkyl chains reaches a limit at the phase transition temperature.

Based on the results of these previous investigations, Gonnet and co-workers[272] prepared phases by inserting free alkanes between bonded alkyl chains to achieve a ligand density value of 8 μmol/m². This value is similar to the density of solid two-dimensional films formed by polar fatty compounds and represents the maximum compactness of fatty chains. The liquid chromatographic properties of these phases were examined by using alkane-saturated mobile phases. Partial separation of an amine mixture was possible for C_{18} and C_{22} bonded phases. However, the separation of various amines was completely suppressed

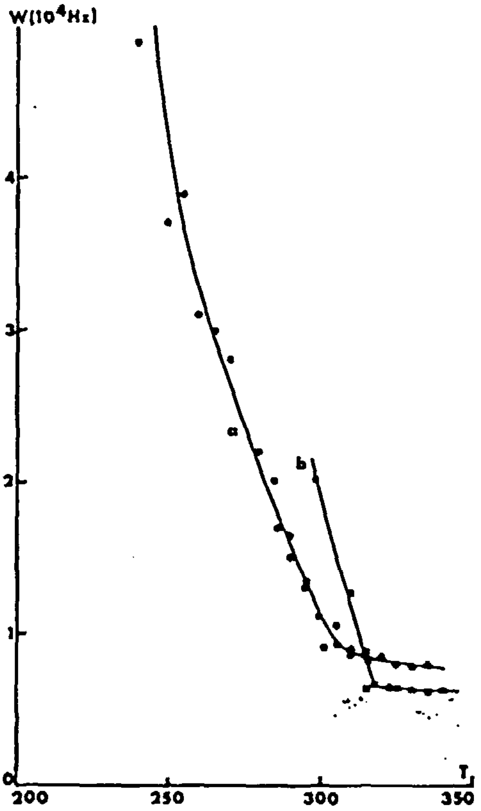


FIGURE 56. Variation in NMR peak width (at quarter height) as a function of temperature. (a) C_{18} bonded Spherosil XOB-075, 4.15 μmol/m²; (b) C_{22} bonded Spherosil XOB-075, 4.4 μmol/m². (Reproduced from Claudy, P., Letoffé, J. M., Gaget, C., Morel, D., and Serpinet, J., *J. Chromatogr.*, 329, 331, 1985. With permission.)

by the addition of the long chain alkane to the mobile phase. This indicates perfect masking of the silica surface with the mixed film phase. The preparation of other types of mixed film phases are in progress.

Gilpin and Squires[273,274] have studied changes in bonded phase structure that occur in aqueous environments as a function of temperature. Octyl, nonyl, and decyl phases were prepared using trichlorosilanes. Phase loading was controlled by varying the degree of surface hydration of the silica. Although not mentioned by the authors, the phases so produced must be polymeric in nature. Retention properties of these midlength phases were studied in a totally aqueous environment using resorcinol or phenol probes. The columns were conditioned with 100 mL acetonitrile followed by 100 mL water, and solute retention was measured as a function of increasing temperature. Plots of ln k′ vs. 1/T were initially linear, but at a given temperature referred to as the "on-set temperature" retention decreased in a sigmoidal fashion (see Figure 57). After cooling, solute retention was again measured as a function of temperature. Retention was observed to differ significantly after this "heat conditioning". Instead of the sigmoidal behavior, plots of ln k′ vs. 1/T were linear over the temperature range examined. At higher temperatures plots from both the conditioning and rerun steps merged and solute retention was equivalent. The authors envisioned a stationary phase model in which the alkyl chains can exist in a collapsed or extended state. Prior to heating, the phase is viewed as existing in a collapsed state. Mutual alkyl chain association is favored

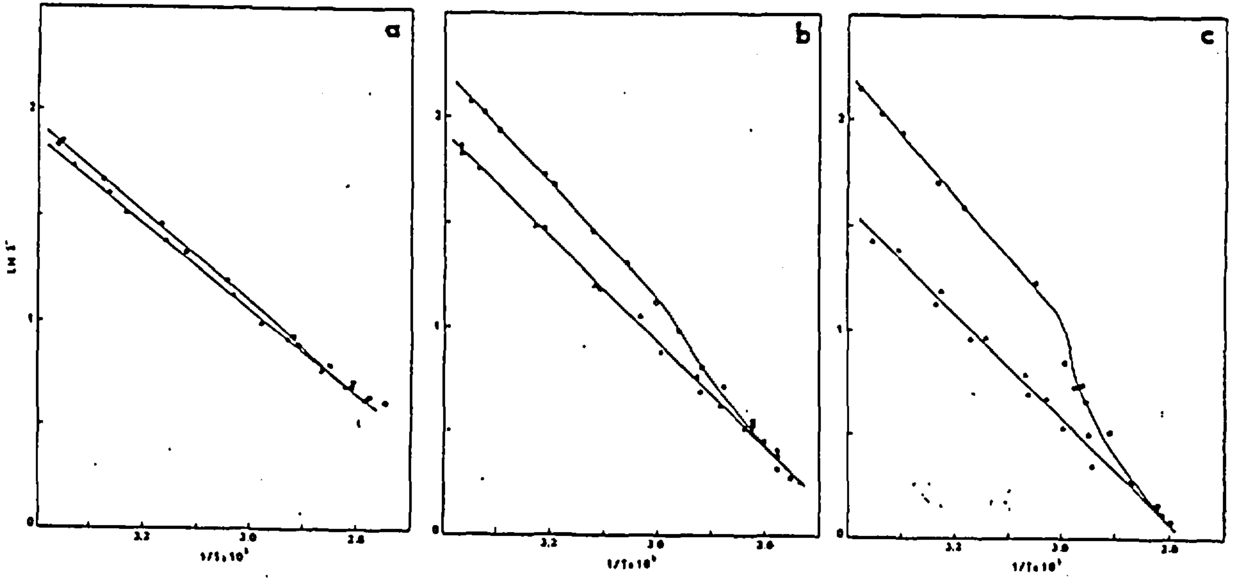


FIGURE 57. Liquid chromatographic retention (log k′) of resorcinol plotted as a function of temperature for phases of different loadings. Curved plots resulted for the first temperature runs; subsequent temperature runs were linear. (a) Low carbon phase; (b) moderate carbon phase; (c) high carbon phase. (Reproduced from Gilpin, R. K. and Squires, J. A., *J. Chromatogr. Sci.*, 19, 915, 1981. With permission.)

by the aqueous environment. Upon heating, sufficient energy is imposed on the phase to overcome this association and cause chain extension. Water molecules are forced between the chains and prevent chain reorganization (collapse) after cooling. Thus, retention is altered for the rerun portion of the experiment. The collapsed orientation can be regenerated by equilibration with acetonitrile followed by water. Apparently, the acetonitrile removes water molecules trapped between the alkyl ligands. The on-set temperature is strongly dependent on alkyl chain length, but only slightly dependent on phase loading. On the other hand, the difference in retention for the collapsed and extended-phase orientations (the "percent break") is strongly affected by the phase loading. The on-set temperatures of the C_8, C_9, and C_{10} phases were determined to be 40.7, 51.8, and 60.1°C, respectively. The role of the conditioning solvent was further examined.[275] Changes in the retention of collapsed phases were found to be caused by choice of the organic solvent used prior to the aqueous mobile phase. The greatest percent break was observed after conditioning the phase with acetonitrile. It was concluded that the organic solvent is trapped in the collapsed phase, and only after extended conditioning with water is the trapped solvent removed. Actually, the organic phase is removed in two steps corresponding to separate kinetic events.

Hammers and Verschoor[276] verified this type of orientational change in phase structure for LiChrosorb RP-18. Unlike the work of Gilpin and Squires,[273] nonlinear Van't Hoff plots were obtained even below the on-set temperature. An interesting experiment was carried out to demonstrate the dynamic nature of the phase. Solutes were injected onto the C_{18} column at elevated temperatures, and the mobile phase flow was stopped. The column was cooled and the solute was eluted. Again the flow was stopped and the column was heated in excess of the original temperature. Mobile phase flow was resumed and additional solute elution was observed. About 20% of the injected solute was found to be trapped by reorientation of the bonded phase due to cooling.

Using adiabatic calorimetry, van Miltenburg and Hammers[248] observed second-order transitions for C_8 and C_{18} bonded phases over the range of 80 to 300 K and 150 to 305 K, respectively. The authors concluded that the transitions were similar to melting processes in solids, but with reduced transition enthalpy and entropy values compared to solids.

Dawidowicz and co-workers[41,277] examined phase transition in C_{18} bonded phases prepared on controlled pore glasses (CPG). Prior to phase synthesis the CPG samples were heat treated at 700°C for various lengths of time to increase the concentration of boron atoms at the glass surface. Phases prepared on these substrates had relatively high surface coverages, and ligand density increased with the heat treatment procedure. The authors concluded that a significant fraction of the alkyl chains was bonded through B-OH groups. The CPG bonded phases were characterized using the inverse gas chromatography technique of Morel and Serpinet.[266] No evidence of phase transitions was observed in any of the heat-treated CPG samples, however, one untreated C_{18} CPG sample did exhibit such a transition. Since the ligand concentration of the heat-treated CPG samples was similar to the C_{18} phases of Morel and Serpinet (i.e., 4.1 to 5.0 μmol/m^2 vs. 4.1 to 4.6 μmol/m^2, respectively), the absence of phase transitions suggests that chain organization for CPG phases is significantly different than for silica-based phases. This difference is thought to result from the presence of B-OH sites as well as Si-OH sites for surface modification. Inverse gas chromatography was used by Rayss[278] to study carbon-silica adsorbents coated with *n*-octadecanol. Discrimination between film coatings on carbon, and on uncovered silica, was demonstrated.

D. Homologous Series

The study of the retention behavior of homologous series of solutes offers an interesting system for the investigation of stationary and mobile phase effects in reversed-phase LC. For a homologous series a linear relationship exists between log k′ and the number of carbon atoms (n_c) in the alkyl chain of the solute.[279-283] Even though there is no rigorous thermo-

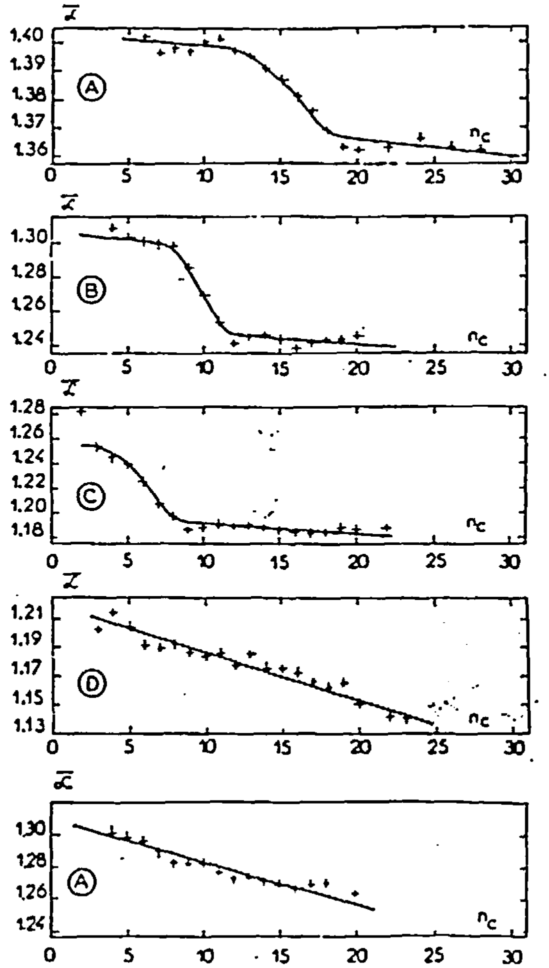


FIGURE 58. Upper figure: mean selectivity plotted as a function of solute chain length n_c on various monomeric phases. (A) C_{18}, (B) C_8, (C) C_6; (D) C_1. Lower figure: as above, but for μBondapak C_{18}. (Reprinted from Tchapla, A., Colin, H., and Guiochon, G., *Anal. Chem.*, 56, 621, 1984. With permission.)

dynamic reason to justify this linear behavior,[283] it has been investigated extensively, particularly by Guiochon and co-workers,[281-284] to determine column dead volume (as discussed later), to evaluate solvent elutropic strength,[281,282] and to optimize separations.[284]

A recent study by Tchapla et al.[283] revealed a discontinuity in the log k′ vs. n_c plots of homologous series occurring at a critical carbon number corresponding to the length of the organic ligand of the stationary phase. The authors pointed out that this discontinuity is not pronounced and requires that a sufficient number of experimental points must be obtained below and above the break point. Since the logarithmic function tends to have a leveling effect, it was difficult to observe a discontinuity in the log k′ vs. n_c plots. However, they found that α vs. n_c plots revealed subtle details. Plots of α vs. n_c for C_{18} (LiChrosorb RP 18), C_8 (LiChrosorb RP 8), C_6 (Spherisorb C_6), and C_1 (LiChrosorb RP 2) are illustrated in Figure 58. Each α value is the mean value obtained for five homologous series, i.e., *n*-alkanes, *n*-alkychlorides, *n*-methylesters of carboxylic acids, *n*-alcohols, and 2-*n*-alkanones. These plots indicate that a particular phenomenon occurs, in all but the C_1 case, at a carbon number depending on the bonded phase chain length, i.e., at around 12 to 18 for C_{18}, 12 to 14 for C_{14}, 8 to 11 for C_8, and 4 to 8 for the C_6 phase. All of these phases were denoted as "monomeric" by the authors. A similar plot for another C_{18} material (μBondapak C_{18}) revealed no discontinuity. The authors explained that this C_{18} phase was "polymeric" based on the method of preparation, although they had received contradictory information on the nature of this phase. However, others have reported this material to be a low, loaded monomeric phase.[285] The authors suggested that these results indicate that the bonded phase structure plays a critical role. With monomeric phases, the alkyl chains of the solute penetrate into the "fur" of the bonded bristles via their alkyl chains. As the length of the alkyl chain of the homologs is shorter than that of the bonded phase, an increase in the number of methylene groups in the solute results in a constant increment in the dispersive forces associated with the solute-ligand complexes. As the length of the solute alkyl chain exceeds that of the bonded phase, a certain number of methylene groups are "out" of the stationary phase, resulting in weaker dispersive interaction with the stationary phase and, thus, less retention than expected. For the C_1 phase, no penetration of the solute can occur and the solutes lie on the surface of the phase.

For the so-called "polymeric" phase, they postulated that since a complex network exists, the chains lose their individual identities. When a solute molecule penetrates this network, its alkyl chain is "intertwined" within the polymeric network, and, therefore, there is no reason to observe any particular phenomenon with increasing solute chain length. The mechanism described above is reasonable if the designation of monomeric and polymeric phases is correct. However, some doubt exists as to whether the μBondapak C_{18} is monomeric or polymeric; in addition, the LiChrosorb RP materials have been described as being prepared using dichlorosilanes (see Table 7) and, thus, would be designated by some workers as "polymeric". It would be of interest to evaluate the behavior of the homologous series on "true" polymeric phases, i.e., those prepared with di- or trichlorosilane with the addition of water to the synthesis as described earlier.

E. Solute Shape Selectivity

The retention model defined by the hydrophobic effect adequately accounts for many of the trends observed in reversed-phase liquid chromatography. For example, hydrophobic retention theory accurately predicts the dependence of solute retention on mobile phase composition and on homologous series length. Stationary phase contributions to selectivity, however, are less easily explained. Separations of structural isomers and other closely related molecules are routinely carried out on reversed-phase C_{18} columns, even though the underlying solute-stationary phase interactions that induce such separations are not well understood. It is clear that both shape and polarity of the solute must be considered in evaluating retention. The effect of stationary phase shape on selectivity has been discussed in earlier sections (see Section II.A and II.D).

Wise and co-workers[86] have studied extensively the retention and selectivity of polycyclic aromatic hydrocarbons (PAH) on monomeric and polymeric C_{18} columns. Retention data were presented in the form of retention indices calculated as described by Popl et al.:[286]

$$\log I_x = \log I_n + \frac{\log R_x - \log R_n}{\log R_{n+1} - \log R_n} \tag{13}$$

where I_x = retention index for solute, I_n = retention index for standard n, R_x = corrected retention volume (retention volume − void volume) for solute, and R_n = corrected retention volume for first (or second, n + 1) standard. This definition of retention indices for liquid chromatography is similar to the Kováts retention index used in GC. Wise et al.[86] selected PAH standards on the basis of overall shape, and with a concern that most *n*-ring PAH

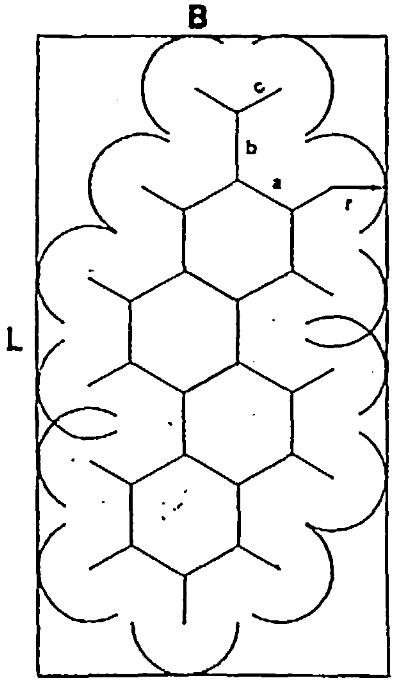


FIGURE 59. Determination of the length-to-breadth ratio for 2-methylchrysene. Bond distances are as follows: (a) 1.4 Å (C-C aromatic); (b) 1.54 Å (C-C aromatic to aliphatic); (c) 1.1 Å (C-H single bond); (r) 1.2 Å. (From Wise, S. A., Bonnett, W. J., Guenther, F. R., and May, W. E., *J. Chromatogr. Sci.*, 19, 457, 1981. With permission.)

solutes should elute between the standards designated n and n + 1. The following standards were assigned: (1) benzene, (2) naphthalene, (3) phenanthrene, (4) benz[a]anthracene, (5) benzo[b]chrysene, and (6) dibenzo[a,h]pyrene. Because the choice of these standards is somewhat arbitrary, retention indices calculated using Equation 13 should only be used for comparison of solutes eluting between the same pair of standards. Wise et al. found that compounds with log I values differing by about 0.07 units were baseline resolved for a 10-µm particle diameter, 25-cm column. With higher efficiency columns, baseline separations resulted at even smaller log I differences.

A shape parameter known as the length-to-breadth (L/B) ratio was calculated for each of the PAH studied and correlated with reversed-phase retention data.[86] Janini et al.[287-289] first studied the correlation of PAH shape with gas chromatographic retention on liquid crystal stationary phases. Radecki et al.[289a] calculated a shape parameter denoted "η", defined as the length-to-breadth ratio of a rectangle of minimum area drawn about the molecule. Wise and co-workers[86] calculated L/B in a slightly different way. Instead of calculating L/B from a rectangle of minimum area, an algorithm was developed based on L/B maximization (see Figure 59). In general, when isomeric PAH are compared, the LC retention increases with increasing L/B ratio. Retention data and L/B ratios for a number of groups of PAH isomers are summarized in Table 9. The reversed-phase LC separation of 11 isomers of molecular weight 278 is shown in Figure 60. The position of the methyl group on a PAH can significantly change the L/B ratio for the solute. A plot of log I vs. L/B for methyl-substituted benzo[c]phenanthrene, benz[a]anthracene, and chrysene isomers is shown in Figure 61 (correlation coefficient 0.936). Better correlation with LC retention was found for L/B calculated

Table 9
SUMMARY OF LENGTH-TO-BREADTH RATIOS AND REVERSED-PHASED LC RETENTION FOR UNSUBSTITUTED PAH[11]

			LC retention (log I)	
Compound	MW	L/B	Polymeric	Monomeric
Phenanthrene	178	1.46	3.00	3.00
Anthracene	178	1.57	3.20	3.14
Fluoranthene	202	1.22	3.37	3.43
Pyrene	202	1.27	3.58	3.65
Benzo[c]fluorene	216	1.34	3.49	3.64
Benzo[a]fluorene	216	1.68	3.72	3.76
Benzo[b]fluorene	216	1.78	3.84	3.77
Triphenylene	228	1.12	3.70	3.83
Benzo[c]phenanthrene	228	1.22	3.64	3.91
Benz[a]anthracene	228	1.58	4.00	4.00
Chrysene	228	1.72	4.10	3.99
Naphthacene	228	1.89	4.51	—
Benzo[e]pyrene	252	1.12	4.28	4.48
Perylene	252	1.27	4.33	4.50
Benzo[a]pyrene	252	1.50	4.53	4.58
Benzo[a]fluoranthene	252	1.16	4.22	4.45
Benzo[j]fluoranthene	252	1.39	4.24	4.37
Benzo[b]fluoranthene	252	1.40	4.29	4.46
Benzo[k]fluoranthene	252	1.48	4.42	4.52
Benzo[ghi]perylene	276	1.12	4.76	5.36
Anthanthrene	276	1.35	5.08	5.61
Indeno[1,2,3-cd]pyrene	276	1.40	4.84	5.23
Indeno[1,2,3-cd]fluoranthene	276		4.93	5.05
Dibenzo[c,g]phenanthrene	278	1.12	4.07	4.51
Dibenz[a,c]anthracene	278	1.24	4.40	4.73
Benzo[g]chrysene	278	1.32	4.27	4.71
Dibenzo[b,g]phenanthrene	278	1.33	4.33	4.80
Benzo[c]chrysene	278	1.47	4.45	4.85
Dibenz[a,j]anthracene	278	1.47	4.56	4.84
Pentaphene	278	1.73	4.67	4.96
Benzo[a]naphthacene	278	1.77	4.99	4.50
Dibenz[a,h]anthracene	278	1.79	4.73	4.86
Benzo[b]chrysene	278	1.84	5.00	5.00
Picene	278	1.99	5.18	5.02

in this way than for the shape parameter η. In addition, C_{18} phases were found to provide a better correlation of retention (log I) and L/B than were monomeric phases. Since most researchers agree that gas chromatographic separations on liquid-crystalline phases are based to some degree on the shape of the solute, Wise et al.[290] recently compared relative retention measurements on polymeric C_{18} phases in reversed-phase LC and on liquid crystalline polysiloxane phases in capillary gas chromatography. Similar behavior was observed in both chromatographic systems and anomalies in elution order were generally found to be consistent in both systems.

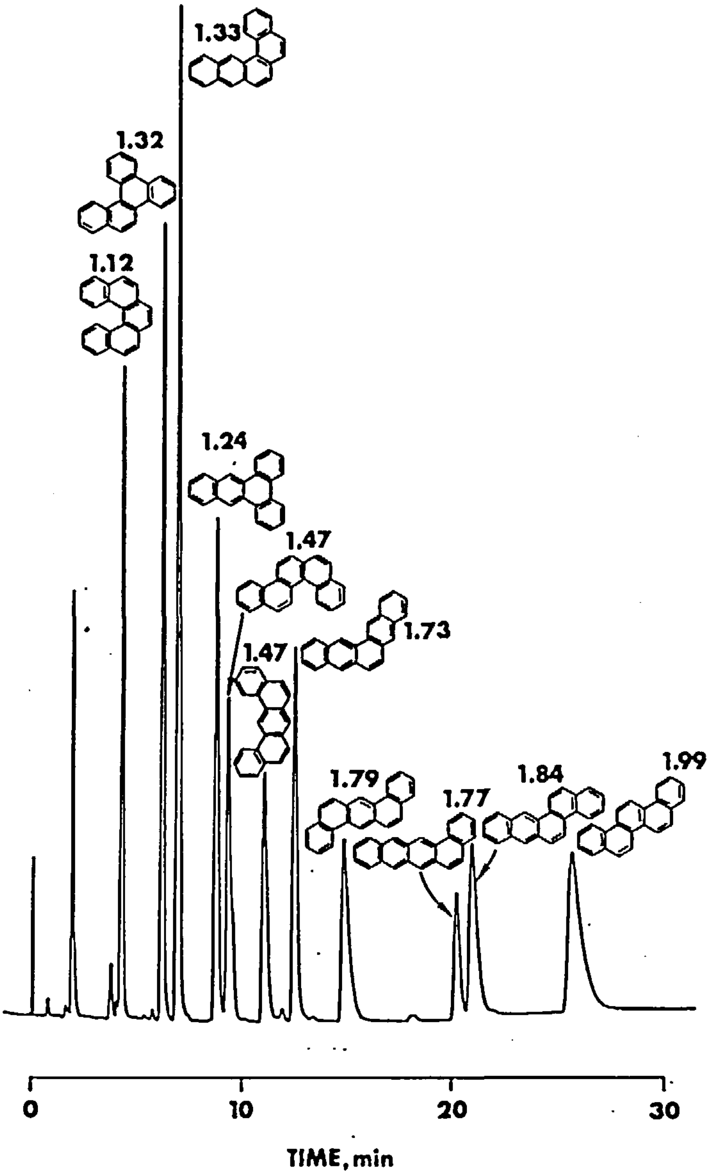


FIGURE 60. Separation of polycyclic aromatic hydrocarbon isomers (MW 278) on a wide pore, heavily loaded polymeric C_{18} column. (From Wise, S. A. and Sander, L. C., *J. High Res.*, *Chromatogr. Chromatogr. Comm.*, 8, 248, 1985. With permission.)

Sander and Wise[35] compared monomeric and polymeric C_{18} phases synthesized on wide-pore substrates for the separation of PAH. In general, better overall separations of complex PAH mixtures were observed with polymeric C_{18} phases. Column selectivity was found to be related to phase density, while absolute retention was more dependent upon the total amount of carbon contained within the column. The influence of phase density on selectivity was examined in more detail by Wise and May.[162] Plots of α_{BaP} vs. k'_{BaP} (where k'_{BaP} was related to the C_{18} surface coverage) were linear and selectivity was usually greatest on the densely loaded polymeric phases (see Figure 62). The separation of 11 PAH isomers of molecular weight 278 on four C_{18} polymeric phases with varying surface coverage is illus-

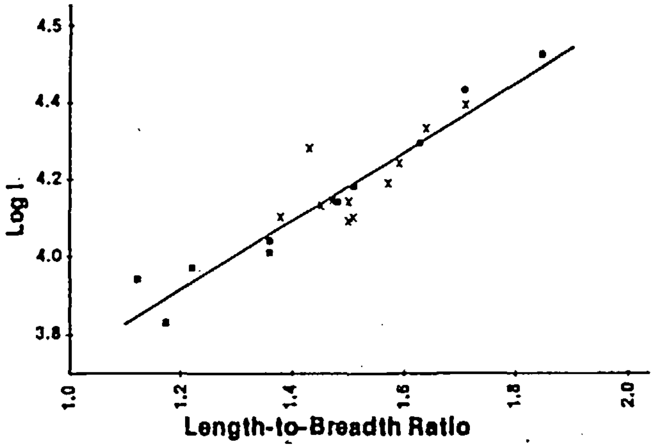


FIGURE 61. Linear correlation of length-to-breadth ratio with LC retention (log I) for (■) methyl-substituted benzo(c)phenanthrenes; (x) benz(a)anthracenes; (●) chrysenes. (From Wise, S. A., Bonnett, W. J., Guenther, F. R., and May, W. E., *J. Chromatogr. Sci.*, 19, 457, 1981. With permission.)

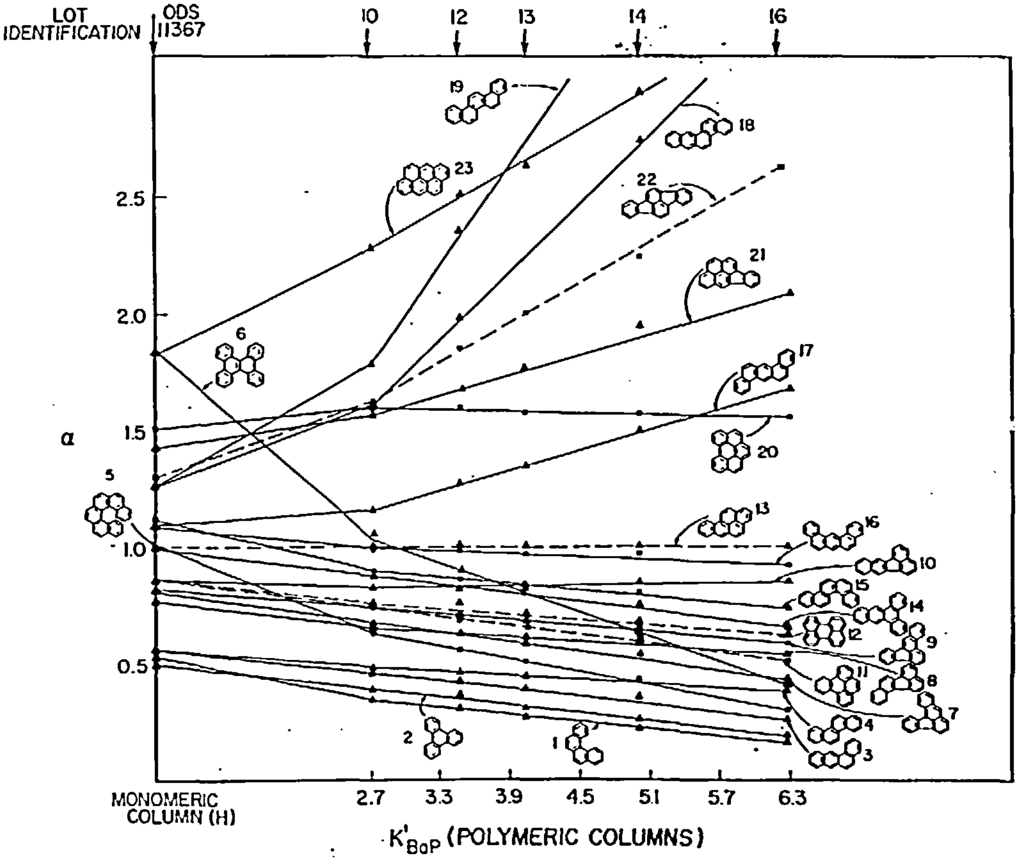


FIGURE 62. Column selectivity factors (α, k'_{solute}/k'_{BaP}) vs. k'_{BaP}) vs. k'_{BaP} for monomeric and polymeric C_{18} phases. The position of the monomeric phase values on the x axis is arbitrary. (From Wise, S. A. and May, W. E., *Anal. Chem.*, 55, 1479, 1983. With permission.)

Table 10
SELECTIVITY FACTORS (α) FOR SELECTED PAH ON MIXED VS. COUPLED COLUMNS[163]

Solute	Mixed	Coupled
m-Tetraphenyl	0.288	0.291
Phenanthro[3,4-c]phenanthrene	0.436	0.438
9,10-Diphenylanthracene	0.454	0.459
m-Quinquephenyl	0.557	0.564
Tetrabenzonaphthalene	0.676	0.676
Dibenz[a,c]anthracene	0.728	0.730
Dibenz[a,j]anthracene	0.939	0.941
Dibenz[a,h]anthracene	1.42	1.43

trated in Figure 27. Overall selectivity toward PAH is easily seen to increase with phase density.

Modification of polymeric phase selectivity for PAH has been approached in two ways.[163] Polymeric bonded phases of different phase densities were combined by (1) blending the bulk packing materials in various proportions, and (2) coupling columns of various lengths. Selectivity of the blended and coupled columns was determined for a variety of representative PAH (see Table 10). Selectivity factors for the two-column systems were the same within experimental error, and overall selectivity was intermediate to that of the individual phases. This study demonstrates an important concept: solute retention for linked columns is additive. Selectivity appears to be related to a weighted average of the individual phase selectivities (although small variations from this relation were noted). Thus, these results suggest that "custom columns" of desired selectivity can be created without the difficulty of phase synthesis and column packing by combining short columns of differing selectivity.

The origins of selectivity differences occurring among different types of C_{18} phases have been reviewed and summarized by Sander and Wise.[11,291] An extensive data set of retention indices was presented for over 150 PAH on monomeric and polymeric C_{18} phases under various conditions. This reference set has proven useful, not only in understanding trends in solute retention, but also in more practical aspects of component identification and prediction of elution order. Because the elution order of certain PAH change with phase type, this suggests the possibility of using carefully selected PAH probes to characterize phases as monomeric or polymeric. Such a mixture has been proposed (see Section III.A), consisting of benzo[a]pyrene (BaP) phenanthro[3,4-c]phenanthrene (PhPh), and 1,2:3,4:5,6:7,8-tetrabenzonaphthalene (TBN).[35] For monomeric C_{18} phases, the elution order is BaP, PhPh, and TBN; while for polymeric C_{18} phases, the order is PhPh, TBN, and BaP. Columns of intermediate selectivity have the elution order PhPh, BaP, and TBN (see Figure 28). The vast majority of commercial C_{18} columns can be classified as monomeric using this criterion. This simple empirical classification is useful for rapid screening of columns for suitability in the separation of more complex PAH mixtures.

The unusual retention behavior of these three components is believed to be due, at least in part, to the planarity (or lack thereof) of the probe molecules. BaP is planar, while PhPh is nonplanar. Due to steric hindrance of opposing hydrogens, PhPh is forced to conform to a helical shape (hence, the alternate name, hexahelicene). TBN is thought to conform to a saddle shape (alternate benzene rings above and below the plane of the molecule), but this has not been proven. To examine the influence of solute structure on retention, a series of planar and nonplanar PAH with similar overall shape was selected as probes[164] (see Figure 63). Linear and nonlinear isomers were also included. In each case, planar and linear PAH were retained longer than nonplanar and nonlinear analogs. Furthemore, phase selectivity

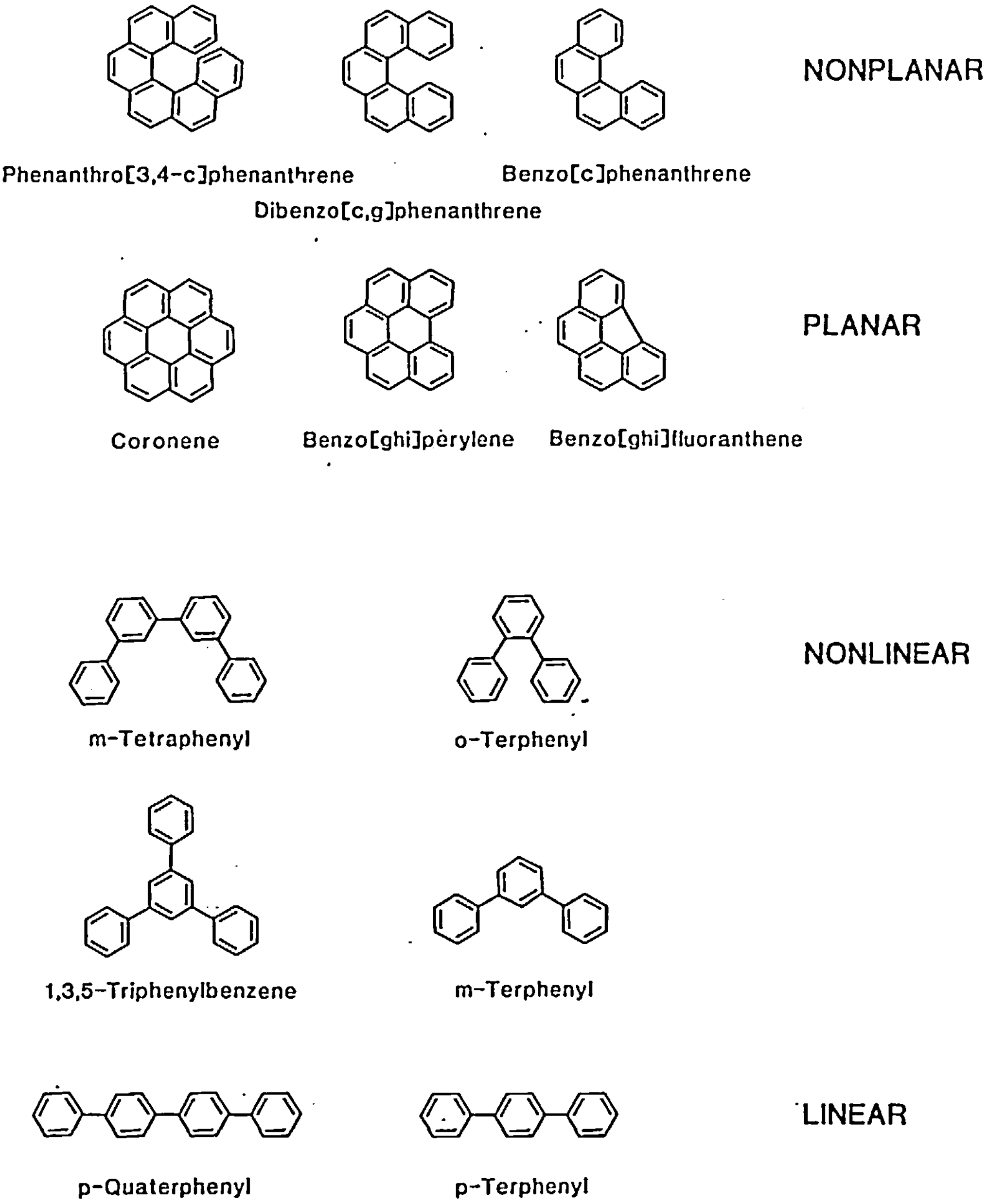


FIGURE 63. Structures of planar/nonplanar and linear/nonlinear PAH pairs. (From Wise, S. A. and Sander, L. C., *J. High Res. Chromatogr. Chromatogr. Comm.*, 8, 248, 1985. With permission.)

factors $\alpha_{planar/nonplanar}$ and $\alpha_{linear/nonlinear}$ increased with increasing polymeric nature of the phase (see Table 11). Stated differently, the greatest shape discrimination resulted from heavily loaded polymeric phases. As an example, the selectivity factor α for *p*-quaterphenyl/1,3,5-triphenylbenzene was 1.5 for the monomeric C_{18} phase, while for the polymeric phase α was 46, a factor of over 30 difference!

Wise and Sander[164] have proposed a schematic model of solute retention referred to as the "slot model" that qualitatively explains trends observed for various PAH on monomeric and polymeric phases. The stationary phase is envisaged as a surface with a number of narrow "slots" into which the solute molecules can penetrate (see Figure 64). Planar mol-

Table 11
PLANAR/NONPLANAR SELECTIVITY COEFFICIENTS[164]

	85% Acetonitrile/water				100% Acetonitrile			
	Monomeric	Polymeric			Monomeric	Polymeric		
		Low	Normal	Heavy		Low	Normal	Heavy
Compounds								
p/*m*-Terphenyl	1.2	1.3	2.1	3.0	1.2	1.3	2.3	3.5
p/*o*-Terphenyl	1.3	1.7	2.6	3.8	1.4	1.5	2.9	4.3
p/*m*-Tetraphenyl	1.4				1.4	4.4	11.7	34.5
p/1,3,5-Triphenylbenzene	1.5				1.5	3.0	14.2	46.0
Benzo[ghi]fluoranthene/ Benzo[c]phenanthrene	1.1	1.2	1.4	1.6	1.2	1.4	1.7	1.8
Benzo[ghi]perylene/ Dibenzo[c,g]phenanthrene	1.8	2.8	4.5	4.1	2.1	3.3	7.8	7.5
Coronene/ Phenanthro[3,4-c]phenanthrene	2.7	5.1	9.6		3.4	6.2	12.1	17.1

Note: Selectivity coefficient = k′ ratio of planar/nonplanar or linear/nonlinear solutes.

ecules would be able to fit into such slots more easily than nonplanar molecules, and in so doing would interact with the stationary phase more strongly than nonplanar molecules. It can further be postulated that long, narrow molecules would penetrate more deeply into the phase than square molecules; since even planar, square solutes would have restricted access to narrow slots. Viewed in this way, the property of nonplanarity is simply an extension of the molecular descriptor of length to breadth to include a thickness parameter. The fact that polymeric C_{18} phases are more shape selective than monomeric phases suggests that polymeric phases are more rigid or more ordered than monomeric phases.

In their "Unified Theory of Retention and Selectivity", Martire and Boehm[292] presented a rigorous mathematical description of reversed-phase retention that included contributions from such effects as bonded phase length, chain stiffness, surface coverage, and mobile phase composition. Aspects of solvophobic retention theory are included in this model, but unlike solvophobic retention, the dependence of solute retention on bonded phase variables is specifically addressed. The bonded phase is viewed as "quasi-liquidlike", and chain conformation is thought to vary with changes in mobile phase composition. The picture advanced is of a bonded phase surface that "breathes" depending on the polarity of the environment. Interestingly enough, methanol and acetonitrile, and water mixtures of each, appear to induce near collapse of alkyl ligands. On the other hand, theory seems to indicate that tetrahydrofuran causes partial extension of the chains. This predicted change in phase conformation may explain certain selectivity changes observed when THF is used in the mobile phase. The question of the effect of solute shape on retention has also been examined by Martire and Boehm.[292] Solutes were classified into three groups: rigid-rod, flexible chain, and cubic (globular) solutes. For both collapsed and extended phase models the predicted order of elution for molecules of roughly the same size is cubic < flexible chain < rigid rod. This is the same order observed by Wise and Sander[164] for linear and nonlinear solutes and is consistent with observations for planar and nonplanar molecules. In the collapsed chain limit, shape selectivity increases with phase density, as rodlike solutes are preferentially retained over flexible chain solutes on the more ordered bonded phase. Bonded phases

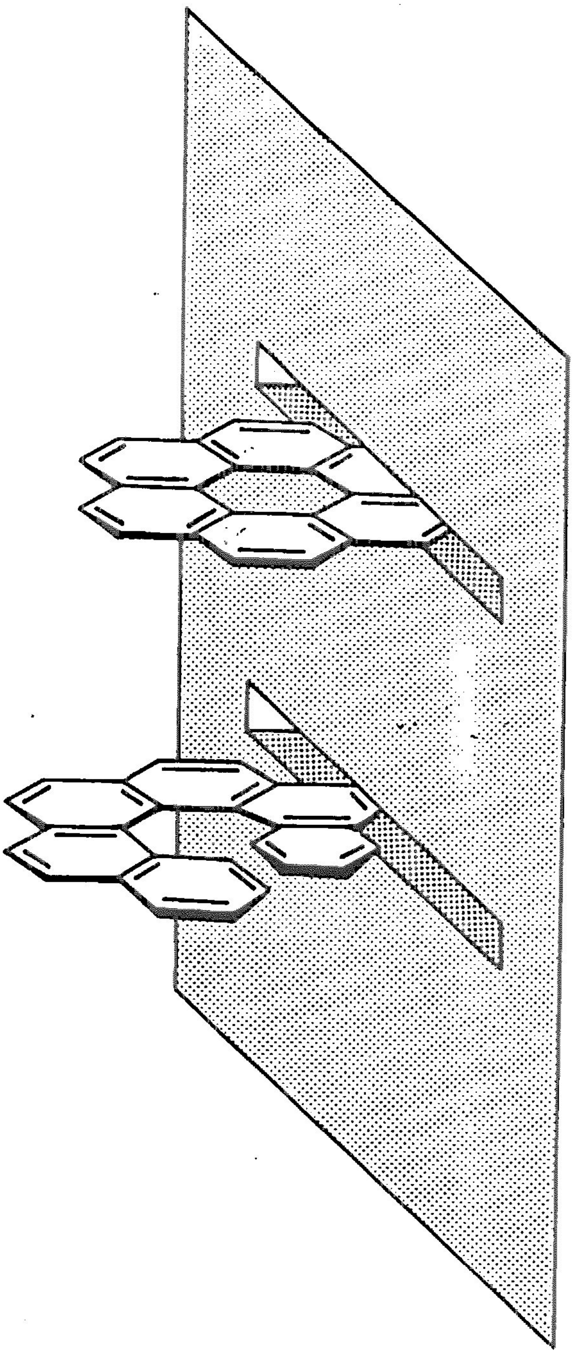


FIGURE 64. Schematic representation of the "slot model" for retention of PAH on polymeric C_{18} stationary phases. (From Wise, S. A. and Sander, L. C., *J. High Res. Chromatogr. Chromatogr. Comm.*, 8, 248, 1985. With permission.)

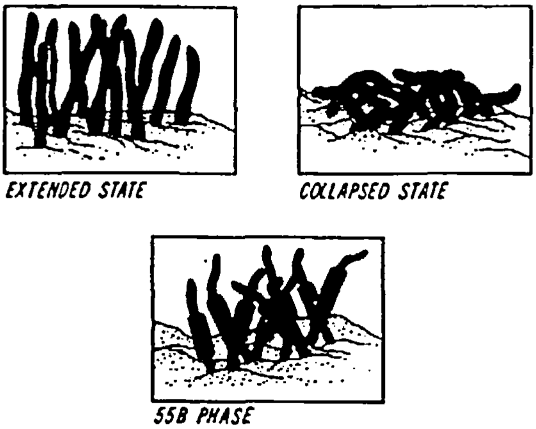


FIGURE 65. Pictorial representation of extended and collapsed states of bonded alkyl chains. The 55B phase is represented with a rigid cylinder (biphenyl) inserted midway along the alkyl chain. (Reprinted from Lochmüller, C. H., Hunnicutt, M. L., and Mullaney, J. F., *J. Phys. Chem.*, 89, 5770, 1985. With permission.)

prepared with rigid bonded ligands may exist in the extended conformation. Such phases are expected to exhibit additional shape selectivity over collapsed phases, because penetration of rodlike molecules between the protruding ligands is favored over penetration by globular solutes. This theory, when applied to the work of Sander and Wise on polymeric phases, suggests that the polymeric C_{18} phases are denser, more rigid, and/or more ordered than are monomeric C_{18} phases.

Lochmüller and co-workers[292a] prepared an extended, rigid bonded phase based on 4-4'-dipentylbiphenyl ligands to test the predictions of the model of Martire and Boehm.[292] The phase, shown schematically in Figure 65, is represented as a rigid cylinder with flexible ends. The "unified theory" of Martire and Boehm predicts that such a rigid phase should be more selective toward rodlike solutes than conventional alkyl phases, which, under reversed-phase conditions, are believed to exist in the collapsed state. Lochmüller et al. found that rodlike molecules (e.g., *p*-terphenyl) were strongly retained by the dipentylbiphenyl phase. The shape selectivity enhancement observed for this phase compared to the C_{18} alkyl phase was greatest for rodlike solutes, intermediate for platelike solutes (e.g., planar PAH), and least for alkyl solutes. These trends are in accord with proposed theory. The difference in shape selectivity between the phases was observed to decrease with increasing organic content of the mobile phase. This suggested to the authors that alkyl chain extension and ordering occur as the phase becomes better solvated, thus, increasing phase shape selectivity.

F. Void Volume Determination

The elution volume for an unretained component is termed the column void volume (also, "dead volume" and "hold-up volume"). The measurement of this quantity may at first appear trivial; however, theoretical considerations concerning void volume determination have made the subject controversial. Methodology can be addressed at two levels. From a practical point of view, the question of void volume determination is not demanding. All that is usually required is a technique that is reproducible and that gives results that approximate the "true" void volume. Small errors in the void volume determination will give rise to errors in k' that will also be small if solute retention is moderate. Relative comparisons of k′ values (such as selectivity factors) so calculated are only slightly affected by inaccuracies. However, from a theoretical point of view, the basis of the void volume determination is fundamental to the assessment of thermodynamic properties. The capacity factor k′ is defined as:

$$k' = g_s/g_m \tag{14}$$

where g_s = the mass of solute in the stationary phase (zone) at equilibrium, and g_m = the mass of solute in the mobile phase (zone) at equilibrium. It follows that g_s for an unretained solute (k′ = 0) must be zero. The definition of void volume, in turn, relies on the definitions of the stationary and mobile phase zones. The situation is complicated when binary or ternary solvent systems are employed, since layers of organic constituents are absorbed at the bonded phase surface. Kováts et al.[293] have reported that, in fact, it is not possible to experimentally measure the "true" stationary phase volume unless the division between the mobile and stationary phase zones can be precisely defined. They recommend use of an imaginary Gibbs' dividing plane fixed by convention. It is easy to see how thermodynamic parameters depend on these definitions. The capacity factor is related to the equilibrium coefficient (K) by the following relation:

$$k' = K\phi \tag{15}$$

where $\phi = V_s/V_m$, the phase ratio, V_s = volume of the stationary phase zone, and V_m = volume of the mobile phase zone. It can be further shown that

$$\ln k' = -\Delta H/RT + \Delta S/R + \ln \phi \tag{16}$$

where ΔH = enthalpy of transfer, mobile phase to stationary phase; ΔS = entropy of transfer, mobile phase to stationary phase; T = absolute temperature, K; and R = gas constant. A plot of ln k′ vs. 1/T (Van't Hoff plot) is commonly used in the assessment of enthalpies and entropies of transfer. Both quantities are dependent on the definition and methodology of void volume determination; however, entropies of transfer are doubly affected by T_0 and by the phase ratio term, ln φ. For these reasons, it is usual to report relative (rather than absolute) entropies of transfer.

Several independent approaches have been taken in the assessment of column void volume. A review of such techniques is beyond the scope of this paper; however, for completeness these technques will be described briefly and applicable references cited. Smith and coworkers[294] recently reviewed much of the progress to date concerning the determination of column void volume. Methods for the determination of column void volume have been compared by several research groups.[295-299] The various approaches can be grouped into the following categories: (1) elution of small, unretained UV absorbing solutes;[295,297-299] (2) elution of labeled mobile phase components;[297,299,300] (3) linearization of homologous series;[295,297,298,301-303] (4) static column measurements;[295,297] (5) step gradients;[296] and (6) isotherm analysis.[296,304]

The first approach involving the elution of unretained solutes is the most expedient method for estimating the void volume, and may be adequate for practical uses. For thermodynamic studies, however, assurance cannot be given on a theoretical basis that the measured value is representative of the true void volume. The observation that a given test probe appears to give the lowest retention volume does not justify its use as a void volume marker. Indeed, it is quite possible for a species to elute before the void volume if the species is excluded from the substrate pores.

The elution of labeled mobile phase components is more useful for theoretical studies, but certain problems still exist. The elution of deuterium- or tritium-labeled eluent is expected to reflect the void volume for systems using single component mobile phases, e.g., pure methanol or pure water. The situation is more complex for binary or trinary solvent systems in which preferential adsorption and displacement effects can occur. An additional problem is difficulty in detecting the labeled component. It has been shown that the separation of deuterium-substituted PAH and-unsubstituted PAH is possible in reversed-phase systems.[305,306] In light of these studies, deuterium (or tritium) substitution on small molecules might be expected to alter retention making this technique invalid.

Plots of ln k′ vs. chain length for homologous series are, as a rule, linear for reversed-phase retention. A method has been proposed for the determination of column void volume that is based on the assumption that this linear relation must be valid. The value of T_0 that results in the best linear fit is assumed to reflect the true void volume. While there is some theoretical basis for the assumption that ln k′ vs. chain length plots must be linear, this assumption has not been proven.

Static void volume determinations are carried out by weighing the column filled with solvents of different density. From the weight difference of the columns and knowing the densities of the solvents, a void volume can be calculated. Guiochon and co-workers[297] have reported that due to adsorbed mobile phase layers, this method does not result in the correct void volume. It does, however, appear to give an upper limit to this value.

Column void volumes have also been estimated by measuring the elution volume after switching the composition of the mobile phase. This approach has been referred to as the step gradient or breakthrough method. Complications arise from the adsorption or displacement of mobile phase components during the solvent switch. A related technique involves the measurement and extrapolation of isotherms for solvent components.[296,304]

V. CONCLUSIONS

The objectives of bonded phase research can be generalized as the increased understanding of physical, chemical, and supramolecular properties of bonded phases, and as the knowledge of interaction processes that occur during solute retention. These goals should ultimately permit the design of new types of stationary phases tailored for specific separation problems. To a large extent the progress and refinements reflected by the current state of bonded phase technology have been made possible only by such studies. Indeed, "basic research" has led to the commercial development of specialized columns for chiral separations and for direct serum analysis, as well as more general purpose columns based on immobilized cyclodextrin molecules.

This review has endeavored to present some of the more important advances made in bonded phase research during the past decade. A few observations about the physical and chemical properties of bonded phases and about retention behavior are listed below.

A. Physical and Chemical Properties

1. Distinct types of silanols exist at the silica surface with different reactivities toward silanes.
2. Alkyl chain mobility of immobilized ligands varies under a variety of conditions.
   A. Mobility increases with the distance from the point of attachment.
   B. Mobility decreases with increasing phase density.
   C. Mobility decreases in the presence of water.
   D. Mobility decreases after endcapping.
   E. Chain motion of bonded ligands differs significantly from bulk liquids.
3. Alkyl chain conformations of immobilized ligands reflect a state of disorder similar

to bulk liquids. Unlike liquids, however, conformational disorder is present even at low temperatures. Formation of an ordered crystal lattice (as with paraffin solids) is precluded by loss of freedom from immobilization. Chain order decreases with increasing temperature.

4. Surface modification appears to be inhomogeneous, i.e., with regions of high and low ligand density. Some evidence exists supporting a "patch" model of surface modification.
5. Substrate surface area values, as determined by nitrogen adsorption techniques, decrease after modification with nonpolar ligands. This decrease is due, in part, to a change in the nitrogen adsorption energy for the nonpolar surface.
6. Apparent surface area values for alkyl modified substrates decrease as a function of overall carbon loading. This trend appears to be independent of alkyl length, ligand density, and phase type.
7. The pore structure of silica substrates is completely accessible to solvents (no closed pores). The pore network remains accessible to wetting solvents after surface modification.
8. The reaction of di- or trifunctional silanes with silica will result in a net gain of silane silanol groups due to silane hydrolyss. Endcapping is effective and desirable for such phases.
9. Phases produced from monofunctional silanes with moderate to high ligand densities do not benefit greatly from endcapping. C_{18} ligand cleavage occurs to some extent during endcapping reactions.
10. The choice of TMCS or HMDS for endcapping will affect the end properties of the phase, especially for the retention of basic and acidic solutes. It is best to use TMCS for acidic or neutral solutes, and HMDS for basic solutes.
11. Phase stability appears to increase with chain length, e.g., C_{18} phases and more stable to cleavage than C_8 phases. Phase hydrolysis and cleavage for aromatic phases are troublesome, since they result in a high UV background and/or artifact peaks.
12. Polymeric C_{18} phases have significantly different selectivity toward PAH and other solutes than monomeric C_{18} phases. The efficiency of polymeric C_{18} phases prepared on totally porous, wide-pore silica substrates is not significantly different than the efficiency of monomeric C_{18} phases. Many separations not possible with monomeric C_{18} are easily performed with polymeric C_{18} phases.

B. Retention Behavior

13. In general, "like retains like."
    - A. Branched solutes are preferentially retained by branched BPs.
    - B. Planar solutes are preferentially retained by planar BPs.
    - C. Fluorinated solutes are preferentially retained by fluorinated ligands.
    - D. Nitro-substituted PAH are separated from PAH on nitroaromatic phases.
    - E. Bulky (cyclic) solutes are separated from extended solutes by cyclic BPs.
14. Better separations of polycyclic aromatic hydrocarbons are usually possible with polymeric C_{18} phases than with monomeric C_{18} phases.
15. The order of elution of PAH isomers is usually correlated with L/B. Long, narrow molecules are retained longer than square molecules. Similarly, planar and linear molecules are almost always retained longer than nonplanar or nonlinear isomers. Polymeric C_{18} phases show enhanced selectivity for solute shape compared to monomeric C_{18} phases.
16. Phase transitions of at least two types occur at chemically modified surfaces. Both types of phase transition can result in anomalous retention behavior.

A. Alkyl phases undergo a reversible conformational change in aqueous environments. Upon heating, the phase changes from a "collapsed state" to an "extended state". In the extended state, water molecules are forced between the chains and maintain the conformation even after cooling. The collapsed state can be regenerated by conditioning with organic solvents to remove the "trapped water".

B. Gas chromatographic measurements on dry bonded phases show evidence for the existence of phase transtions near room temperature. These transitions decrease in magnitude as a function of decreasing ligand density. Phase transitions are also observed by liquid chromatographic measurements and "mark a change from adsorption dominated retention at the surface of a solid monolayer to a solution-dominated retention in a liquid expanded-like layer."

17. Solute retention, in general, increases with bonded phase chain length and phase density. Overall retention is related to the phase ratio. Retention increases with the total quantity of carbon contained within the column.

18. Under certain conditions, solute retention does not increase continuously with bonded phase length. For densely loaded phases longer than the "critical chain length", retention is observed to level off. The critical chain length is dependent on solute size (the critical chain length is larger with larger solutes). These observations suggest that solute penetration of the bonded phase may occur only to a limited extent.

APPENDIX 1. BONDED PHASE SYNTHESIS REFERENCES

Ref.	Authors	Phase type	Topics, comments
J. Chromatogr., 294, 175, 1984	Nondek, Ponec	2,4-Dinitroanilinopropyl	Electron acceptor phase (donar acceptor complexes)
Anal. Chem., 46, 11, 1974	Grushka, Kikta	2-Chloromethylphenylethane	Thermodynamic retention theory, efficiency
J. Chromatogr. Sci., 23, 391, 1985	Pharr, Uden, Siggia	3-(*p*-Acetylphenoxy)propyl	Selectivity toward chloroanilines
J. Chromatogr., 125, 115, 1976	Unger, Becker, Roumeliotis	Alkyl (monomeric, bulk)	Reaction conditions, properties, retention theory
J. Liq. Chromatogr., 1, 561, 1978	Berendsen, de Galan	Alkyl (monomeric)	Coverage, endcapping properties, retention theory
J. Liq. Chromatogr., 3, 1437, 1980	Berendsen, Pikaart, de Galan	Alkyl (monomeric)	Reaction conditions, phase coverage, properties
Adv. Coll. Int. Sci., 6, 95, 1976	Boksanyi, Liardon, Kovats	Alkyl (monomeric)	Reaction conditions, phase coverage
J. Chromatogr., 318, 173, 1985	Cheng, McCown	Alkyl (monomeric)	Reaction conditions, phase coverage vs. phase length
J. Chromatogr., 255, 115, 1983	Cooke et. al.	Alkyl (monomeric)	Effect of phase length for protein separations
Chromatographia, 20, 555, 1985	Dawidowicz, Rayes	Alkyl (monomeric)	High density phase synthesis, substrate effects
Coll. Surf., 9, 109, 1984	Erard, Nagy, Kovats	Alkyl (monomeric)	High density phase synthesis, phase coverage
Chromatographia, 13, 5, 1980	Evans, Dale, Little	Alkyl (monomeric)	Reaction conditions, characterization
Anal. Chem., 46, 1314, 1974	Gilpin, Korpi, Janicki	Alkyl (monomeric)	*In situ* preparation of alkyl and phenyl phases
Chromatographia, 10, 726, 1977	Hemetsberger et al.	Alkyl (monomeric)	Reaction conditions, chain length, retention theory
Chromatographia, 9, 303, 1976	Hemetsberger et al.	Alkyl (monomeric)	Effect of phase length
Chromatographia, 12, 71, 1979	Hemetsberger et al.	Alkyl (monomeric)	Reaction conditions, ligand structure, retention theory

Anal. Chem., 51, 1807, 1979	Hirata, Novotny, Tsuda, Ishii	Alkyl (monomeric)	*In situ* synthesis of C_{18}, amino, cyano, other phases
J. Chromatogr., 153, 543, 1978	Little, Dale, Evans	Alkyl (monomeric)	Characterization
J. Chromatogr., 171, 431, 1979	Little et al.	Alkyl (monomeric)	Effect of solvent on synthesis
J. Chromatogr., 171, 435, 1979	Little et al.	Alkyl (monomeric)	Reaction conditions, temperature, endcapping
J. Chromatogr. Sci., 17, 574, 1979	Lochmüller, Wilder	Alkyl (monomeric)	Effect of phase length, "liquid droplet model"
J. Chromatogr., 166, 21, 1978	Hennion, Picard, Caude	Alkyl (monomeric)	Effect of phase length, reaction conditions, ret theory
J. Chromatogr., 149, 211, 1978	Roumeliotos, Unger	Alkyl (monomeric)	Silane functionality, properties, retention theory
J. Liq. Chromatogr., 3, 1465, 1980	Spacek, Kubin, Vozka, Porsch	Alkyl (monomeric)	Comparison of *n*-alkyl phases and mixed phases
Chromatographia, 20, 677, 1985	Suprynowicz et al.	Alkyl (monomeric)	High density phase, characterization
J. Chromatogr., 279, 439, 1983	Takeuchi, Ishii	Alkyl (monomeric)	*In situ* synthesis of C_{18} phases
J. Chromatogr., 332, 57, 1985	Tanaka et al.	Alkyl (monomeric)	High density phase, on column BP synthesis
J. Chromatogr., 199, 327, 1980	Tanaka, Sakagami, Araki	Alkyl (monomeric)	Effect of phase length, planar, and nonplanar test probe
Chem. Lett., 587, 1980	Tanaka, Sakagami, Araki	Alkyl (monomeric)	Effect of phase length
Angew. Makro. Chemie, 34, 81, 1973	Fery, Laible, Hamann	Alkyl (polymeric)	Detailed reaction conditions for polymeric modification
J. Chromatogr., 189, 179, 1980	Hibi, Ishii, Tsuda	Alkyl (polymeric)	*In situ* synthesis of C_{18} phases in open-tubular columns
J. Chromatogr. Sci., 10, 129, 1972	Kirkland	Alkyl (polymeric)	Classical reference on polymeric BP synthesis
PAH: 8th Int. Symp., 1133, 1983	Sander, Wise	Alkyl (polymeric)	Selectivity toward PAH, comparison with monomeric phase
Anal. Chem., 56, 504, 1984	Sander, Wise	Alkyl (polymeric)	Reaction conditions, selectivity toward PAH
J. Chromatogr., 316, 163, 1984	Sander, Wise	Alkyl (polymeric)	Pore size effects on phase synthesis, PAH selectivity
J. Chromatogr., 282, 27, 1983	Schomburg et al.	Alkyl (polymeric)	Radiation or peroxide induced cross-linking of silanes
J. Chromatogr., 158, 227, 1978	Tsuda et al.	Alkyl (polymeric)	*In situ* synthesis of C_{18} phases in open-tubular columns
J. Chromatogr., 158, 227, 1978	Tsuda et al.	Alkyl (polymeric)	*In situ* polymerization for open tubular microcapillary
J. Chromatogr., 254, 117, 1983	Verzele, Mussche	Alkyl (polymeric)	Properties of monomeric and polymeric phases
J. HRC CC, 8, 248, 1985	Wise, Sander	Alkyl (polymeric)	Polymeric vs. monomeric phase synthesis, PAH selectivity
Chemically Modified Surfaces, Vol. 1, Leyden, D. E., Ed., Gordon and Breach Science Publishers, N.Y., 1986, 349	Wise, Sander, May	Alkyl (polymeric)	Polymeric vs. monomeric phase synthesis, PAH selectivity

Chromatographia, 19, 231, 1985	Den, Kettrup	Aromatic BP	Characterization by NMR, for separation of PAH
Chromatographia, 20, 242, 1985	Jinno, Okamoto	Aromatic BP	Retention theory, PAH solutes, pore size effects
Chromatographia, 18, 495, 1984	Jinno, Okamoto	Aromatic BP	Retention theory, shape recognition
Chromatographia, 18, 677, 1984	Jinno, Okamoto	Aromatic BP	Retention theory, PAH solutes
Chromatographia, 20, 641, 1985	Melander et al.	Aromatic BP	Selectivity comparison for aromatic phases
J. Chromatogr., 283, 61, 1984	Okamoto, Yamada	Aromatic BP	Phase coverage, separation of PAH
Chromatographia, 20, 239, 1985	Verzele, Van de Velde	Aromatic BP (anthracene)	Selectivity toward PAH, comparison with alkyl phases
J. Chromatogr., 247, 167, 1982	Okamoto, Yamada	Aromatic BP (benzyldimethyl)	Phase coverage, separation of anthracene and naphthalene
J. Chromatogr. Sci., 21, 444, 1983	Lochmüller, Hunnicutt, Beaver	Aromatic BP (pyrene)	For separation of nitro-PAH, endcapping effects
Anal. Chem., 50, 1337, 1978	Gilpin, Sisco	Butyl, cyanopropyl, misc.	*In situ* preparation of phases for normal phase
J. Chromatogr., 319, 432, 1985	Felix, Bertrand	Caffeine BP	Selectivity toward PAH
Chromatographia, 20, 155, 1985	Felix, Bertrand, Bastel	Caffeine BP	Selectivity toward PAH
J. Chromatogr., 119, 41, 1976	Colin, Eon, Guiochon	Carbon phase	Carbon phase prepared by benzene pyrolysis
J. Chromatogr., 122, 223, 1976	Colin, Eon, Guiochon	Carbon phase	Carbon phase prepared by benzene pyrolysis
J. Chromatogr., 167, 41, 1978	Colin et al.	Carbon phase	Temperature effects in retention
J. Chromatogr., 126, 43, 1976	Colin, Guiochon	Carbon phase	Pyrocarbon-coated silica
J. Chromatogr., 137, 19, 1977	Colin, Guiochon	Carbon phase	Reproducibility of carbon phases
Chromatographia, 17, 627, 1983	Dawidowicz et al.	Carbon phase	Reaction conditions, characterization, selectivity
J. Chromatogr., 352, 3, 1986	Knox, Kaur, Millward	Carbon phase	Characterization of porous glassy carbon phase
J. Chromatogr., 148, 53, 1978	Patzelova, Jansta, Dousek	Carbon phase	Carbon phase from reduction of PTFE by alkali-metal
J. Chromatogr., 147, 137, 1978	Plzak, Dousek, Jansta	Carbon phase	Carbon phase from reduction of PTFE by alkali-metal
Anal. Chem., 53, 812, 1981	Zwier, Burke	Carbon phase	Reaction conditions, characterization, retention theory
Chromatographia, 16, 224, 1982	Akanya, Hitchen, Taylor	Chiral phase	Resolution of D/L amino acid esters
J. Liq. Chromatogr., 7(S-2), 353, 1984	Armstrong	Chiral phase	Review of various types of chiral phases
J. Chromatogr., 325, 103, 1985	Grierson, Adam	Chiral phase	*In situ* phase synthesis, for resolution of amino acids
J. Chromatogr., 130, 511, 1978	Lochmüller, Ryall	Chiral phase	Resolution of helicenes
J. Chromatogr., 322, 309, 1985	Pirkle, Hyun	Chiral phase	Reaction conditions, separation of chiral amino acids
J. Chromatogr., 322, 295, 1985	Pirkle, Hyun	Chiral phase	Reaction conditions, urea-linked chiral phases

J. Chromatogr., 322, 287, 1985	Pirkle, Hyun	Chiral phase	For separation of *N*-(3,5-dinitrobenzoyl)-a-amino acids
J. Chromatogr., 316, 585, 1984	Pirkle, Hyun, Bank	Chiral phase	Approach to designing chiral phases
Chromatographia, 18, 459, 1984	Sinibaldi et al.	Chiral phase	Demonstration of phase to separate various enantiomers
L. C. Mag., 3, (No. 4), 348, 1985	Allenmark	Chiral phase (proteins)	Proteins as stationary phases for chiral separations
J. Chromatogr., 200, 3, 1980	Aue, Wickramanayake	Cyclic organosilicons	Polymeric phase of type also used in GC
Anal. Chem., 57, 237, 1985	Hinze et al.	Cyclodextrin	Separation of enantiomers
Anal. Chem., 55, 1852, 1982	Kawaguchi et al.	Cyclodextrin	Retention of disubstituted benzene isomers
J. Chromatogr., 267, 285, 1983	Tanaka, Kawaguchi, Shono	Cyclodextrin	Retention of disubstituted benzene isomers
J. Chromatogr. Sci., 22, 411, 1981	Armstrong, DeMond	Cyclodextrin BP	For separation of amino acid enantiomers, barbiturates
J. Liq. Chromatogr., 8, 261, 1985	Armstrong et al.	Cyclodextrin BP	For routine separations, e.g., PAH, quinones, etc.
J. Chromatogr., 347, 51, 1985	Chang et al.	Cyclodextrin BP	Retention behavior of organometallic solutes
J. Chromatogr., 333, 59, 1985	Feitsma, Bosman, Drenth, Zeeuw	Cyclodextrin BP	For separation of carboxylic acid enantiomers
Anal. Chem., 55, 446, 1983	Fujimura, Ueda, Ando	Cyclodextrin BP	For separation of PAH, retention theory
Anal. Chem., 55, 1852, 1983	Kawaguchi et al.	Cyclodextrin BP	For separation of aromatic compounds, benzene isomers
J. Chromatogr., 267, 285, 1983	Tanaka et al.	Cyclodextrin BP	Modification by acetylation, separation of benzene isomers
J. Chromatogr., 246, 207, 1982	Tanaka et al.	Cyclodextrin BP	Retention theory, separation of disubstituted benzene isomers
J. Chromatogr., 249, 19, 1982	Walters	Diol BP	Affinity chromatography, pore size effects
J. Chromatogr., 323, 281, 1985	Rudzinski et al.	Diphenylphosphine-C_{18}	Reaction conditions, NMR characterization
J. Chromatogr., 326, 79, 1985	Rassi, Horvath	Ethylene diamino etc.	For separation of tRNAs
Chromatographia, 16, 178, 1982	Ecknig, Trung, Radeglia, Gross	Fluorinated BP	Group separation of alkyl-substituted PAH
J. HRC CC, 8, 362, 1985	Felix, Bertrand	Fluorinated BP	Comparison of normal and reversed phase retention
Chromatographia, 14, 341, 1981	Haas, Köhler, Hemetsberger	Fluorinated BP	Separation of aromatic and halogenated aromatic solutes
Chromatographia, 20, 161, 1985	Shih-Hsien et al.	Hydrosilylation	Hydrosilylation reaction and subsequent BP synthesis
J. HRC CC, 2, 507, 1979	Matlin, Tinker	Hydroxyalkyl	Synthesis and retention characteristics
J. Chromatogr. Sci., 19, 296, 1981	Chmielowiec	Metal (organomericuric) BP	For separation of heteroatom PAH
J. Liq. Chromatogr., 8, 281, 1985	Djerki, Laub, Milonjic	Metal modified phase	Separation of substituted anilines reported

J. HRC CC, 5, 616, 1982	Dewaele, Mussche, Verzele	Methyl (monomeric)	Endcapping reactions, chromatographic characterization
Anal. Chem., 45, 1383, 1973	Gilpin, Burke	Methyl (monomeric)	Silica pretreatment, reaction conditions, theory
Adsorp. Sci. Technol., 1, 77, 1984	Gobet, Kovats	Methyl (monomeric)	Silica pretreatment, phase coverage
Anal. Chim. Acta, 142, 63, 1982	Lochmüller, Marshall	Methyl (monomeric)	Endcapping reactions, luminescence and PAS, polarity
Chromatographia, 20, 308, 1985	Nawrocki	Methyl (monomeric)	Endcapping reactions, mixed silane reactions
J. Liq. Chromatogr., 3, 201, 1980	Rassi, Gonnet	Methyl (monomeric)	Silica pretreatment, phase coverage
J. HRC CC, 8, 337, 1985	Tomellini et al.	Methyl (monomeric)	Endcapping reactions, retention theory, phase coverage
J. Liq. Chromatogr., 1, 403, 1978	Berendsen, de Galan	Methyl, phenyl (monomeric)	Coverage, geometric model of phase surface
J. Chromatogr. Sci., 19, 130, 1981	Lochmüller, Hangac, Wilder	Misc. alkyl	Comparison of *n*-alkyl and cyclic alkyl BP
J. Chromatogr., 191, 71, 1980	Rehak, Smolkova	Misc. alkyl	Comparison of *n*-alkyl and branched alkyl BP
J. Chromatogr., 239, 761, 1982	Tanaka et al.	Misc. alkyl and aromatic	Retention theory: BP structure
Chromatographia, 16, 349, 1982	Crowther, Hartwick	Multifunctional	Mixed mode retention
J. Liq. Chromatogr., 4, 1361, 1981	Engelhardt, Ahr, Hearn	*n*-Acetylaminopropyl	For size exclusion of polypeptides and proteins
J. Chromatogr., 178, 298, 1979	Lochmüller, Ryall, Amoss	Nitroaromatic	Selectivity
J. Chromatogr., 351, 506, 1986	Van Damme, Verzele	Phenyl (polymeric)	Normal phase characteristics of polyphenyl silica
J. HRC CC, 3, 33, 1980	Matlin, Louch, Bryan	Picramidopyopyl	Retention characteristics of an electron acceptor phase
J. Chromatogr., 83, 25, 1973	Novotny et al.	Polar BP (polymeric)	Classical reference on polymeric BP synthesis
J. Chromatogr., 332, 15, 1985	Rokushika et al.	Polyallylamine (polymeric)	Coated and cross-linked phase
J. Chromatogr., 319, 396, 1985	Chang, Rassi, Horvath	Polyethyleneglycol	For separation of proteins
J. Chromatogr., 351, 393, 1986	Figge, Deege, Köhler, Schomburg	Polymer-coated phases	Preparation of mechanically coated polymeric phases
J. Chromatogr., 108, 85, 1975	Lochmüller, Amoss	Tetranitrofluorenimino	Charge transfer stationary phase, selectivity toward PAH

APPENDIX 2. CHARACTERIZATION REFERENCES

Ref.	**Authors**	**Technique**	**Topics, comments**
Adsorp. Sci. Technol., 1, 111, 1984	Gobet, Kováts	Area	Area determination by chemisorption
Chromatographia, 16, 204, 1982	Larsen, Schou	Area	Area determination by chemisorption, liquid scintillation
J. Chromatogr., 186, 435, 1979	Verzele et al.	Area	Practical techniques for BET area measurements
J. Chromatogr., 321, 319, 1985	Diamondstone et al.	Carbon	Technique for carbon analysis of bonded phases

J. Chromatogr., 289, 367, 1984	Crowther et al.	Cleavage	HF cleavage of bonded phase for quantitation
Anal. Chem., 54, 193, 1982	Erard, Kovats	Cleavage	HF cleavage of bonded phase for quantitation
J. Chromatogr., 244, 368, 1982	Genieser et al.	Cleavage	Phase cleavage by fused alkyl treatment
J. Chromatogr., 269, 127, 1983	Genieser et al.	Cleavage	Phase cleavage by fused alkyl treatment, det. of ligand
J. HRC CC, 8, 513, 1985	Temmerman et al.	Cleavage	Phase cleavage by alkaline hydrolysis
J. Chromatogr., 190, 331, 1980	Verzele et al.	Cleavage	Alkaline hydrolysis, det. of ligand type, endcapping
J. Chromatogr., 149, 199, 1978	Wehrli et al.	Cleavage	Study of phase stability in basic environments
J. Chromatogr. Sci., 21, 93, 1983	Rayss et al.	DTA	Differential thermal analysis of C_{18} films on carbon-silica
Ber Bunseges Phys. Chem., 79, 882, 1975	Sistovaris et al.	ESR	Mobility of silica bound nitroxide spin labels
Surf. Sci., 67, 251, 1977	Wagner et al.	ESR	Study of silica surface by bound spin labels
J. Anal. Appl. Pyrol., 4, 273, 1983	Mussche, Verzele	GC	Pyrolysis GC of bonded phases, determination of phase type
J. Chromatogr., 262, 183, 1983	Trojer, Hansson	GC	Pyrolysis GC of bonded phases, determination of phase type
J. Chromatogr., 318, 173, 1985	Cheng, McCown	Gravimetry	Determination of carbon loading by gravimetry
J. Liq. Chromatogr., 1, 561, 1978	Berendsen et al.	IR	IR spectra of various bonded phases
J. Chromatogr., 260, 1, 1983	Bush et al.	IR	Quantitative study of silanols by IR
Anal. Chem., 55, 1068, 1983	Sander et al.	IR	FTIR study of alkyl chain conformation of C_{18} ligands
Chem. Lett., 1373, 1981	Watanabe	IR	Diffuse reflectance FTIR of bonded species
Anal. Chim. Acta, 100, 545, 1978	Leyden et al.	IR, PAS	Study of *n*-β-aminoethyl-γ-aminopropylsilane
J. Chromatogr., 352, 399, 1986	Goworek et al.	Isotopes	Test for silanol activity based on isotopic exchange
J. Chromatogr., 321, 1, 1985	Antle et al.	LC	Characterization of retention selectivity in reversed phase
L. C. Mag., 2(11), 840, 1984	Antle, Snyder	LC	Test mixtures for column evaluation
Chromatographia, 18, 81, 1984	Daldrup, Kardel	LC	Test mixture for column evaluation for toxicological use
Chromatogr. Newslett., 10, 12, 1982	DiCesare, Dong	LC	Test mixtures for column evaluation
Chromatographia, 15, 657, 1982	Domagalska, Loscombe	LC	Test mixtures for column evaluation
Chromatographia, 16, 11, 1982	Engelhardt et al.	LC	Test mixtures for column evaluation
J. Chromatogr., 218, 395, 1981	Engelhardt, Müller	LC	Test mixtures for column evaluation
J. Chromatogr., 352, 91, 1986	Jandera	LC	Characterization of selectivity based on homologous series
J. Chromatogr., 352, 111, 1986	Jandera	LC	Characterization of selectivity based on homologous series
J. Chromatogr. 122, 3, 1976	Karch et al.	LC	Test mixtures for column evaluation
J. Liq. Chromatogr., 3, 1151, 1980	Katz, Ogan	LC	Characterization of column selectivity toward PAH
J. Chromatogr., 188, 115, 1980	Ogan, Katz	LC	Characterization of column selectivity toward PAH

J. Chromatogr. Sci., 21, 314, 1983	Sadek, Carr	LC	Test for silanol activity based on tetraaza compounds
J. Chromatogr., 316, 163, 1984	Sander, Wise	LC	Test mixture to assess monomeric vs. polymeric phases
Anal. Chem., 56, 504, 1984	Sander, Wise	LC	Test mixture to assess monomeric vs. polymeric phases
J. Chromatogr., 142, 213, 1977	Scott, Kucera	LC	Criteria for silanol determination
J. Chromatogr., 217, 399, 1981	Verzele, Dewaele	LC	Test mixture for trace metal activity
Chromatographia, 19, 457, 1984	Welsch et al.	LC	Criteria for silanol determination
Anal. Chem., 55, 1479, 1983	Wise, May	LC	Characterization of column selectivity toward PAH
HRC CC, 8, 248, 1985	Wise, Sander	LC	Test mixture to assess monomeric vs. polymeric phases
J. Liq. Chromatogr., 6, 2709, 1983	Wise, Sander, May	LC	Characterization of column selectivity toward PAH
J. Chromatogr., 352, 275, 1986	Köhler et al.	Misc	Comprehensive characterization of BP by various techniques
J. Magn. Res., 62, 428, 1985	Albert, Evers, Bayer	NMR	Motional study of alkyl ligands
Surf. Sci., 47, 661, 1975	Chang et al.	NMR	^{13}C NMR study of silanized chrysotile asbestos
J. Coll. Int. Sci., 86, 26, 1982	Chiang, Liu, Koenig	NMR	^{13}C study of aminosilane coupling agents
Chromatographia, 19, 231, 1985	Den, Kettrup	NMR	^{13}C NMR of phenyl pentyl phase
J. Organomet. Chem., 210, 295, 1981	Engelhardt et al.	NMR	^{29}Si NMR study of organosilicon polymers
J. Magn. Res., 53, 140, 1983	Gangoda, Gilpin	NMR	Motional study of alkyl ligands
Anal. Chem., 56, 1470, 1984	Gilpin, Gangoda	NMR	Motional study of alkyl ligands, ^{13}C enrichment
J. Chromatogr. Sci., 21, 352, 1983	Gilpin, Gangoda	NMR	^{13}C NMR of alkyl ligands, solvation, and mobility studies
Appl. Surf. Sci., 10, 247, 1982	Hays et al.	NMR	^{13}C NMR characterization of various bonded phases
J. Chromatogr., 213, 33, 1981	Holik, Matejkova	NMR	Determination of hydroxyl groups in silica
J. Am. Chem. Soc., 108, 1746, 1986	Kelusky, Fyfe	NMR	NMR study of alkyl chain motion in bonded phases
Anal. Chim. Acta, 126, 207, 1981	Leyden et al.	NMR	Initial ^{13}C NMR study of silane ligands
J. Chromatogr., 205, 438, 1981	Maciel et al.	NMR	^{29}Si and ^{13}C NMR characterization of bonded phases
J. Am. Chem. Soc., 102, 7606, 1980	Maciel, Sindorf	NMR	^{29}Si NMR assignments of silanol and siloxane groups
J. Chromatogr., 331, 23, 1985	McNally, Rogers	NMR	^{13}C NMR study of chain mobility, mobile phase effects
J. Chromatogr., 319, 9, 1985	Miller et al.	NMR	Quantitative measurement of silanol activity by ^{29}Si NMR
Sep. Sci., 5, 23, 1970	Pickett et al.	NMR	NMR of molecules adsorbed onto silica
Sep. Sci., 5, 11, 1970	Pickett et al.	NMR	NMR of molecules adsorbed onto silica
J. Phys. Chem., 84, 2204, 1980	Resing et al.	NMR	Motional study of phenyl groups
J. Chromatogr., 323, 281, 1985	Rudzinski et al.	NMR	^{13}C, ^{29}Si, ^{31}P NMR of diphenylphosphine-C_{18} phase
J. Coll. Int. Sci., 89, 293, 1982	Shinoda, Saito	NMR	Hydrogen bonding and dynamics of bound amino ligands

J. Am. Chem. Soc., 105, 1487, 1983	Sindorf, Maciel	NMR	^{29}Si NMR study of silica hydration, dehydration
J. Am. Chem. Soc., 105, 1848, 1983	Sindorf, Maciel	NMR	^{13}C CP-MAS study of C_{18} motion
J. Am. Chem. Soc., 105, 3767, 1983	Sindorf, Maciel	NMR	Study of silane bonding with silica, spectral assignments
J. Phys. Chem., 86, 5208, 1982	Sindorf, Maciel	NMR	Study of trimethylsilane to probe surface geometry
Chem. Lett., 179, 1979	Tanaka et al.	NMR	Study of γ-aminopropyl and *N*-β(aminoethyl)γ-aminopropyl
Chromatographia, 20, 582, 1985	Claessens	NMR, FTIR	Study of ageing processes in alkyl bonded phases
Anal. Chem., 54, 101, 1982	Leyden et al.	NMR, PAS	NMR, PAS, and IR study characterizing acetoacetamide ligands
Anal. Chem., 52, 19, 1980	Lochmüller et al.	PAS	PAS of charge transfer bonded phase
Anal. Chim. Acta, 116, 19, 1980	Lochmüller, Wilder	PAS	Qualitative study of bonded phases by PAS
Anal. Chim. Acta, 118, 101, 1980	Lochmüller, Wilder	PAS	Quantitative study of bonded phase by PAS
Spect. Lett., 11, 581, 1978.	Low, Parodi	PAS	Infrared PAS study of methyl bonded silica
Appl. Spect., 34, 76, 1980	Low, Parodi	PAS	Infrared PAS of modified silica
Anal. Chem., 56, 2204, 1984	Miller et al.	PAS, ESCA	Quantitative analysis of C_{18} bonded silica
J. HRC CC, 153, 1984	Nondek, Reissova	Reaction	Determination of silanols based on reaction and evolved methane
J. Liq. Chromatogr., 9(7), 1387, 1986	Smith et al.	Review	Review of methods for determination of void volume
Anal. Chem., 57, 2079, 1985	Glinka et al.	SANS	Characteristics of BP substrates by small angle neutron scattering
Anal. Chem., 57, 2593, 1985	Beaufils et al.	Scattering	Neutron scattering to assess alkyl chain motion
Anal. Chem., 49, 1183, 1977	Freeman, Poinescu	SEC	Pore size distribution by size exclusion chromatography
Anal. Chem., 53, 1235, 1981	Freeman, Schram	SEC	Pore size distribution by size exclusion chromatography
J. Chromatogr., 206, 449, 1981	Kuga	SEC	Pore size distribution by size exclusion chromatography
J. Chromatogr. Sci., 18, 207, 1980	Nikolov et al.	SEC	Pore size distribution by SEC, effects of bonding
J. Liq. Chromatogr., 3, 403, 1980	Schram, Freeman	SEC	Pore size distribution by size exclusion chromatography
Chromatographia, 13, 271, 1980	Werner, Halász	SEC	Pore size distribution by size exclusion chromatography
Anal. Chem., 58, 2895, 1986	Jennings, Brownlee	Spectroscopy	Determination of silanols based on ammoniacal copper complex
Chromatographia, 17, 157, 1983	Dawidowicz et al.	Transition	Study of phase transitions by GC retention studies
Chromatographia, 20, 555, 1985	Dawidowicz, Rayss	Transition	Study of phase transitions by GC retention studies
J. Chromatogr. Sci., 22, 371, 1984	Gilpin	Transition	Study of phase transitions by LC retention studies
Am. Lab., March, 104, 1982	Gilpin	Transition	Study of phase transitions by LC retention studies
J. Chromatogr. Sci., 20, 345, 1982	Gilpin et al.	Transition	Study of phase transitions by LC retention studies

J. Chromatogr., 330, 227, 1985	Gonnet et al.	Transition	Study of phase transitions by GC retention studies
J. Chromatogr., 282, 41, 1983	Hammers, Verschoor	Transition	Study of phase transitions by LC retention studies
J. Chromatogr. Sci., 21, 560, 1983	Hansen, Callis	Transition	Differential scanning calorimetry of C_{18} and C_{22} phases
J. Chromatogr., 268, 147, 1983	Miltenburg, Hammers	Transition	Adiabatic calorimetry study of bonded phase transitions
J. Chromatogr., 248, 231, 1985	Morel, Serpinet	Transition	Study of phase transitions by GC retention studies
J. Chromatogr., 214, 202, 1981	Morel, Serpinet	Transition	Study of phase transitions by GC retention studies
Chromatographia, 15, 517, 1982	Rayss	Transition	Study of phase transitions of (coated) C_{18} by GC retention

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