



ELSEVIER

Journal of Chromatography A, 868 (2000) 153–167

JOURNAL OF
CHROMATOGRAPHY A

www.elsevier.com/locate/chroma

Studies of retention and stability of a horizontally polymerized bonded phase for reversed-phase liquid chromatography

Li Li^a, Peter W. Carr^{a,*}, John F. Evans^b

^aUniversity of Minnesota, Smith and Kolthoff Halls, 207 Pleasant St., S.E. Minneapolis, MN 55455-0431, USA

^bUniversity of Minnesota-Duluth, 10 University Drive, Duluth, MN 55812-2496, USA

Received 7 May 1999; received in revised form 9 November 1999; accepted 16 November 1999

Abstract

We have studied the novel horizontally polymerized mixed trichloropropyl–trichlorooctadecyl silane bonded phase described by Wirth. These materials can be reproducibly prepared and give very high bonded phase density ($>7.5 \mu\text{mol m}^{-2}$). They show significantly improved alkaline stability and chromatographic selectivity towards PAHs similar to conventional monomeric phases. Study of retention by the Linear Solvation Energy Relationship approach as well as measurement of dead volume and retention of methanol indicate that less mobile phase is sorbed by a horizontally polymerized phase than by conventional phases. Silanophilic interaction of amines are decidedly weaker on a silica modified by horizontal polymerization compared to a conventionally modified phase. In addition, this work provides additional support for the “partition-like” retention mechanism of bonded phase RPLC. © 2000 Elsevier Science B.V. All rights reserved.

Keywords: Stationary phases, LC; Surface coverage; Shape selectivity; Silanophilicity; Column stability

1. Introduction

Reversed-phase high-performance liquid chromatography is in common use for the separation of a wide variety of compounds [1]. It is well known that chemically bonded phases on silica gel are unstable at pH below 2–3 and above 8–9 due to the hydrolytic instability of siloxane bonds and the solubility of silica at high pH [2–4], respectively. A great deal of research has been done to design new stationary phases which are more robust. For example, Kirkland et al. [5] and Hartwick et al. [6] used silanizing

agents with bulky side groups to modify silica gel, and these stationary phases show improved stability under acidic mobile phase conditions. It was claimed that the bulky side group sterically protect the siloxane bonds. Chromatographers have also tried to improve stability by use of fully hydroxylated silica [7] and high-coverage synthetic conditions [8]. Another strategy to improve stability is to use polyfunctional silanes to make polymeric phases, due to the ability of the surface modifier to form multiple covalent bonds with the surface. However, the chromatographic performance of polymeric phases can be limited by low column efficiency due to slow intraparticle mass transfer [9–11], poor peak shape [12,13], and difficulties in reproducing the phase synthesis [14]. Wirth et al. used a mixture of

*Corresponding author. Tel.: +1-612-64-0253; fax: +1-612-66-7541.

E-mail address: carr@chem.umn.edu (P.W. Carr)

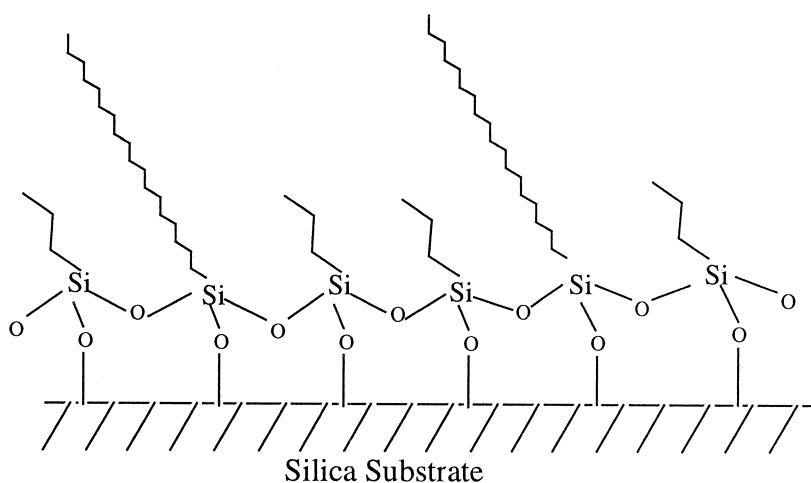
trifunctional silanes (*n*-octadecyltrichlorosilane and *n*-propyltrichlorosilane) to silanize silica gel by the so-called “horizontal polymerization silanization method” [15–22]. Their phase is different from typical phases in four respects. First, even though trifunctional silanes were used, the phase behaves chromatographically just as does a monomeric phase [16]. Second, the surface coverage of hydrocarbon chains including the sum of both the octadecyl and propyl chains is close to $8 \mu\text{mol m}^{-2}$. This is nearly equal to the total silanol concentration on silica’s surface [17]. Obtaining such high coverage suggests complete reaction of all silanol groups originally present on the silica gel. However, this agreement in coverage may be coincidental in that some new silanol groups might be introduced during the bonding reaction. Furthermore, others have indicated that not all of the original surface silanols are sterically accessible to the silane reagent during bonding [23]. In any event, these coverages are unquestionably much higher than those achieved previously with other monolayer phases [24]. Third, the hydrolytic stability of the phase is improved; the phase is claimed to be stable to at least 2800 column volumes of pH 1.8 solution and stable to at least 900 column volumes of pH 10.0 solution [16]. Fourth, it is claimed [16] that there is significant Si–O–Si bridging of the silane layer parallel to the silica substrate (see Fig. 1A) which is the basis of the “horizontal polymerization” concept. In a conventional polymeric bonded phase, some alkyl chains are not directly attached to the substrate (see Fig. 1B). This is termed “vertical polymerization”. The key structural differences are that horizontal polymerization provides a higher hydrocarbon chain density at the silica surface, and multilayered polymeric structures are not formed.

In the preparation of horizontally polymerized stationary phases, trifunctional silanes are reacted with silica gel under rigorously anhydrous solvent conditions. However, it is vital to note that a nearly complete monolayer of preadsorbed water is present on the silica’s surface. As such, the reaction is restricted to the surface, and only a monolayer of silane chains should be formed on the silica surface [16]. However, ideally anhydrous solution conditions are not easy to maintain. A small amount of dissolved water could initiate vertical polymerization. It is important to characterize the chromatographic

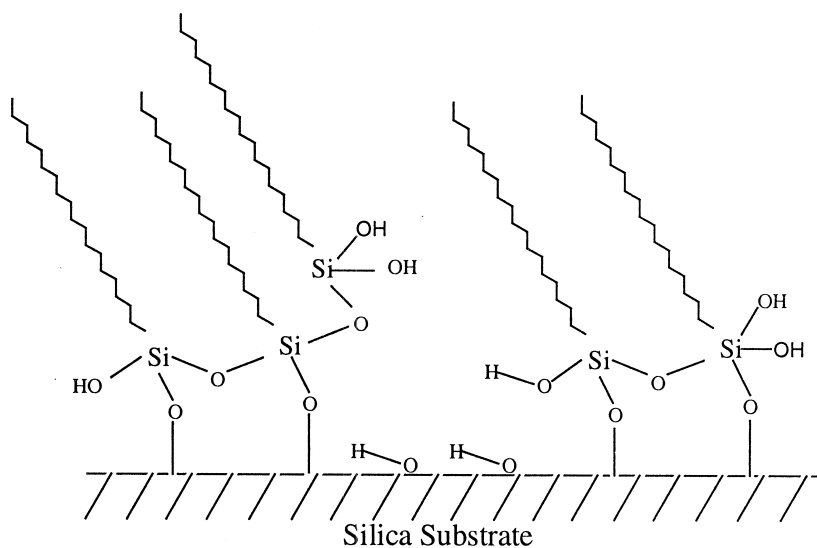
behavior of this new type of stationary phase, and to compare its behavior to conventional materials, i.e. those prepared by using silanes which are incapable of polymerization (e.g. dimethyloctadecylchlorosilane). On the other hand, the unusually high surface coverage of these phases ($8 \mu\text{mol m}^{-2}$) leads to another question: Will silanophilic interactions still exist in such phases, since almost all of the silanol groups are thought to have been reacted during the synthesis and any silanols introduced during synthesis are expected to be less accessible? Also in previous studies [16], the stability tests at high pH were not conducted in a manner that is entirely convincing. Specifically, the purely aqueous mobile phases which do not readily wet the RPLC phase were used, and thus, one might obtain an erroneous impression of the phase stability.

Horizontally polymerized stationary phases provide surfaces which are mostly covered by alkyl chains. Thus, the influence of the substrate, i.e. silica gel, should be greatly diminished. In view of the reduced of solute–silanol interactions, the study of retention mechanisms on these materials should be more straight forward. One of the oldest debates among chromatographers is whether or not a solute partitions into the layer of the bonded phase or adsorbs at the interface between the bonded phase and the eluent. Many retention models involving partition and adsorption mechanisms have been proposed [25–27], and even the definition of partitioning and adsorption have been debated. In this study, we quantitatively evaluate the performance of this new material in the context of Dill and Dorsey’s models of partition and adsorption [28].

There is considerable evidence that the retention of nonpolar solutes in RPLC is partition-like. First, the surface density of bonded chains should not affect the retention of nonpolar solutes, if adsorption were the principal mechanism. However, previous studies [29,30] have shown a strong dependence of retention on the surface density of bonded chains. Second, in their studies of homologous series, Tchaplak et al. [31] found a change in the slope of plots of $\log k'$ vs. carbon number, at a point where the carbon number of the homolog series was almost equal to the length of the bonded chains. This suggests that the solute alkyl chains insert themselves into the bonded phase. Third, the logarithmic capacity factor in RPLC was found to be propor-



A. Horizontal Polymerization.



B. Vertical Polymerization.

Fig. 1. Schematic of a horizontally (A) and vertically (B) polymerized phase.

tional to the logarithmic oil/mobile phase partition coefficient with a slope close to unity [32]. A slope of $1/z$ (z is the lattice coordination number) would have been expected if solutes were retained via an adsorption-like mechanism.

In a previous study from this laboratory, Tan and Carr [33–36] found that the ratio of the free energy of transfer for a methylene group of a nonpolar

solute from mobile phase to conventional bonded phase and the free energy of transfer a methylene group of a nonpolar solute from the same mobile phase to bulk hexadecane is close to unity over a wide range of mobile phase composition, which suggests a partition process. Furthermore, they showed that the ratio was closer to unity for conventional vertical polymeric phase supports as compared

to more ordered monomeric phases of the same chain length. Last, they showed that the ratio became independent of bonded phase length provided the length exceeded ten to twelve methylene units [33]. However, when the volume fraction of methanol in the mobile phase was increased above 70% (v/v), the difference between these two free energies increased significantly. In the current study, the free energy of transfer a methylene group of a nonpolar solute from mobile phase to horizontally polymerized stationary phase and the free energy of transfer a methylene group of a nonpolar solute from the same mobile phase to bulk hexadecane are shown to be nearly the same at high concentrations of methanol [50–100% (v/v)] in the mobile phase. This provides additional strong evidence that partitioning is the principal retention mechanism of the nonpolar solutes for this stationary phase, and by implication, for other alkyl silane phases.

The chromatographic selectivities of tetrabenzonaphthalene (TBN) and benzo[a]pyrene (BaP) were tested according to the method of Sanders and Wise [37–41]. Their approach provides a means of differentiating between conventional polymeric and monomeric bonded phases. The extent of solute accessible residual silanol coverage was also examined using 5,14-dimethyl-7,12-diphenyl-1,4,8,11-tetraazacyclotetradecane (DMDPC) as a probe solute. This molecule is extremely sensitive to the presence of unreacted silanol groups on a reversed-phase packing material [42]. Also, the phases were characterized by using the linear solvation energy relationship method [43–45]. Finally, the stability in basic solution was tested under more rigorous conditions.

2. Experimental

2.1. Synthesis of the stationary phases

Silica gel (diameter 10 μm , pore diameter 60 \AA , surface area 550 $\text{m}^2 \text{g}^{-1}$) was purchased from EM Merck. The primary synthesis procedures are slight modifications of the method of Wirth et al. [16].

2.1.1. Synthesis of the horizontally polymerized phase

Silica gel was cleaned by boiling in concentrated

nitric acid overnight, rinsed with pure water till neutral, and dried at 120°C. The silica gel was then exposed to 50% water saturated air for at least 24 h. This condition was generated and maintained by means of a 42% v/v aqueous sulfuric acid solution. A monolayer of water is thus adsorbed onto the clean silica gel surface [16]. After transfer of this material to a nitrogen-purged dry box (Vac Atmospheres), a solution of 10 ml *n*-octadecyltrichlorosilane, 2.5 ml of *n*-propyltrichlorosilane and 25 ml of *n*-heptane was added to 5 g of the pretreated silica gel. Immediately after addition of this solution to the silica, hydrogen chloride gas was evolved for about 15 min. The reaction was allowed to continue for at least 16 h in the dry box. The reactive ligands were removed by pipeting out the reaction solution and then the silanized silica gel was washed with fresh heptane, hexane and hot isopropanol at least 10 times with 20 ml of each solvent. Finally, the silica gel was dried overnight at 85°C in a vacuum oven.

2.1.2. Synthesis of the conventional phase

A conventional monomeric phase was prepared for comparison on the same silica used above by using Kinkel and Unger's method for the bonding of dimethyloctadecylchlorosilane [46].

2.2. Chromatographic studies

All columns were packed by the stirred slurry, upward packing method. The slurry and pushing solvent was isopropanol and the packing pressure is 5000 p.s.i. A HP 1090 chromatographic system and data system was employed to collect all chromatographic data. Prior to use, each column was equilibrated with degassed mobile phase and all mobile phases were filtered by using a 0.45 μm Millipore membrane filter. The dead volumes of all the columns were measured by using Knox and Kaliszan's method [47].

2.2.1. Basic stability test

The mobile phase was made by mixing equal volumes of acetonitrile and 0.05 *M* triethylamine in water (pH=10 adjusted with acetic acid) was used to test the stationary phase stability. The retention times of both toluene and pentylaniline were measured every 20 min at a mobile phase flow-rate of 2 ml min^{-1} .

2.2.2. Silanophilic interaction

DMDPC, which was synthesized by the modified procedure of Hideg [48], was investigated as a marker compound for characterization of residual silanol groups by measuring its retention time in a mobile phase of 100% methanol for both the conventional and the horizontally polymerized phases.

2.2.3. Monomeric and polymeric selectivity

The elution order of benzo[a]pyrene (BaP), phenanthro[3,4-c]-phenanthrene (PhPh) and 1,2:3,4:5,6:7,8-tetrabenzonaphthalene and the selectivity TBN/BaP in 85% acetonitrile/15% water were measured to determine the monomeric-like vs. polymeric-like retention properties of the horizontally polymerized phase.

3. Results and discussion

3.1. Surface coverage

Silica gels with different average particle diameters were used to test the reproducibility of the silanization method. The surface coverages obtained as measured by elemental analysis are given in Table 1. The surface coverages of the different particles and different runs using the same particle size are all close to $7.8 \pm 0.1 \mu\text{mol m}^{-2}$. Thus, the silanization method of Wirth reproducibly provides much higher surface coverages than do traditional silanization methods. It is important to point out that this phase is a mixture of octadecyl and propyl chains. The mole ratio of these two groups on the surface is 1:1.6 [16] corresponding to an octadecyl chain surface concentration of about $3 \mu\text{mol m}^{-2}$. The remaining silanol groups are covered by propyl chains. More importantly, the horizontal polymerization method provides a better way to control the polymerization direction. Most organic chains are directly attached to the silica gel surface. Therefore, most of the silanol groups on the silica gel surface are derivatized or covered with a close packed layer of alkyl chains (in a self-assembled layer). Probably, a monomeric-like phase is obtained. Typically, monomeric phases can be only obtained by using monofunctional silanes such as dimethyloctadecylchlorosilane as the silanizing reagent and the highest theoretical surface carbon coverage obtained for this type of

Table 1

Reproducibility of the surface coverage^a for horizontally polymerized phases based on carbon and hydrogen elemental analysis

| Particle diameter, μm | Silane surface coverage $\mu\text{mol m}^{-2}$ |
|----------------------------------|--|
| 75 – 250 ^b | 8.06 |
| 75 – 250 ^b | 7.24 |
| 75 – 250 ^b | 8.07 |
| 75 – 250 ^b | 7.95 |
| 75 – 250 ^b | 7.35 |
| 75 – 250 ^b | 7.88 |
| 10 ^{c,d} | 7.35 |

^a The surface coverage was determined from the following equation:

$$\mu\text{mol m}^{-2} = \frac{\%C \times 10^6}{1200[3x + 18(1-x)] - \%C[xMW_3 + (1-x)MW_{18}]} \frac{1}{S}$$

$x = \mu\text{mol m}^{-2}$ of propyl, and $1-x = \mu\text{mol m}^{-2}$ of octadecyl. x and $(1-x)$ are the mole fraction of chains that are propyl groups and octadecyl groups, respectively. The ratio of x to $(1-x)$ is 1.6 ± 0.2 . MW_3 and MW_{18} are the molecular weight of $(-\text{O}_3)\text{Si}(\text{CH}_2)_2\text{-CH}_3$ and $(-\text{O}_3)\text{Si}(\text{CH}_{17})\text{CH}_3$, respectively. S is the surface area of silica gel [16].

^b Mean pore diameter 60 Å; surface area $266 \text{ m}^2 \text{ g}^{-1}$.

^c Mean pore diameter 60 Å; Surface area $550 \text{ m}^2 \text{ g}^{-1}$.

^d This material was used to characterize the chromatographic properties of this new phase.

phase typically is about $4.2 \mu\text{mol m}^{-2}$ [49]. It is evident that the horizontal polymerization method of Wirth provides a new way to synthesize more ideal hydrocarbon phases with very high surface coverages.

3.2. Silanophilic interactions

A very sensitive method for testing for the presence of silanophilic interactions was previously developed in this laboratory [42]. The retention of DMDPC (a silanophilic interaction probe) relative to chrysene (a hydrophobic interaction probe) was used to characterize this new phase. Both probes are eluted with pure methanol without any amine blocker present in the eluent. Since both compounds are similar in size, they should have similar retention when there are no silanophilic interactions. However, DMDPC is a tetraazamacrocyclic compound which is extremely sensitive to the presence of surface silanol groups [42]. Whenever there are accessible silanol groups, the retention time of DMDPC

changes compared to the case that there are no silanol sites available to the solutes. By convention we take R as:

$$R \equiv [(k' \text{ of DMDPC}) - (k' \text{ of Chrysene})] / (k' \text{ of Chrysene})$$

Small R values, correspond to weak silanophilic interactions. R values ranged from as low as 0.26 to as high as 2000 for a large number of commercially available columns [42]. In this study, the k' value for chrysene was found to be 1.27. The R value for the horizontally polymerized phase was 1.1 and the R value for the conventional phase prepared on the same silica gel was 4.3. The peak shape for DMDPC is better for the horizontally polymerized phase, but it was still tailed. We conclude that some silanophilic interactions still take place on the new phase.

In order to explain the above phenomena, it is important to compare the structural differences between the horizontally polymerized C_3/C_{18} phase with conventional monomeric phases. Schematics illustrating these concepts are shown in Fig. 2A and B. The concentration of silanol groups on a typical silica gel surface is approximately $8 \mu\text{mol m}^{-2}$. This implies that the average distance between two silanols is about 4 Å. The chain density of a conventional monomeric phase is typically less than

$4 \mu\text{mol m}^{-2}$. If the alkyl chains of a conventional monomeric phase were evenly spaced, the average distance between two unreacted silanol groups, as well as between two alkyl chains would be about 5.7 Å. For a horizontally polymerized C_3/C_{18} phase, the situation is quite different. This phase has a very high chain density, about $8 \mu\text{mol m}^{-2}$. This is nearly equal to the close packed self-assembled monolayer obtained for a silica plate [50]. The long chain (octadecyl) density is about $3 \mu\text{mol m}^{-2}$, which is similar to that of a conventional monomeric phases. Most substrate silanol groups are reacted. For the horizontally polymerized phase, the average distance between any two alkyl chains is approximately 4 Å.

A typical conventional monomeric phase can be viewed as being comprised of three layers: the modifier chain layer, the substrate silanol layer and the substrate layer. The horizontally polymerized C_3/C_{18} phase can be divided to four layers or regions: the long modifier chain layer, the short modifier chain layer, the modifier and substrate silanol layer and the substrate layer. It is very important to note that the octadecyl chains are sufficiently far from each other that the long chain region can be considered unstructured or fluid like. However, the distance between chains in the short chain region is very small (about 4 Å). Although it is possible that silanol groups are introduced into the stationary phase through the silanization reaction [17], the accessibility of these modifier silanols to solutes in the chromatographic process should be less than that of the substrate silanols in a conventional monomeric phase. Because the average distance between two modifier chains is sufficiently short, adjacent silanols can form a hydrogen bond network (see Fig. 2B) in the horizontally polymerized C_3/C_{18} phase. Therefore, the activity of these silanols should be significantly reduced. Furthermore, the short interchain distance inhibits access to the modifier silanol groups even for a small solute, such as methanol. Thus, both the reactivity and accessibility of the surface modifier silanol groups should be reduced in a horizontally polymerized C_3/C_{18} phase.

On the other hand, due to the presence of the short chain layer in the horizontally polymerized phase, the retention behavior should also be changed compared to a conventional monomeric phase. On conventional phases, polar solutes should be stabilized

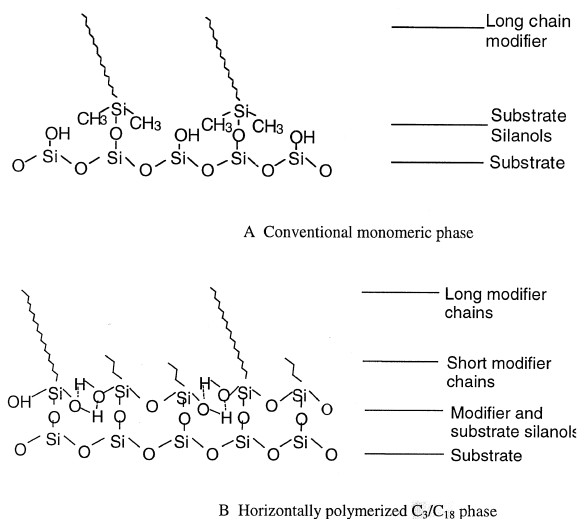


Fig. 2. Detailed view of a conventional (A) and horizontally polymerized (B) phase indicating different structural features.

in the stationary phase by their interaction with sorbed mobile phase components. However, on a horizontally polymerized phase a polar solute will have to overcome the barrier created by the very dense chain region before it can interact with the silica substrate and encounter the unreacted silanol groups. A polar solute will prefer to interact with the silanol groups through hydrogen bond formation. Thus, in contrast to a conventional monomeric phase, in a horizontally polymerized C_3/C_{18} phase polar solutes will encounter the short chains before they can access silanol sites. Thus, the total silanophilicity should be less for a horizontally polymerized C_3/C_{18} phases than for a conventional monomeric phases.

3.3. Polyfunctional-like vs. the monofunctional-like behavior of the new phase

Sander and Wise established an empirical test for differentiating between polymeric and monomeric packings [37–41]. The elution order and the selectivity factors for BaP, PhPh and TBN depend on the phase type. With monomeric phases, the elution order is usually $BaP \leq PhPh < TBN$, and the ratio of k' of TBN to BaP is greater than 1.7. With polymeric phases, the elution order is usually $PhPh < TBN \leq BaP$, and the ratio of k' of TBN to BaP is less than 1. We found that for the horizontally polymerized phase, PhPh and BaP coelute, and the ratio of k' of TBN over BaP is 1.76. Both observation indicate that the horizontally polymerized stationary acts like a monofunctional phase, even though trichlorosilanes were used to prepare the stationary phase. This suggests that the horizontally polymerized phase has the same type of chain organization in the outer region as does a monomeric phase.

3.4. Retention mechanism of nonpolar solutes in horizontally polymerized stationary phase: adsorption vs. partitioning

In this study, we used the definition of “adsorption” and “partition” as per Dill and Dorsey [28,51]. According to their model, a “partition” process corresponds to a situation in which the solute is approximately fully embedded within the stationary phase. Physically this means that the entire surface of the solute contacts the bonded alkyl

chains. On the other hand, “adsorption” is the process in which a solute is only in limited contact with the nonpolar alkyl chain of the stationary phase and is not fully embedded. Dill treated the solute as a simple cube. Thus, a solute has six surfaces and its lattice coordination number is 6. For a partitioning mechanism, all six solute–mobile phase interactions are replaced by six solute–stationary phase interactions. For an adsorption-like mechanism, only 1/6 of the solute–mobile phase interactions are replaced. Therefore, in an adsorption process the total free energy of transfer of a solute should be about 1/6 that of a partitioning process.

We define the F value [33] as:

$$F = \frac{\Delta G^0(-CH_2)_{C_{16}/m}}{\Delta G^0(-CH_2)_{s/m}} \quad (1)$$

$\Delta G^0(-CH_2)_{C_{16}/m}$ refers to the free energy of transferring a methylene group from bulk hexadecane to mobile phase and $\Delta G^0(-CH_2)_{s/m}$ refers to the free energy of transferring a methylene group from stationary phase to mobile phase. However, it is important to note that the retention mechanism need not be strictly either partition or adsorption. Intermediate situations are evident. Previous studies have shown that bulk hexadecane is a useful model of the bonded phase for nonpolar solutes [52]. We consider that the transfer of a solute from an aqueous organic mobile phase to bulk hexadecane serves as a reference for a pure partitioning process. Thus, if one observes that the ratio of the free energy of transfer of a methylene unit from mobile phase to bonded phase to the same quantity into bulk hexadecane is close to unity, then a partition-like mechanism predominates. However, it is important to note that the attachment of the silane to the surface imposes order on the alkyl chains which is not present in a bulk solution [53,54]. Consequently, if a solute is inserted into alkyl chains, and even if the solute is fully embedded into it, the free energy change might not be the same as in the bulk process. If the F value is substantially greater than unity, the retention mechanism has characteristic of both partitioning and adsorption.

Fig. 3 is a plot of the F value vs. mobile phase composition from this study and from a previous study [33]. From Fig. 3, we conclude that the ratio of

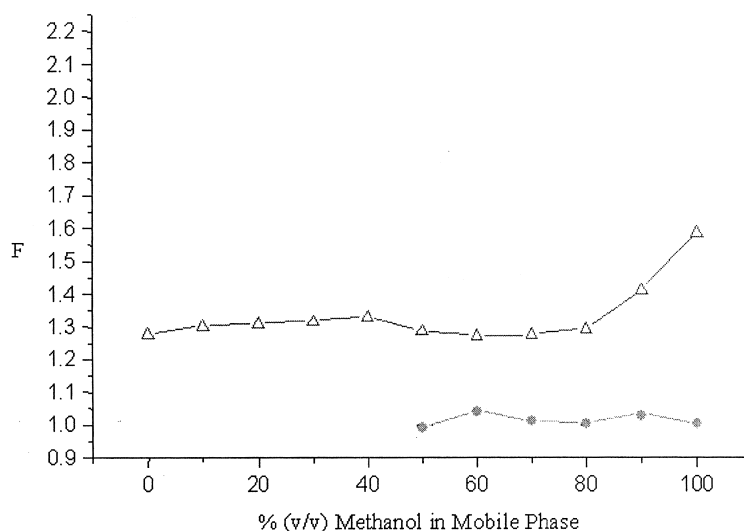


Fig. 3. Comparison of F -ratio for conventional and horizontally polymerized phase on a function of mobile phase composition ●: Horizontally polymerized stationary phase Δ: Nucleosil conventional polymerized C_{18} phase.

the standard free energy of transfer of a methylene group from the mobile phase to the stationary phase to that of the transfer to bulk hexadecane is extraordinarily close to unity for all mobile phase compositions tested. Hence, we infer that the retention mechanism for this new phase is nearly *pure partitioning*.

In previous studies [33,55], similar F values (1.1–1.2) were obtained from other polymeric octadecyl columns when the eluent was varied from 10% to 70% (v/v) methanol. However, when the methanol concentration was greater than 70%, the F value increased rapidly as shown in Fig. 3. For some octadecyl phases, the F value was as high as 2 at methanol contents in excess of 70% (v/v). A previous study [33] suggested, among other possibilities, that the larger F , or smaller $\Delta G^\circ(-CH_2)_{s/m}$ in 100% MeOH eluent, might be caused by the sorption of mobile phase components into the bonded phase. If large amounts of mobile phase components are sorbed within the bonded chains, the chemical properties of the bonded phase will become more like those of the mobile phase. Thus, the free energy of transfer a nonpolar solute from the mobile phase to the stationary phase will be smaller in this case, and the F value should be larger as seen in Fig. 3. At the same time, the sorption of methanol ought to induce the *trans* conformation of the bonded chains,

thereby causing them to become more extended [52]. Thus, a smaller fraction of the solute surface would be in touch with the bonded chains and F should increase. The new phases prepared by horizontal polymerization have very high hydrocarbon chain coverages (see Table 1). We believe that this will reduce the amount of mobile phase component that can be sorbed into the bonded phase compared to what happens with a conventional phase. This in turn makes F closer to unity and less dependent of mobile phase composition. On the other hand, the greater hydrophobicity of the horizontally polymerized phase may also provide a better environment for a partitioning non-polar solute. Thus, the F value is close to unity in one hundred percent methanol. In any case the difference in the behavior of the horizontally polymerized phase and conventional polymerized phase in terms of the absolute F -ratio and the difference in dependent of the F -ratio on eluent composition are hard to explain unless the solute is nearly fully embedded in the bonded chains.

3.5. Linear solvation energy relationships (LSER)

According to the LSER formalism [56,57], the logarithmic capacity factors, $\log k'$ of RPLC can be separated into several molecular interaction terms as follows:

$$\log k' = \log k'_0 + vV_2 + s\pi_2^{*H} + a\sum \alpha_2^H + b\sum \beta_2^H + rR_2 \quad (2)$$

where $\log k'_0$, v , s , a , b and r are the fitting coefficients. These coefficients can be obtained by multi-factor simultaneous least square linear regression of $\log k'$ vs. the solute parameters: e.g., molecular volume (V_2), dipolarity/polarizability (π_2^{*H}), hydrogen-bond acidity ($\sum\alpha_2^H$) hydrogen-bond basicity ($\sum\beta_2^H$) and excess molecular polarizability (R_2) [58]. The signs and magnitudes of the coefficients reflect the contribution and relative strengths of the different types of solute–stationary and solute–mobile phase interactions which affect retention for a given system. When the mobile phase is fixed, any variations in the coefficients v , s , a , b and r are due to changes in the properties of the

stationary phase. In the work of Tan and Carr [52], it was shown that the r coefficient is statistically zero for long chain aliphatic phases. Consequently, we did not include it in the present work.

Two mobile phase systems were used for this study: 50:50 and 40:60 (v/v) methanol–water. The test solutes, which are used to test the horizontally polymerized phase, and the solute parameters are given in Table 2. For the LSER studies on a conventional C_{18} phase, Yugi [59] measured the $\log k'$ value of 23 compounds. We fitted their $\log k'$ values to the LSER equation and the fitting coefficients v , s , a and b measured from these studies are compared to that of the horizontally polymerized stationary phase (see Table 3). The correlation coefficient values and average standard deviation are comparable and we conclude that the retention

Table 2
Test solutes and solute parameters for C_3/C_{18} horizontally polymerized stationary phase^a

| Number | Compounds | $V_2/100$ ^b | π_2^{*H} ^c | $\sum\alpha_2^H$ ^c | $\sum\beta_2^H$ ^c |
|--------|--------------------------------|------------------------|---------------------------|-------------------------------|------------------------------|
| 1 | <i>p</i> -Chlorotoluene | 0.9797 | 0.67 | 0 | 0.07 |
| 2 | Bromobenzene | 0.8914 | 0.73 | 0 | 0.09 |
| 3 | Chlorobenzene | 0.8388 | 0.65 | 0 | 0.07 |
| 4 | Benzylbromide | 1.0323 | 0.98 | 0 | 0.2 |
| 5 | <i>p</i> -Nitrobenzyl Bromide | 1.2065 | 1.5 | 0 | 0.4 |
| 6 | Nitrobenzene | 0.8906 | 1.11 | 0 | 0.28 |
| 7 | <i>o</i> -Nitrotoluene | 1.0315 | 1.11 | 0 | 0.27 |
| 8 | <i>p</i> -Nitrotoluene | 1.0315 | 1.11 | 0 | 0.28 |
| 9 | Methyl Benzoate | 1.0726 | 0.85 | 0 | 0.46 |
| 10 | Ethyl Benzoate | 1.2135 | 0.85 | 0 | 0.46 |
| 11 | Anisol | 0.916 | 0.75 | 0 | 0.29 |
| 12 | <i>p</i> -Chlorophenol | 0.9875 | 1.08 | 0.67 | 0.2 |
| 13 | <i>o</i> -Cresol | 0.916 | 0.86 | 0.52 | 0.3 |
| 14 | <i>p</i> -Cresol | 0.916 | 0.87 | 0.57 | 0.31 |
| 15 | <i>p</i> -Ethylphenol | 1.0569 | 0.9 | 0.55 | 0.36 |
| 16 | Propiophenone | 1.1548 | 0.95 | 0 | 0.51 |
| 17 | Butylphenone | 1.2957 | 0.95 | 0 | 0.51 |
| 18 | 2-Phenylethanol | 1.0569 | 0.91 | 0.3 | 0.64 |
| 19 | 3-Phenyl Propanol | 1.1987 | 0.9 | 0.3 | 0.67 |
| 20 | <i>p</i> -Tolunitrile | 1.012 | 1.1 | 0 | 0.34 |
| 21 | <i>N,N</i> -Dimethylaniline | 1.098 | 0.84 | 0 | 0.42 |
| 22 | Benzyl Cyanide | 1.012 | 1.15 | 0 | 0.45 |
| 23 | Benzonitrile | 0.8711 | 1.11 | 0 | 0.33 |
| 24 | <i>p</i> -Nitrobenzyl Chloride | 1.1539 | 1.34 | 0 | 0.4 |
| 25 | Benzene | 0.7164 | 0.52 | 0 | 0.14 |
| 26 | Toluene | 0.8573 | 0.52 | 0 | 0.14 |
| 27 | Ethylbenzene | 0.9982 | 0.51 | 0 | 0.15 |
| 28 | propylbenzene | 1.1391 | 0.5 | 0 | 0.15 |

^a In this study, we use V_x , π_2^{*H} , $\sum\alpha_2^H$ and $\sum\beta_2^H$ for V_2 , π_2^{*H} , α_2 and β_2 in Eq. (2), respectively.

^b V_x represents a calculated solute molar volume using McGowan's method [68,69].

^c Values of π_2^{*H} , $\sum\alpha_2^H$ and $\sum\beta_2^H$ were obtained from Ref. [70].

Table 3
Summary of the LSER results^a

| Stationary | % (v/v) | log k'_0 | m | s | a | b | $-s/m$ | $-a/m$ | $-b/m$ | n | sd | r |
|---------------------------------|------------------|-------------|-----------|------------|------------|------------|--------|--------|--------|-----|-------|-------|
| - | Organic modifier | | | | | | | | | | | |
| HP-C ₁₈ ^a | 50 | -0.058±0.15 | 2.73±0.16 | -0.96±0.07 | -0.54±0.08 | -2.44±0.14 | 0.352 | 0.198 | 0.894 | 28 | 0.082 | 0.987 |
| C ₁₈ ^b | 50 | -0.59±0.16 | 2.99±0.16 | -0.59±0.07 | -0.45±0.08 | -2.36±0.13 | 0.197 | 0.151 | 0.789 | 22 | 0.085 | 0.980 |
| HP-C ₁₈ ^a | 40 | 0.003±0.17 | 3.20±0.21 | -1.04±0.09 | -0.55±0.09 | -2.69±0.17 | 0.325 | 0.172 | 0.841 | 28 | 0.089 | 0.982 |
| C ₁₈ ^b | 40 | 0.40±0.17 | 3.18±0.22 | -0.60±0.08 | -0.43±0.08 | -2.49±0.14 | 0.189 | 0.135 | 0.783 | 22 | 0.087 | 0.981 |

^a Phase HP-C₁₈ is a mixed propyl and octadecyl phase synthesized by the horizontal polymerization method.

^b Commercial octadecyl phase. Data taken from Ref. [59].

behavior of the solutes on this type of new phase is well correlated by the LSER model.

The large positive v and the large negative b are the main factors that control retention on this phase (see Table 3). This agrees with previous results [58,60] for conventional columns. However, there are differences between the two data sets. The v coefficient results from a combination of cavity and dispersion interaction effects. Although, the horizontally polymerized phase is a mixture of octadecyl and propyl groups, its v coefficient is very similar to a conventional octadecyl phase. This suggests that the octadecyl group of the horizontally polymerized phase is the major player in the stationary phase. Because the C₃ region is extremely densely packed (about 8 $\mu\text{mol m}^{-2}$), there is very limited amount of free-volume which can be penetrated by the solute. This means that the region does interact with the solutes is just the outer 15 carbons of the C₁₈ chains. The density of these chains is about the same as the density of a conventional phase. Thus, the v coefficient found in this LSER studies is very similar to those in other studies.

At this point, we are compelled to point out a very interesting observation. In preliminary work, a horizontally polymerized phase comprised of only *trichlorooctadecylsilane* was twice synthesized and tested chromatographically. The columns prepared from the material, despite the very high carbon content (27.7% w/w, corresponding to a surface density of 4.5 $\mu\text{mol m}^{-2}$ of bonded chains), showed very low retention and extremely poor peak shapes. The column could not be used. We believe that this poor performance resulted from the extremely low free-volume between the octadecyl chains. *It is hard*

to reconcile this result with an adsorption-like mechanism of retention.

The coefficient s indicates the effect of the solute dipolarity/polarizability on retention. The mobile phase is a highly dipolar medium as both its components, water and methanol, are strongly dipolar substances. In contrast, in the stationary phase, the bonded alkyl chains are incapable of dipole/induced dipole interactions ($\pi^*=0$), but the sorbed organic modifier ($\pi^*_{\text{methanol}}=0.6$) and water molecules ($\pi^*=1.17$) substantially increase the stationary phase's π^* value [61,62]. The net effect of sorption of mobile phase into the bonded phase is that there is only a small difference in the dipolarities of the stationary and the mobile phase [63]. Thus, a dipolar solute will only experience a relatively small decrease in π^* upon transfer from the mobile phase to the stationary phase. The resulting s coefficient should, therefore, be small and negative. The less negative is the s value, the less solvated is the stationary phase. The s coefficients observed (see Table 3) for the horizontally polymerized phase are statistically larger than those observed for the conventional phase in this study and in previous studies of alkyl bonded phase [59]. Thus, the difference of dipolarity/polarizability between the new phase and mobile phase should be, as experimentally found, larger than that of an ordinary octadecyl phase. This also suggests that the new stationary phase is less solvated by the mobile phase.

The a coefficient reflects the difference in the strength of interactions of hydrogen bond donor solute with the hydrogen bond acceptor mobile and stationary phases. The mobile phases used here are only moderately basic, and the bonded alkyl chains

themselves have no HBA basicity. However, sorbed eluent and residual silanol groups impact the hydrogen bond acceptor strength. Because the mobile phase is only slightly more basic than is a stationary phase equilibrated with mobile phase [61], based solely on its HB acidity, a solute should have only a slight preference for the mobile phase over the stationary phase, if we consider only its HB donor acidity. Thus, a negative and relatively small a coefficient is experimentally observed for both the horizontally polymerized phase and conventional stationary phase. The a coefficients are almost equal for both types of phases (see Table 3). The horizontally polymerized phases have a slightly more negative a coefficient suggesting that it is slightly weaker HB acceptor than the conventional phase.

The b coefficient, along with v , is the other main factor which controls retention. It reflects the difference in the strength of interaction of a hydrogen bond acceptor solute with a hydrogen bond donor mobile and stationary phase. The mobile phases used here is highly acidic, as bulk water ($\alpha = 1.17$) is an extremely strong HBD acid [58]. The bonded alkyl chains themselves have no HBD acidity. The large negative b coefficient observed in all cases indicates that the bonded phases are much weaker HBD acids compared to the mobile phase. The similar b coefficient for both the horizontally polymerized phase and ordinary C_{18} phases implies that this phase has the same hydrogen bond donating ability as do ordinary C_{18} phases.

From this LSER study, we conclude that horizontally polymerized phases have very similar cavity and dispersion interactions compared to conventional RPLC phases. The hydrogen bonding donor ability is similar to conventional octadecyl phases. They are slightly weaker hydrogen bond donors compared to conventional alkyl bonded phases, and tend to sorb less eluent.

3.6. The amount of adsorbed organic modifier of mobile phase in the stationary phase

It is very important to note that both the LSER study and the free energy study support our hypothesis that the amount of sorbed mobile phase components in a horizontally polymerized phase is less than in conventional monomeric phase. Accord-

ing to Martire, et al. [64], water is indirectly sorbed by reversed-phase materials. The solvent mixture cannot wet the reversed-phase at high water content, indicating that there is no intimate contact between the solvent mixture and the alkyl bonded phase. Water molecules are brought into the stationary phase by hydrogen bonding to the sorbed organic modifier. Thus, it seems valid to assume that the total amount of both sorbed mobile phase components is proportional to the amount of sorbed organic component.

Yonker et al. used a combination of gas and liquid chromatography [65,66] to measure the total amount of sorbed mobile phase components. Martire and Zepeda [64] have measured the total amount of sorbed mobile phase components by injecting isotopically labeled mobile phase components. In this study, we did not measure the exact amount of adsorbed mobile phase components. Instead, we merely compare the amount of sorbed methanol, hence the total amount of sorbed mobile phase components, in a conventional monomeric phase to that in a horizontally polymerized phase.

It is known that the retention of methanol is related to the amount of methanol sorbed in the stationary phase. The larger is the retention volume, the more methanol that is sorbed in the stationary phase. Because methanol is a constituent of the eluent, it does not follow the normal retention behavior of dilute solutes in RPLC. Thus, it is not wise to measure the retention volume of methanol by directly injecting methanol. To avoid this problem we used a homolog series of longer straight chain primary alcohols as our solutes, and measured their retention volumes. A plot of $\log k'$ vs. the number of the methylene groups gives a straight line from which the capacity factor of methanol, behaving as if it were infinitely dilute, can be obtained by extrapolation. Comparing the capacity factors of methanol obtained in this way for both types of phases (see Fig. 4), shows that the amount of adsorbed methanol, hence the amount of total sorbed mobile phase is larger for a conventional monomeric phase than for a horizontally polymerized phase.

It is also interesting to note the change in pattern of the dead volumes of both columns (see Fig. 5). The same batch of silica gel was used to make the conventional monomeric phase and the horizontally

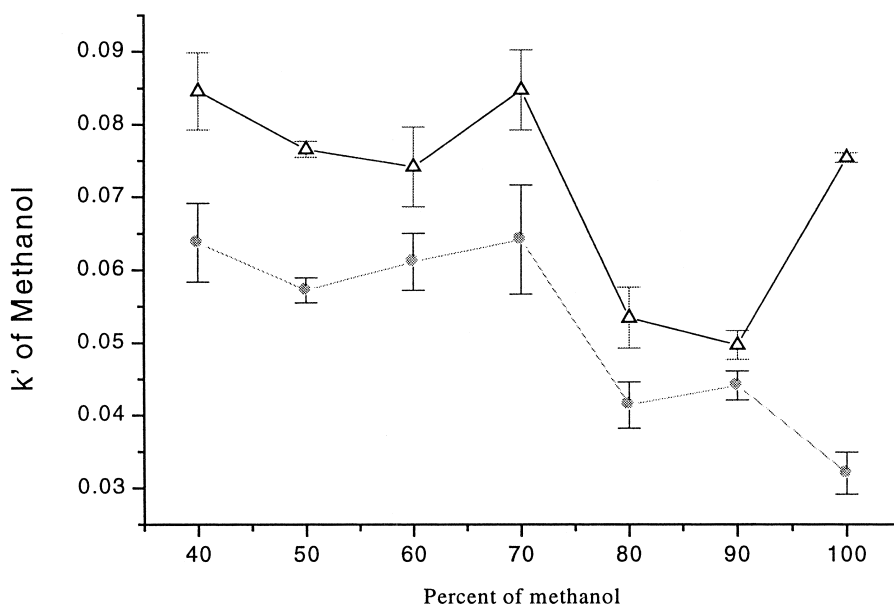


Fig. 4. Comparison of retention of methanol as a dilute solute on a conventional and horizontally polymerized phase. Retention is estimated by extrapolation of homolog series of alcohols as described in the text. Δ : k' of methanol of conventional ODS phase. \bullet : k' of methanol of horizontally polymerized C_3/C_{18} phase.

polymerized phase, and both were then packed in same size columns. Because the percent carbon loading is very similar (about 26.3% for the conventional monomeric phase vs. about 23.5% for the horizontally polymerized C_3/C_{18} phase), the dead volume of both columns should be similar. When the concentration of methanol in the mobile phase is 60% or less, the dead volumes are similar (see Fig. 5) with the dead volume of the horizontally polymerized column being slightly larger than that of conventional phase. This is mostly due to the packing process. However, when the concentration of methanol in mobile phase reached 70%, the dead volume difference increased and the dead volume of the horizontally polymerized column is smaller not larger than that of conventional phase. We interpret this change as indicating the more extensive sorption of mobile phase components on the conventional column. This is consistent with the data in Fig. 4 for retention of methanol discussed in the preceding section.

3.7. Alkaline stability

Toluene and *n*-pentylaniline were used as probe

solutes to evaluate the alkaline stability of the horizontally polymerized phase. If the stationary phase were hydrolytically removed by the high pH eluent, then due to loss of the bonded phase the retention time of toluene and *n*-pentylaniline should decrease and increase [67] respectively. At the same time, the peak shape of pentylaniline should become more tailed due to an increase in concentration of exposed silanol groups. Upon flushing the column with 2800 column volumes of pH 10 mobile phase (50:50 v/v 50 mM triethylamine–acetonitrile), there was no sign of stationary phase degradation, e.g. retention time change (Fig. 6) and no deterioration in peak shape. Clearly, this new phase is much stabler than a typical conventional alkyl bonded phase.

4. Conclusions

The horizontally polymerized material described by Wirth is a unique type of alkyl bonded stationary phase. By controlling the amount of adsorbed water on the silica gel surface and scrupulously removing water from the reaction solvent, reproducible surface polymerization of trichlorosilanes can be achieved

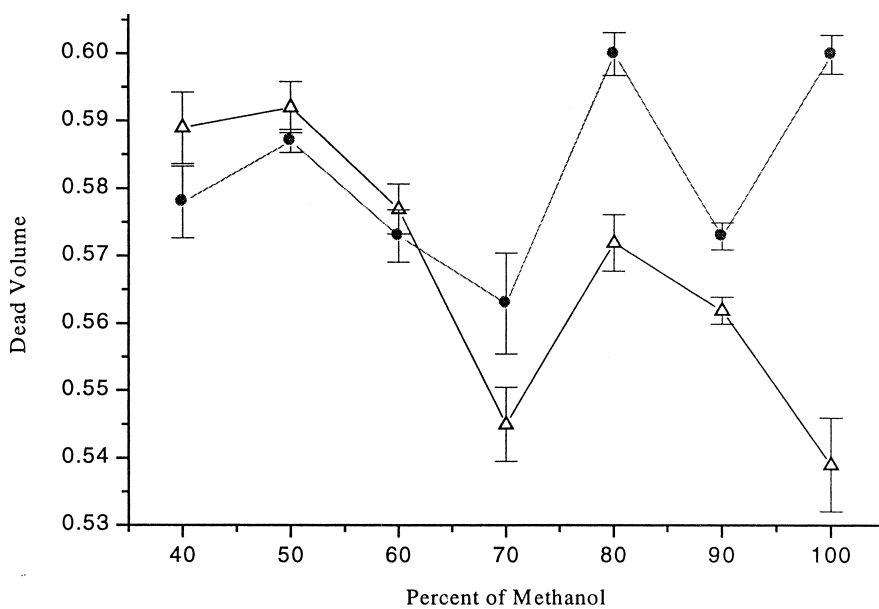


Fig. 5. Comparison of dead volume of conventional and horizontally polymerized phase as a function of mobile phase compositions. ●: horizontally polymerized C₃/C₁₈ column. △: conventional ODS column.

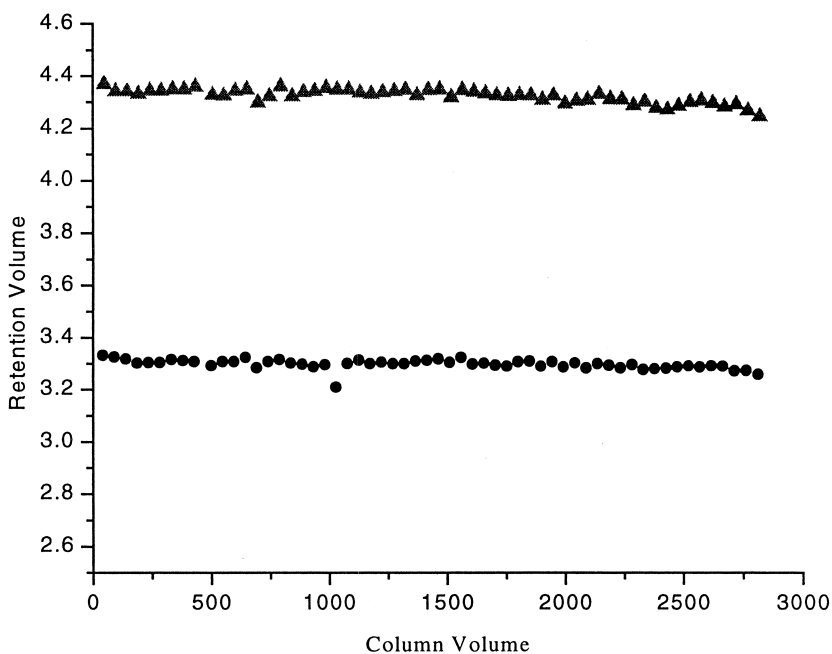


Fig. 6. Test of alkaline stability of a horizontally polymerized stationary phase. Mobile phase: 50% ACN and 50% 50 mM triethylamine buffer solution at pH 10. Eluent: (a) ●: toluene; (b) ▲: pentylaniline.

and a high surface coverage obtained fully confirming Wirth's results. This high surface carbon coverage significantly diminishes the influence of substrate, i.e., silica gel, on retention. Access of both the eluent and analyte to the surface silanol groups is reduced for this new material. Thermodynamic studies show that the ratio of the free energy of transfer of a nonpolar solute methylene group from mobile phase to this bonded phase to that for transfer of a solute from mobile phase to bulk hexadecane phase is very close to unity, over a wide range in mobile phase composition for methanol–water mixtures including methanol rich medium. We conclude that the principal retention mechanism of a nonpolar solute on this material is partitioning. Although trichlorosilanes were used for silanization, the new phase shows the same selectivity towards PAHs as do the so-called monomeric phases. The base stability of this new stationary phase is much better than that of conventional bonded phases. It is stable up to at least pH 10. LSER studies show that the horizontally polymerized phases have stronger dispersion interactions with solutes than do ordinary stationary phases. LSER studies also show that the new phase is a weaker hydrogen bond donor than ordinary alkyl bonded phases. Due to the lower accessibility of silanol groups, it tends to sorb less mobile phase than do ordinary stationary phase.

Acknowledgements

This work was supported in part by a grant from the National Science Foundation.

References

- [1] R.E. Majors, *LC·GC Mag.* 9 (1992) 686.
- [2] N. Saglian, R.A. Hartwick, R.E. Patterson, B.A. Woods, J.L. Bass, N.T. Miller, *J. Chromatogr.* 458 (1988) 225.
- [3] M.J.J. Hetem, J.W. de Haan, H.A. Claessens, I.J.M. van de Ven, C.A. Cramers, *Anal. Chem.* 62 (1990) 2288.
- [4] N.T. Miller, J.M. DiBussolo, *J. Chromatogr.* 499 (1990) 317.
- [5] J.J. Kirkland, J.L. Glajch, R.D. Farlee, *Anal. Chem.* 61 (1989) 2.
- [6] N. Sagliano Jr., T.R. Floyd, R.A. Hartwick, *J. Chromatogr.* 443 (1988) 155.
- [7] J. Köhler, J.J. Kirkland, *J. Chromatogr.* 385 (1987) 125.
- [8] J. Köhler, D.B. Chase, R.D. Farlee, A.J. Vega, J.J. Kirkland, *J. Chromatogr.* 352 (1986) 275.
- [9] J.J. Kirkland, J.J. DeStefano, *J. Chromatogr. Sci.* 8 (1970) 309.
- [10] R.E. Majors, *J. Chromatogr. Sci.* 12 (1974) 767.
- [11] J.J. Kirkland, *J. Chromatogr. Sci.* 9 (1971) 206.
- [12] J.H. Knox, G. Vasvari, *J. Chromatogr.* 83 (1973) 181.
- [13] E. Grushka, E.J. Kikta, *Anal. Chem.* 46 (1974) 1370.
- [14] M. Novotny, S.L. Bektesh, K.K. Grohmann, *J. Chromatogr.* 83 (1973) 25.
- [15] M.J. Wirth, O.H. Fatumbi, *Anal. Chem.* 64 (1992) 2783.
- [16] M.J. Wirth, O.H. Fatumbi, *Anal. Chem.* 65 (1993) 822–826.
- [17] H.O. Fatunmbi, M.D. Bruch, M.J. Wirth, *Anal. Chem.* 65 (1993) 2048.
- [18] M.J. Wirth, R.W. Peters Fairbank, H.O. Fatunmbi, *Science* 275 (1997) 44.
- [19] H.O. Fatunmbi, M.J. Wirth, *LC·GC* 12 (1994) 222.
- [20] R.W.P. Fairbank, Y. Xiang, M.J. Wirth, *Anal. Chem.* 67 (1995) 3879.
- [21] R.W.P. Fairbank, M.J. Wirth, *J. Liq. Chromatogr. Rel. Tech.* 19 (1996) 2799.
- [22] R.W.P. Fairbank, M.J. Wirth, *J. Chromatogr. A* 830 (1999) 285.
- [23] K.K. Unger, *Porous Silica*, Elsevier, Amsterdam, 1979.
- [24] J.G. Dorsey, K.B. Sentell, K.W. Barnes, Patent number: 4,919,804.
- [25] M.R. Böhmer, L.K. Koopal, R. Tijssen, *J. Phys. Chem.* 59 (1982) 257.
- [26] D.E. Martire, R.E. Boehm, *J. Phys. Chem.* 87 (1983) 1045.
- [27] R.P.W. Scott, C.F. Simpson, *Faraday Symp. Chem. Soc.* 15 (1980) 69.
- [28] J.G. Dorsey, K.A. Dill, *Chem. Rev.* 89 (1989) 331.
- [29] K.B. Sentell, J.G. Dorsey, *Anal. Chem.* 61 (1981) 930.
- [30] P. Claudy, J.M. Letoffe, D. Morel, J. Serpinet, *J. Chromatogr.* 329 (1985) 331.
- [31] A. Tchaplal, H. Colin, G. Guiochon, *Anal. Chem.* 56 (1984) 621.
- [32] A. Nahum, C. Horvath, *J. Chromatogr.* 192 (1980) 315.
- [33] L.C. Tan, P.W. Carr, *J. Chromatogr. A* 755 (1997) 1.
- [34] P.W. Carr, J. Li, A.J. Dallas, D.I. Eikens, L.C. Tan, *J. Chromatogr. A* 656 (1993) 113.
- [35] P.W. Carr, L.C. Tan, J.H. Park, *J. Chromatogr. A* 724 (1996) 1.
- [36] J.H. Park, Y.K. Lee, Y.C. Weon, J.W. Li, L. Li, J.F. Evanse, P.W. Carr, *J. Chromatogr. A* 767 (1997) 1.
- [37] L.C. Sander, S.A. Wise, *J. Chromatogr. A* 656 (1993) 335–351.
- [38] L.C. Sander, S.A. Wise, *J. Microcolumn Separations* 6 (1994) 115.
- [39] L.C. Sander, S.A. Wise, *Anal. Chem.* 67 (1995) 3284.
- [40] L.C. Sander, S.A. Wise, *Retention Selectivity Liquid Chromatography* 57 (1995) 337.
- [41] L.C. Sander, S.A. Wise, in: *Chromatographic Separation Based on Molecular Recognition*, 1997, pp. 1–64.
- [42] P.C. Sadek, P.W. Carr, *J. Chromatogr. Sci.* 21 (1983).
- [43] M.J. Kamlet, R.W. Taft, *J. Am. Chem. Soc.* 98 (1976) 377.
- [44] M.J. Kamlet, R.W. Taft, *J. Am. Chem. Soc.* 98 (1976) 2886.

- [45] M.J. Kamlet, R.W. Taft, *Acta Chem. Scand. B* 39 (1985) 611.
- [46] J.N. Kinkel, K.K. Unger, *J. Chromatogr.* 316 (1984) 193.
- [47] J.H. Knox, R. Kaliszan, *J. Chromatogr.* 349 (1985) 211.
- [48] K. Hideg, D. Lloyd, *J. Chem. Soc., C* (1971) 3441.
- [49] B. Buszewski, M. Jezierska, M. Welniak, D. Berek, *J. High Resol. Chromatogr.* 21 (1998) 267.
- [50] S.R. Wasserman, Y.T. Tao, G.M. Whitesides, *Langmuir* 5 (1989) 1074.
- [51] K.A. Dill, *J. Phys. Chem.* 91 (1987) 1980.
- [52] P.W. Carr, J. Li, A.J. Dallas, D.I. Eikens, L.C. Tan, *J. Chromatogr. A* 656 (1993) 113.
- [53] D.E. Martire, R.E. Boehm, *J. Phys. Chem.* 87 (1983) 1045.
- [54] R. Tijssen, P.J. Schoenmakers, M.R. Böhmer, L.K. Koopal, H.A.H. Billiet, *J. Chromatogr. A* 656 (1993) 135.
- [55] J. Li, in: Ph.D Thesis, University of Minnesota, 1992.
- [56] M.H. Abraham, M. Roses, *J. Phys. Org. Chem.* 7 (1994) 672.
- [57] M.J. Kamlet, M.H. Abraham, P.W. Carr, R.M. Doherty, R.Taft, *J. Chem. Soc. Perkin Trans. II* (1988) 2087.
- [58] L.C. Tan, P.W. Carr, M.H. Abraham, *J. Chromatogr. A* 752 (1996) 1.
- [59] F. Yugi, Z. Pengling, H. Zhide, *Chromatographia* 25 (1988) 382.
- [60] L.C. Tan, P.W. Carr, *J. Chromatogr. A* 799 (1998) 1.
- [61] M.H. Abraham, G.S. Whiting, R.M. Doherty, W.J. Shuely, *J. Chromatogr.* 587 (1991) 213.
- [62] A.J. Dallas, Ph.D Thesis, University of Minnesota.
- [63] J.L. Jones, S.C. Rutan, *Anal. Chem.* 63 (1991) 1318.
- [64] A.Z. Aurello Zepeda, D.E. Martire, *J. Chromatogr.* 550 (1991) 285.
- [65] C.R. Yonker, T.A. Zwier, M.F. Burke, *J. Chromatogr.* 241 (1982) 257.
- [66] C.R. Yonker, T.A. Zwier, M.F. Bueke, *J. Chromatogr.* 241 (1982) 269.
- [67] J. Köhler, D.B. Chase, R.D. Farlee, A.J. Vega, J.J. Kirkland, *J. Chromatogr.* 352 (1986) 275.
- [68] J.C. McGowan, *J. Chem. Technol. Biotechnol.* 34A (1984) 38.
- [69] M.H. Abraham, J.C. McGowan, *Chromatographia* 23 (1987) 243.
- [70] M.H. Abraham, G.S. Whiting, R.M. Doherty, W.J. Shuely, *J. Chromatogr.* 587 (1991) 213.