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Chromatographic evaluation of alkyl-bonded phases prepared through olefin hydrosilylation on a hydride–silica intermediate

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Abstract

The chromatographic characterization of alkyl-bonded silica-based stationary phases for HPLC is described. A previously established method involving catalytic hydrosilylation of terminal olefins on a hydride-modified intermediate was used to obtain octyl and octadecyl packings. Generally speaking, the resulting phases were found to be chromatographically equivalent (comparable retention and selectivity) to sorbents produced in a conventional manner using organosilanization. The new bonded phases are, however, significantly more stable toward hydrolysis than conventional bonded silicas. This highly desirable feature of the new bonded phases not only reduces the need for frequent column replacement but also provides enhanced long-term reproducibility of retention data. The new bonded phases exhibit good separation of polycyclic aromatic hydrocarbons, basic solutes and polypeptides.

1. Introduction

Advances in the surface modification of sorbents, especially silica, have led to the development of HPLC into a powerful analytical and preparative separation technique. Alkyl-bonded silicas have had great popularity for HPLC separations of solutes ranging from low-molecular-mass organic compounds to biopolymers. Currently, the most common approach to prepare alkyl-bonded silicas involves organosilanization, a reaction between fully hydroxylated silica and dimethylalkylchlorosilane, as follows:

$$|\text{Si–OH} + \text{ClSiMe}_2\text{R} \rightarrow |\text{Si–O–SiMe}_2\text{R} + \text{HCl} \qquad (1)$$

This reaction produces a uniform monolayer of non-polar moieties covalently attached to the substrate's surface through a siloxane linkage, Si–O–SiC. In a related approach, "polymeric" bonded phases are prepared by reaction of trifunctional organosilanes of the type X_3SiR with silica in the presence of a measured amount of water and a suitable solvent

$$|\text{Si–OH} + \text{X}_3\text{SiR} \xrightarrow[\text{Solvent}]{\text{H}_2\text{O}} |\text{Si–O–}\underset{\displaystyle \text{O}\atop\displaystyle |\atop\displaystyle \text{O}}{\overset{\displaystyle \text{Y}\atop\displaystyle |\atop\displaystyle \text{Y}}{\text{Si}}}\text{–R} \qquad (2)$$

where X is an easily hydrolyzable group (e.g., halide, alkoxy, acyloxy, etc.) and Y represents –H or –Si≡. Although the thickness of the attached organic layer may vary according to the reaction conditions, it has been suggested that

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most polymeric phases with surface coverages approaching those of materials made through reaction 2 are actually monolayer in nature [1].

Despite their wide utility, the bonded phases resulting from organosilanization suffer from two major drawbacks. Due to the steric hindrance of the R group, residual acidic silanols remain in varying degrees on the surface of alkyl-silicas. These unreacted species often result in strong interactions with certain basic solutes which lead to partial solute recovery, poor peak symmetry and non-reproducible retention. Perhaps the most stringent limitation of commercial alkyl-silicas lies in their unsatisfactory hydrolytic stability towards certain mobile phase compositions. Chemical deterioration of the bonded phase results in decreased retention and increased exposure of silanols, which in turn lead to poor long-term precision and degraded solute elution respectively.

To overcome the problems associated with the relatively poor hydrolytic stability of the siloxane linkage, alternate approaches involving the formation of direct Si–C linkages have been developed. The reaction of chlorinated silica with certain organometallic reagents has been used to accomplish this [2–4]

$$|\text{Si–Cl} + \text{RM} \xrightarrow[\text{ether}]{} |\text{Si–R} + \text{MCl} \qquad (3)$$

where M = MgBr or Li. Despite the hydrolytic advantage of the resulting Si–C linkage, bonded stationary phases prepared by this approach have not found widespread applicability, probably because the extreme reactivities of both the chlorinated silica and the organometallic reagent (Grignard or alkyl–lithium) make the process extremely cumbersome to carry out on a commercial scale.

We have recently developed a completely different approach for producing Si–C linkages on silica supports [5–7]. In this method, catalytic olefin hydrosilylation is used as the primary bonding reaction, according to

$$|\text{Si–H} + \text{CH}_2{=}\text{CH–R} \xrightarrow{\text{catalyst}} |\text{Si–CH}_2\text{–CH}_2\text{–R} \qquad (4)$$

The reaction involves the addition of surface silicon hydride to a terminal olefin. Silicon hydride groups are anchored to the silica surface by reaction with the hydrolysis products of triethoxysilane [7]

$$|\text{Si–OH} + (\text{EtO})_3\text{Si–H} \xrightarrow[\text{Dioxane}]{\text{HCl},\text{H}_2\text{O}} |\text{Si–O–}\underset{\displaystyle \text{O}\atop\displaystyle |\atop\displaystyle \text{Y}}{\overset{\displaystyle \text{Y}\atop\displaystyle \text{O}\atop\displaystyle |}{\text{Si}}}\text{–H} + 3\text{EtOH} \qquad (5)$$

where Y is defined as in reaction 2 above. While the bonded materials as well as the hydride intermediate have been previously evaluated by spectroscopic, thermal and chemical methods [5–7], there is still a need for an evaluation of the chromatographic performance of the new stationary phases. Such an evaluation is the subject of this work.

2. Experimental

2.1. Materials

Proteins and peptides, as well as 1-octadecene were obtained from Sigma (St. Louis, MO, USA). The standard mixture of polycyclic aromatic hydrocarbons (SRM869) was obtained from the National Institute of Standards and Technology (Gaithersburg, MD, USA). 1-Octene, dibenzo-18-crown-6, dibenzo-24-crown-8, trifluoroacetic acid (TFA), 1-phenylheptane, N,N-diethylaniline, N,N-dimethylaniline, aniline and phenol were purchased from Aldrich (Milwaukee, WI, USA). The barbiturate mixture was obtained from Alltech (Deerfield, IL, USA). A 100 m*M* hexachloroplatinic acid (37.5% as Pt, Aldrich) solution in 2-propanol was used as the catalyst for hydrosilylation. Bonded phases were prepared from a variety of commercially available silica sorbents whose physical properties are described in Table 1. LC-grade organic solvents were purchased from EM Science (Cherry Hill, NJ, USA). Deionized water was prepared on a

Table 1
Native silica sorbents used in this work.

Brand name	Lot	Particle diameter (μm)[a]	Pore diameter (nm)[b]	Specific surface area (m^2/g)[b]	Manufacturer
Vydac TP	890414	5.8	29.1	88.8	1
Vydac TP	900201	6.6	38.0	106.5	1
Vydac HS	900423	6.7	10.4	261.2	1
YMC-Gel	630212	10	13.8	314.0	2
Nucleosil	1021	7	30[a]	100[a]	3

Manufacturers: 1 = The Separations Group (Hesperia, CA, USA); 2 = Yamamura Chemical Laboratories Co., Ltd. (Kyoto, Japan); 3 = Macherey–Nagel (Düren, Germany).
[a] Data supplied by manufacturer.
[b] BET nitrogen adsorption method.

Millipore (Bedford, MA, USA) purification system.

2.2. Instrumentation

All chromatographic measurements were made with a Hewlett-Packard (Avondale, PA, USA) Model 1050 liquid chromatographic system equipped with quaternary gradient pump, automatic injector, variable-wavelength UV detector and computer data station. Columns were slurry-packed [10% (w/v) bonded silica in CCl_4–methanol (9:1, v/v)] into 15 cm × 0.46 cm I.D. stainless-steel tubes (Alltech) using a Haskel (Burbank, CA, USA) pneumatic pump at 40 MPa with methanol as the driving solvent. When required, column temperature was controlled by a Model CH-30 (Fiatron System, Oconomowoc, WI, USA) column heater. A Perkin-Elmer (Norwalk, CT, USA) Model 240C elemental analyzer equipped with a Perkin-Elmer Model 56 recorder was utilized for carbon determinations on bonded silicas. The specific surface area and mean pore diameter (Brunauer–Emmett–Teller, BET, nitrogen adsorption method) were determined at Chevron Research and Technology (Richmond, CA, USA) with a Micromeritics Model ASAP 2400. Data analysis and plotting were performed using RS/1 scientific spreadsheet software (BBN Research Systems, Cambridge, MA, USA).

2.3. Synthetic procedures

The C_8- and C_{18}-silicas were synthesized by olefin hydrosilylation as previously described [6]. The hydride intermediate was made according to a recently reported method [7]. Conventional octyl- and octadecyl-dimethylsilyl-silicas were prepared according to the procedures described by Berendsen et al. [8]. The concentration, α_R, of surface-bonded groups was obtained, as reported earlier [6] from the carbon content of the bonded material along with the BET specific surface area of the native silica substrate using the equation proposed by Berendsen and De Galan [9].

3. Results and discussion

3.1. Hydrolytic stability

The limited stability of the siloxane (Si–O–SiR) linkage has been recognized as the major source of hydrolytic instability of conventional silica-based stationary phases used in many HPLC bioseparations. The improved stability obtained through the formation of direct surface-to-carbon linkages strongly suggests that the siloxane and not the Si–C bond is the most hydrolytically labile portion of the organosiloxane structure. Short column lifetime, poor long-term precision and potential fraction contamina-

tion are the most evident deleterious effects arising from phase deterioration. Fraction contamination can be particularly disadvantageous in the case of preparative separations.

Recently, there has been a considerable activity aimed at evaluating stationary phase degradation [10–13]. Kirkland and co-workers [11,13], in their attempts to develop more stable silica-based separation materials, have studied degradation of bonded phases in a systematic way. The bonded phase degradation scheme used here was adapted from that developed by these authors. A mobile phase consisting of 0.10% (v/v) TFA in acetonitrile (solvent A) and 0.10% (v/v) TFA in water (solvent B) was passed at 1.00 ml/min through a packed column maintained at 50°C; the mobile phase composition cycle used is depicted in Fig. 1. The retention of 1-phenylheptane (10-μl injection of 0.1 mM solution in 50% solvent A) was used as an indirect measure of the alkyl coverage remaining on the bonded phase as it becomes degraded. This measurement was made during the initial isocratic period of the cycle and then repeated under the same conditions after subjecting the column to the entire mobile phase cycle. Column void volume was determined using KNO_3 (0.05 mM) as an unretained marker. Normalized retention data for 1-phenylheptane, in the form of fraction of remaining k', as a function of the number of column volumes passed through the bonded silica bed are shown in Fig. 2 for several columns tested. Clearly, all bonded phases undergo degradation under the test conditions, but the hydrosilylation product does so at a significantly slower rate. The loss of retention for the latter was only about 10% during the entire test period while, under the same conditions, conventional phases lost 35% or more. It is also apparent that lightly loaded phases (curve C of Fig. 2) are more rapidly degraded by the TFA-containing mobile phase, as expected. There does not seem

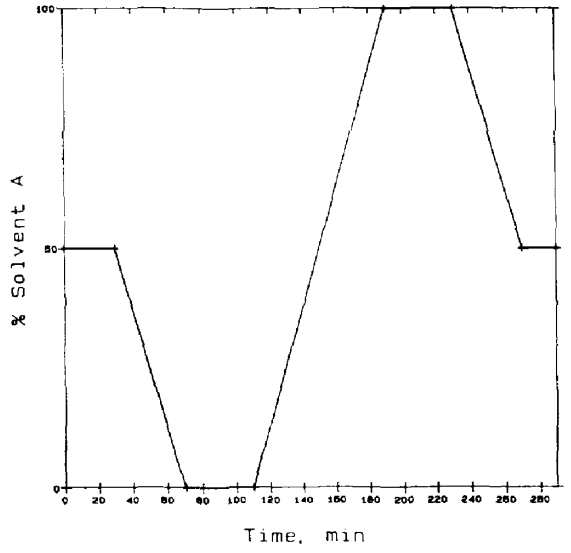


Fig. 1. Solvent composition cycle used for the long-term chromatographic hydrolysis test in TFA-containing mobile phase. Solvent A, 0.10% (v/v) TFA in acetonitrile; solvent B, 0.10% (v/v) TFA in water; flow-rate, 1.0 ml/min; column temperature, 50.0 ± 0.1°C.

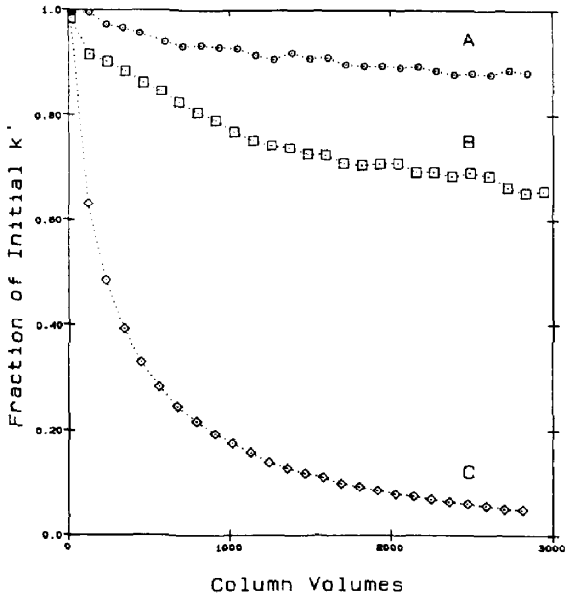
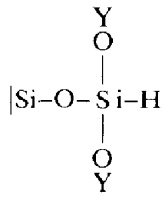


Fig. 2. Relative loss of retention for 1-phenylheptane on C_8-Vydac TP as a function of column volumes of mobile phase passed through the bonded phase during the long-term hydrolysis test. Retention (k') for 1-phenylheptane was measured during the initial isocratic period depicted in Fig. 1. Curves: A = hydrosilylation product (initial $\alpha_R = 3.2$ μmol/m^2, initial $k' = 5.9$, BET surface area $S_{BET} = 89$ m^2/g); B = conventional organosilanization product (initial $\alpha_R = 3.1$ μmol/m^2, initial $k' = 11.7$, $S_{BET} = 106$ m^2/g); C = low-coverage, conventional organosilanization product (initial $\alpha_R = 1.7$ μmol/m^2, initial $k' = 1.9$, $S_{BET} = 89$ m^2/g).

to be, however, a simple first-order rate function that fits any of the experimental data. Additionally, it should be noticed that, although the alkyl surface coverages for the upper and middle curves are comparable, the latter –the conventional phase– exhibits an unusually high retention towards the non-polar probe. One possible reason is that the organosilanization product was prepared from a silica having a higher surface area (106 versus 89 m^2/g). This observation alone, however, cannot account for the almost double retention shown by this phase. Neither can the presence of the two additional methyl groups at the base of the anchored octyl chain. The conventional octyl phases whose data are shown in Fig. 2 (curves B and C) correspond to the upper and lower hydrolytic stability limits found in similar phases prepared from the silica sorbents of Table 1; other conventional C_8 phases show stabilities which lie between the middle and lower curves.

Additional evidence that the bonded alkyl groups were actually lost from the tested sorbents can be obtained from carbon content and coverage data in Table 2. Again, it is quite evident that in the case of the conventional materials, a significant fraction of the bonded phase is lost as a result of the long-term elution with the TFA-containing mobile phase. In contrast, the bonded phase prepared by hydrosilylation via hydride maintains most of its carbon content upon the same hydrolysis regime. The present data also confirms the results from a previous non-HPLC study [6], in which elemental analysis was used to monitor the effects of phase degradation.

It seems clear that, compared to conventional organosilanization, olefin hydrosilylation on an SiH-containing substrate produces significantly more stable bonded phases. The superior stability of the new product is, very likely, a direct result of the greater hydrolytic strength of the Si–C linkage as compared to the siloxane Si–O–SiR linkage of conventional bonded phases. To some readers, the last statement may require further elaboration. While the necessity of representing pictorially the product of reaction 5 as



may lead to an apparent structural equivalence between the hydrosilylation product (reaction 4) and a polymeric phase (reaction 2), such a correspondence must not be taken literally. It should be emphasized that albeit the underlying siloxane layer in the new bonded phase is produced by a trifunctional (hydro)silane, it is far more efficiently attached to the silica surface than that of *any* trifunctional alkylsilane. This, naturally, is in virtue of the *minimal* size of the hydride group. By means of ^{29}Si cross-polarization (CP) NMR we have shown that about 90%

Table 2
Carbon, coverage and retention data for octyl-bonded silicas (Vydac TP) subjected to long-term hydrolysis test with TFA-containing aqueous acetonitrile mobile phases

Bonded phase[a]	Curve Fig. 2	Carbon content (%, w/w)[b]		Surface coverage (μmol/m^2) (initial)	k' for 1-phenylheptane		Column volumes
		Initial	Final		Initial	Final	
1	A	4.14	3.88	3.2	5.9	5.1	2842
2	B	4.22	3.24	3.1	11.7	7.6	2942
2	C	1.71	0.65	1.7	1.9	0.1	2813

[a] 1 = Product of 1-octene hydrosilylation; 2 = conventional octyldimethylsilyl product.
[b] Uncorrected.

of the hydride-bearing species in the intermediate are fully condensed (via formation of three siloxane linkages) whereas its presumably "analogue" polymeric C_8 phase exhibits only about 50% efficiency [7]. For steric reasons, this figure must be lower for a C_{18} phase. Thus, based on known NMR evidence, one can say that representing silica as |Si–OH (single silanol) is roughly 70% accurate [14], while representing the hydride intermediate as |Si–H is about 90% accurate and representing a "polymeric" phase as |Si–R is only 50% accurate if $R = C_8$ [7], or less if $R = C_{18}$. Another possible interpretation is that hydrosilanetriol, $HSi(OH)_3$, appears to be attached to the silica surface in the hydride intermediate more efficiently than silicic acid, $Si(OH)_4$, in native silica (not really surprising since a hydride group is smaller than a hydroxyl group). While this argument may seem like stretching the spectroscopic evidence, very few researchers would question the fact that the outermost layer of the silica surface is indeed an integral part of the substrate. Furthermore, if the new phase had been prepared through the much less attractive chlorination–reduction–hydrosilylation sequence [5,6], that is

$$|\text{Si–OH} \xrightarrow{SOCl_2} |\text{Si–Cl} \xrightarrow{LiAlH_4}$$
$$|\text{Si–H} \xrightarrow{CH_2=CH-R} |\text{Si–CH}_2\text{CH}_2\text{R}$$

neither the attachment of the underlying siloxane layer nor the monomeric nature of the final phase would likely be questioned. The only structural difference between this material and the current product is a higher population of unreacted SiH species underneath the anchored alkyl chains. Since the anchored SiH species in the hydride intermediate are an integral part of the silica surface, the formation of a true *surface*-to-carbon linkage (Si–C) via hydrosilylation should now be readily rationalized. Naturally, one can expect these materials to exhibit the hydrolytic stability of bonded phases prepared by a Grignard or a related method.

The practical importance of this hydrolytic advantage is evident:

(i) After several thousands of column volumes of a mobile phase containing aqueous TFA, a conventional bonded silica loses its anchored organic material to such an extent that the column has to be replaced by a new one. Because of the superior hydrolytic stability of the Si–C linkage (the predominant species in the new bonded packings) the useful lifetime of the column will be significantly increased. Obviously, the hydrolytic advantage means lower operating costs for HPLC separations.

(ii) From an analytical point of view, the long-term precision of retention is compromised in the case of a conventional bonded phase used under the conditions described above. Under the same conditions, the new bonded phases also degrade, but more slowly and therefore the long-term precision should be less limiting.

(iii) Another practical consequence of the loss of bonded phase during use is that significant organosiliceous material must be washed off the column during gradient elution. Elution of such hydrolytic degradation by-products would have deleterious consequences, particularly for preparative or process separations where fraction contamination may become a serious problem.

We also did some exploratory work on the hydrolytic stability of new and conventional bonded phases at basic pH by using neat acetonitrile as solvent A and replacing the aqueous TFA solution with 25 m*M* phosphate pH 9.0 in solvent B. The conventional C_8 phase (Vydac TP, initial $\alpha_R = 3.1$ μmol/m^2, initial $k' = 18.0$) virtually collapsed, as indicated by repeated pressure buildup and subsequent outlet frit clogging. This resulted in complete interruption of the test after only 1200 column volumes and an overall loss in retention for 1-phenylheptane of about 55% after that period. Although the C_8 phase prepared from hydrosilylation (Vydac TP, initial $\alpha_R = 3.2$ μmol/m^2, initial $k' = 4.7$) endured the whole test (about 2800 column volumes) without any abnormal pressure buildup, only about 50% of its retention remained by the end of the experiment. It is obvious that none of the bonded phases tested could withstand prolonged exposure to mobile phases containing aqueous high-pH buffers without undergoing unacceptable retention degradation in the case of our new

phase or disastrous phase collapse in the case of conventional bonded phases. Despite some advantage of the new bonded phases under the test conditions reported here, it seems clear that the inherently low hydrolytic stability of the siloxane linkages in the silica backbone is the primary limiting factor for this type of substrate.

3.2. Selectivity for polycyclic aromatic hydrocarbons

One very important application of reversed-phase (RP) chromatography is in the separation of polycyclic aromatic hydrocarbons (PAHs). Chemically bonded C_{18} phases are the most popular packings for the separation of PAHs. Although neutral solutes such as hydrocarbons do not exhibit elution problems such as peak tailing due to slow desorption kinetics, in some cases, particularly with structurally similar PAHs, separations are often difficult and sometimes impossible.

A simple empirical HPLC test has been developed by Sander and Wise [1,15–17] to assess the monomeric or polymeric nature of a reversed-phase material. The test mixture (known as SRM869) is an acetonitrile solution of three PAHs: benzo[*a*]pyrene (BaP), 1,2:3,4:5,6:7,8-tetrabenzonaphthalene (TBN) also known as dibenzo[*g*,*p*]-chrysene, and phenanthro[3,4*c*]-phenanthrene (PhPh). The elution order of the mixture is thought to be strongly dependent on the type of phase. Under isocratic conditions of acetonitrile–water (85:15, v/v) at 25 ± 2°C and 2.0 ml/min., monomeric C_{18} phases (see reaction 1) are expected to elute in the order BaP ≤ PhPh < TBN, while corresponding polymeric phases (see reaction 2) give the elution order PhPh < TBN ≤ BaP. Phases with intermediate properties (that is, heavily loaded monomeric or lightly loaded polymeric C_{18} phases) show elution order PhPh < BaP < TBN. Semiquantitative comparisons of different C_{18} phases have been made in terms of the selectivity factor of TBN with respect to BaP, that is, $\alpha_{TBN/BaP}$ (defined as the ratio k'_{TBN}/k'_{BaP}, where k' refers to the solute's retention factor). Polymeric phases show $\alpha_{TBN/BaP}$ values less than or equal to one, while monomeric phases have $\alpha_{TBN/BaP}$ greater than 1.7.

In order to avoid direct comparisons with literature figures derived from an empirical generalization, new and conventional bonded phases were prepared from the same silica substrate and the chromatographic tests were carried out under identical conditions. As shown in Table 3, it appears that, albeit the surface octadecyl densities of all phases were comparable and typical of monomeric C_{18}-silicas, the elution order of the mixture in four of the six phases corresponds to that of intermediate materials, that is, "heavily loaded monomeric" phases, according to the nomenclature used by Sanders and Wise. Additionally, the intermediate character of *all* phases shown is indicated by a selectivity of TBN/BaP consistently greater than 1 but smaller than 1.7. Notice also that while the new phases consistently exhibit α values slightly lower (average 1.34 ± 0.06, $n = 3$) than those for the corresponding octa-

Table 3
Retention and selectivity data for the three-component standard SRM869 mixture on octadecyl silicas

Silica	Bonded phase[a]	α_R (μmol/m^2)	k_{BaP}	k_{PhPh}	k_{TBN}	$\alpha_{TBN/BaP}$
Vydac TP	1	2.35	3.35	3.35	5.27	1.57
Vydac TP	2	2.48	2.93	2.70	4.03	1.37
Nucleosil	1	2.60	4.11	4.31	6.82	1.66
Nucleosil	2	2.96	2.60	2.25	3.31	1.27
YMC-Gel	1	2.65	9.25	8.77	14.64	1.58
YMC-Gel	2	2.66	9.42	7.90	13.00	1.38

[a] 1 = Conventional octadecyldimethylsilyl product; 2 = product of 1-octadecene hydrosilylation.

than those for the corresponding octadecyldimethylsilyl products (average 1.60 ± 0.05, $n = 3$), none of the latter were ≥ 1.7 either. The important issue here is, nevertheless, that the selectivity data of the C_{18} phases prepared by hydrosilylation resemble those of the intrinsically monomeric conventional phases prepared by organosilanization, as expected. Fig. 3 illustrates typical separations of the test mixture in two silicas. It is interesting to note that the new phases appear to provide slightly but consistently weaker overall retention than the conventional ones.

Separations of a more complex mixture consisting of sixteen priority pollutant PAHs on several C_{18} phases (not shown) indicate that, in all cases, only partial separation of closely related isomers was achieved, a behavior which appears to be characteristic of monomeric phases [1,6]. It seems reasonable to conclude that selectivity of the octadecyl bonded phases prepared from olefin hydrosilylation is comparable to that exhibited by their conventional octadecyl-dimethylsilyl counterparts.

3.3. Mobile phase effects and silanophilic interactions

It has been proposed by Horváth and coworkers [18,19] that under regular RP conditions, (i.e., those with a mobile phase which is more polar than the stationary phase), retention

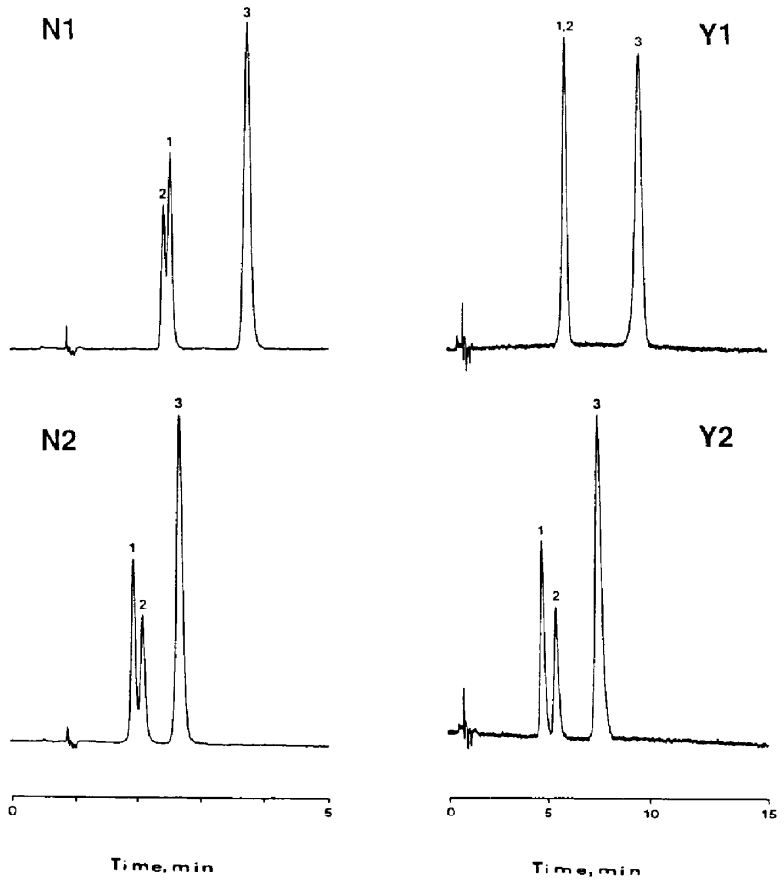


Fig. 3. Typical separation profiles for the SRM869 PAH mixture on C_{18} phases prepared from two brands of silica by two different methods. (N1) Nucleosil, conventional organosilanization product; (N2) Nucleosil, hydrosilylation product; (Y1) YMC-Gel, conventional organosilanization product; (Y2) YMC-Gel, hydrosilylation product. The mixture was chromatographed isocratically with acetonitrile–water (85:15, v/v) at 2.0 ml/min and UV detection at 254 nm. Peaks: 1 = PhPh; 2 = BaP; 3 = TBN.

is due to the superimposed contribution of two mechanisms, characterized by different modes of solute binding to the alkyl-derivatized silica surface: (i) regular solvophobic interactions and (ii) silanophilic interactions. The practical importance of the model is that it rationalizes the unusual behavior (non-linear logarithmic k' versus ψ plots, with ψ being the volume fraction of water in a binary mobile phase) observed with probes which are known to be particularly sensitive to silanophilic interactions. For instance, many biologically important solutes such as peptides having free amino groups exhibit this behavior [20,21].

There are several structural differences between conventional monomeric reversed phases (made via reaction 1) and those resulting from olefin hydrosilylation on hydride–silicas (reaction 4). Differences in the anchored moieties lie in the dimethylsiloxane linkages at the bottom of the attached group. In general, solute binding with these small groups should be rather weak and, therefore, from a solvophobic point of view the hydrocarbonaceous layers of the two alkyl-silicas are virtually equivalent. In conventional bonded phases at least 50% of the original silanols are left unreacted after their preparation. On the other hand, in the new bonded phase we find mostly SiH groups underneath the alkyl moieties. The low polarity of such SiH species should decrease even further the overall polarity of the bonded material. Thus, generally speaking, we expect to have a more hydrophobic bonded phase than the conventional one and, therefore, mostly solvophobic interactions should govern retention on these new bonded phases. This expectation, however, does not necessarily mean that silanols are absent from the new bonded phases. Although during the reaction of the silica with triethoxysilane (TES) (see reaction 5) most of the silanetriols formed upon hydrolysis are fully condensed, there are a few silanols that are not totally removed, as proved by IR and NMR [7].

In order to explore the possibility of obtaining some insight on the extent at which silanophilic and solvophobic interactions are present in both the conventional and the new RP phases, logarithmic k' versus ψ plots were developed for a series of octyl-bonded materials prepared from the same silica substrate. Crown ethers dibenzo-24-crown-8 (DB24C8) and dibenzo-18-crown-6 (DB18C6) were used as silanol-sensitive probes. Triplicate k' measurements at 30°C were made after prolonged column preconditioning, as described elsewhere [18,19]. A single lot of Vydac TP silica was used to eliminate any difference arising from any batch-to-batch substrate variation. Figs. 4 and 5 summarize the experimental results for DB24C8 and DB18C6, respectively. In addition to the C_8 phases, native and hydride sorbents are also included for comparison purposes. Although qualitatively the retention behavior of the crown ethers measured with C_8-Vydac TP silica resembles that found by Horváth and co-workers [18,19] with C_{18}-Partisil (i.e., k' increases with both low as well as high volume fraction of acetonitrile in water), simple analysis of the experimental data in terms of the dual

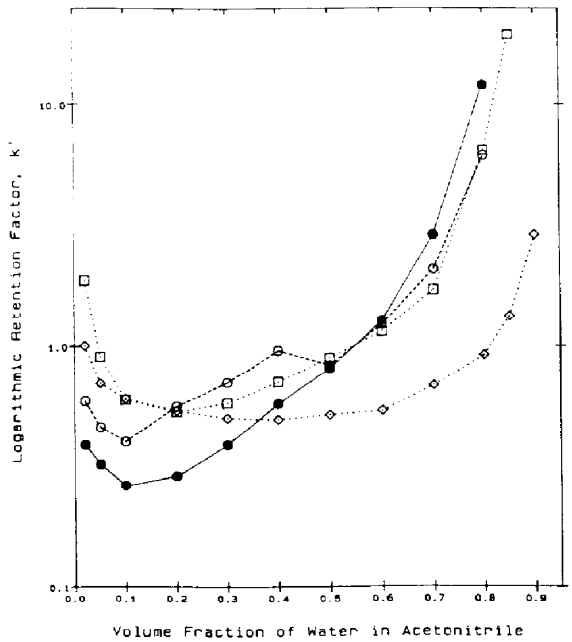


Fig. 4. Plots of the logarithmic retention factor of DB24C8 against the volume fraction of water in aqueous acetonitrile eluent, as a function of the surface chemistry of the sorbent. Curves: ◇ = bare silica; □ = hydride intermediate; ○ = conventional octyldimethylsilyl phase ($\alpha_R = 2.1$ μmol/m^2); ● = octyl phase from hydrosilylation ($\alpha_R = 3.2$ μmol/m^2). Conditions given in the text.

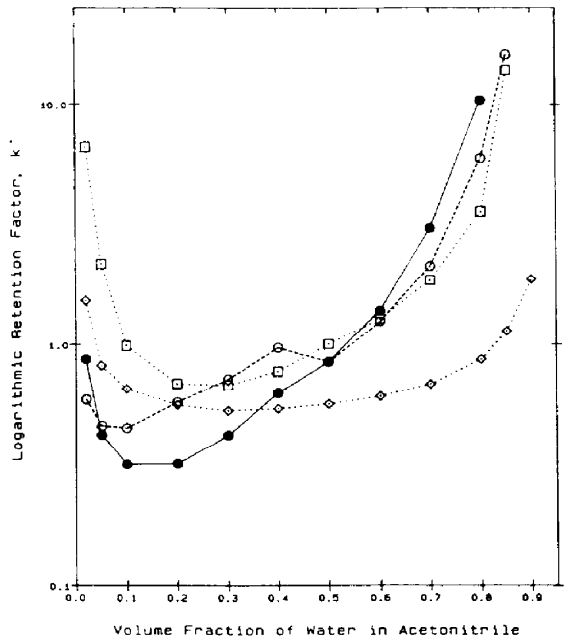


Fig. 5. Plots of the logarithmic retention factor of DB18C6 against the volume fraction of water in aqueous acetonitrile eluent, as a function of the surface chemistry of the sorbent. Curves as in Fig. 4.

retention equations [18,19] was not possible due to the lack of an acceptable fit. Retentions greater than those predicted by the dual model were consistently observed for mobile phase compositions where $0.1 < \psi < 0.6$. Both the conventional and the new C_8-bonded phases exhibit this behavior. These results suggest that, under the conditions used, additional or different binding modes may be operative and, as a consequence, the dependence of k' on eluent composition appears to be a more complex one. Despite this, some important qualitative observations can still be made about the data. Clearly, bare silica exhibits the lowest retention when water is the major component of the mobile phase ($\psi < 0.4$), in agreement with its essentially hydrophilic (silanol-populated) surface. On the other hand, surprisingly, the hydride and not the bare material shows the greatest retention at low water concentration in the mobile phase ($\psi < 0.1$), particularly in the case of DB18C6. Evidently, some very active sites are still present on the hydride surface and are readily accessible for interaction with the crown ring. If it is assumed that isolated silanols on bare silica are essentially removed during silanization with TES (reaction 5), partially condensed species of the type $\equiv$SiH(OH) might be responsible for the unusually high retention on the hydride material. Surprisingly again, at high water concentration in the eluent ($\psi > 0.8$) the hydride exhibits solvophobic retention for crown ethers as strong as that with the conventional C_8-bonded silica. Due to the greater alkyl coverage, larger k' values are shown by the new C_8 phase, as expected. It can be concluded that, although significant departure of the data from the dual model was observed, the general pattern exhibited by silanol-sensitive probes such as crown ethers is qualitatively followed in our case; that is, the logarithmic retention factor is larger at low as well as high water concentrations in the eluent, and a minimum is exhibited at intermediate compositions.

3.4. More on retention of basic solutes

Although crown ethers such as those tested above are sensitive probes for silanophilic interactions, a practical limitation of many RP sorbents lies in the separation of solutes containing basic nitrogen functionalities. These are frequently eluted with varying degrees of peak tailing depending on the accessibility of highly acidic silanol groups on the modified silica surface. To further compare the extent of silanophilic interactions in the new and conventional phases, an empirical HPLC test was utilized. The test, recently proposed by Engelhardt and coworkers [22,23], relies on the combined use of retention factors and peak asymmetries to characterize the chromatographic behavior of selected eluites, including basic solutes. The test solutes include, among others, toluene as a hydrophobic probe, aniline and N,N-dimethylaniline (N,N-DMA) as polar, basic probes and phenol as a neutral, weakly retained reference probe. The later compound has a polar, nonbasic group and therefore should not exhibit any strong acid–base interaction with the stationary phase. Consequently, any peak asymmetry arising from sources other than silanophilic interac-

tions will affect all solutes, while the later will affect only the basic probes. Under the conditions stipulated by the empirical test, isocratic elution with methanol–water [55:45, v/v (49:51, w/w)] at 1.5 ml/min., an RP phase is classified as "good" for the separation of basic solutes if the following requirements are fulfilled [22]: (i) $k'_{\text{aniline}} \leq k'_{\text{phenol}}$; (ii) $k'_{\text{N,N-DMA}} \leq k'_{\text{toluene}}$; and (iii) $A_{\text{s,aniline}}/A_{\text{s,phenol}} < 1.3$ (here A_s represents peak asymmetry as defined elsewhere [24]). Table 4 shows relevant data for two pairs of C_{18}-silicas modified by olefin hydrosilylation and conventional organosilanization. A detailed examination of the data reveals that, according to the empirical test, all bonded phases tested were "well behaved". It appears that the method of surface derivatization does not have a significant effect on the retention characteristics of the solutes under test. Any unfavorable retention of basic solutes in a given bonded phase can rather be attributed to the intrinsic adsorptivity of the native silica support [14,25]. Since it is the derivatization method and not the native silica support what is under study here, this hypothesis was not tested during this work. Furthermore, passing this empirical test does not constitute a sine qua non condition for a good bonded phase. In fact, based on statistical evidence, Schmitz et al. [26] have recently questioned the use of N,N-DMA as a truly silanol-sensitive probe. A cursory literature search reveals that virtually every research group establishes its own set of test compounds for evaluating chromatographic packings. Needless to say, all claim their selection is *the* test mixture, but an objective analysis always arrives at the conclusion that a really good LC column packing is one that gives narrow, symmetric peaks with reproducible retention characteristics for *all* solutes. Naturally, this observation is only a reminder of the many difficulties associated with the lack of standardization in the field of separations, which in turn reflects the complexity of the materials used. Generally speaking, pharmaceutical and related industries select their own product(s), either a basic drug, a peptide or a protein. More commonly, off-the-shelf reagents like simple amines containing a strong chromophore (e.g.: aniline and its derivatives) are the probes of choice. While the former approach responds to a "real world" situation, the second one involves a readily available, structurally simple and, perhaps, easy to model eluite. In our search for a sensitive probe for silanophilic interactions, we found that, compared to aniline and N,N-DMA, N,N-diethylaniline (N,N-DEA) shows a higher susceptibility, even though the latter compound seems intrinsically more hydrophobic than the other two. It must be clear that appropriate mobile phase conditions are required for this behavior to be exhibited. The water content in the mobile phase must be sufficiently high for convenient retention of the polar compounds to occur but not so great as to allow solvophobic interactions to cause the collapse of the anchored alkyl groups ("bristles"). In the absence of mobile phase additives, such a balance occurs at water contents ranging between 30–50% (v/v). At lower water content or with acetonitrile as the organic mobile phase component, retention

Table 4
Retention data for selected probes on octadecyl silicas

Silica	Type[a]	Phenol, k'	Aniline, k'	Aniline, rel. A_s[b]	N,N-DMA, k'	Toluene, k'
Vydac TP	1	0.90	0.82	1.1 ± 0.1	3.03	4.14
Vydac TP	2	0.84	0.86	1.2 ± 0.1	2.83	3.05
YMC-Gel	1	2.12	1.75	1.2 ± 0.1	10.15	14.69
YMC-Gel	2	1.94	1.88	1.4 ± 0.2	10.06	12.33

[a] 1 = Conventional octadecyldimethylsilyl product; 2 = product of 1-octadecene hydrosilylation.
[b] Relative asymmetry factor = $A_{\text{s,aniline}}/A_{\text{s,phenol}}$.

of basic solutes is weaker and with well-shaped elution profiles. We have compared the behavior of these and many other compounds, including basic drugs, peptides and proteins and found N,N-DEA to be one of the most sensitive solutes to these interactions, as evidenced by increased retention concomitant with considerable band tailing. When the four packings of Table 4 were tested with N,N-DEA, they all exhibited broad and asymmetric chromatographic profiles to varying degrees. In some cases this compound eluted with very low recovery, as evidenced by peak area comparisons. Additionally, the probe showed decreasing retention times along with improving peak shape upon consecutive injections. These observations suggest that this compound is strongly adsorbed on scarce but highly active sites which are still accessible on the silica surface. These tailing-producing sites are rendered inactive by strong binding with the organic amine upon consecutive injections. This phenomenon which is much less pronounced in the case of N,N-DMA, aniline and other basic probes, including basic proteins, has also been reported by Kirkland and co-workers [14,25]. It should be pointed out, however, that we were unable to find any correlation between the degree of retention of N,N-DEA and the method of preparing the bonded phase. It seems, nevertheless, that the intrinsic adsorptivity of the silica substrate plays a considerable role in the remarkably slow desorption kinetics of this sensitive chromatographic probe as suggested by this and previous studies [14,25]. When the hydride intermediate was also examined with respect to the elution of N,N-DEA, again, strong retention along with substantial tailing was observed in varying degrees, depending on the native silica as well as the hydride-producing reaction conditions (namely TES, HCl and water concentrations, see Ref. [7]). A more extensive study of the use of N,N-DEA as a silanol-sensitive probe is currently underway. It should be pointed out that this behavior could not be anticipated from our extensive spectroscopic characterization of the hydrosilylation product and its hydride intermediate. Chromatography appears to be considerably more sensitive in this respect than spectroscopy.

The basic probes were also used to evaluate the occasionally observed effect of darkening of the bonded silica product during olefin hydrosilylation [6]. Product darkening is more pronounced at relatively high reaction temperatures (above 110°C) and has been attributed to the reduction of the Pt(II) catalyst complex to elemental platinum by the surface SiH groups [27,28]. The catalytic activity of platinum is significantly decreased or completely lost as a result of this reaction. The test mixture was injected into columns containing darkened C_8- and C_{18}-silicas. The results (not shown) consistently indicate strong tailing for basic probes and, again, particularly for N,N-DEA. The effect of decreasing retention times of this solute (and to a lesser extent that of N,N-DMA) upon repeated injections with concomitant peak shape improvement was more pronounced in the case of darkened bonded phases, indicating a stronger adsorption of the organic amine(s). Clearly, product darkening must be avoided by careful choice of the catalyst and/or the reaction temperature.

The ability of a C_{18}-bonded silica (prepared via olefin hydrosilylation) for the separation of a mixture of alkaloids containing five barbital derivatives is shown in Fig. 6. Clearly, baseline separation of all sample components with good peak shape is obtained in about 10 min. This separation is a typical example of the applicability of the new bonded phases to pharmaceutical analysis.

Another important application involves certain macromolecular solutes such as proteins which, due to their structural complexities, appear to be particularly susceptible to being tenaciously adsorbed on many reversed phases. The incorporation of TFA or low pH phosphate buffers into aqueous acetonitrile or 2-propanol solvent mixtures has resulted in improved peak shapes. In some cases, particularly in preparative separations, TFA is preferred because is easily removed from the separated fraction by lyophilization. We have already demonstrated that the new

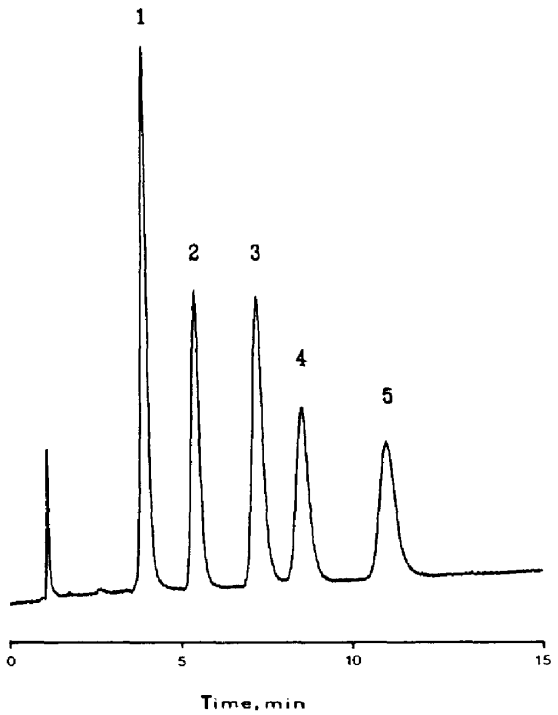


Fig. 6. RP separation of a commercial barbiturate mixture on a C_{18}-YMC-Gel prepared via hydrosilylation. Mobile phase: methanol–water (50:50) at 1.0 ml/min; sample injection: 10 μl; detection: UV at 220 nm. Peaks: 1 = butabarbital; 2 = amobarbital; 3 = secobarbital; 4 = phenobarbital; 5 = hexobarbital.

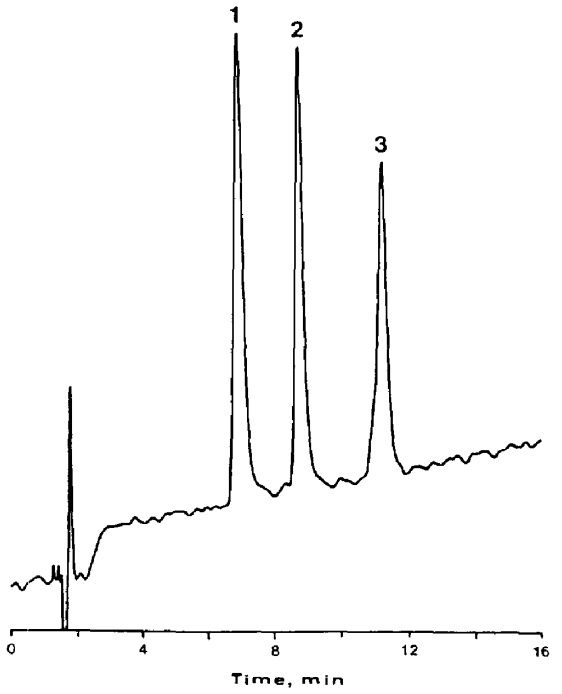


Fig. 7. RP-HPLC separation of peptides on C_{18}-YMC-Gel (10 μm, 13.8 nm) prepared via hydrosilylation. Mobile phase: solvent A: 0.10% (v/v) TFA in acetonitrile–water (75:25); solvent B: 0.10 (v/v) TFA in water. Linear gradient 25–45% A in 15 min. Flow-rate: 1.3 ml/min. UV detection at 214 nm. Injection: 5 μl of 0.5 mg/ml of each peptide. Peaks: 1 = bradykinin; 2 = angiotensin III; 3 = angiotensin I.

bonded phases which are the object of this study are very resistant to hydrolytic deterioration under these conditions. Gradient elution on C_4- and C_8-bonded, wide-pore silicas appears to provide satisfactory separation conditions for proteins. Smaller polypeptides are more commonly separated on narrow-pore C_{18} phases. The potential of C_{18}-YMC Gel (prepared also by olefin hydrosilylation) for the separation of three peptides is shown in Fig. 7. As illustrated in Fig. 8, a standard protein mixture of basic proteins can be separated with a C_8-Vydac TP phase. In all cases, similar chromatographic profiles (not shown) were obtained from columns packed with the same silica substrates modified in a conventional manner.

4. Conclusions

Evaluation of the alkyl-bonded phases prepared via olefin hydrosilylation on hydride-modified silica supports showed that, generally speaking, the selectivity of the new chromatographic phases resembles that of their conventional counterparts, alkyldimethylsilyl-bonded phases. Compared to conventional phases under particularly aggressive eluent conditions, more specifically those involving TFA-containing aqueous–organic solvents, the new bonded materials showed superior hydrolytic stability, as expected from a direct Si–C linkage formed upon olefin addition. Besides improving column longevity and long-term precision in retention,

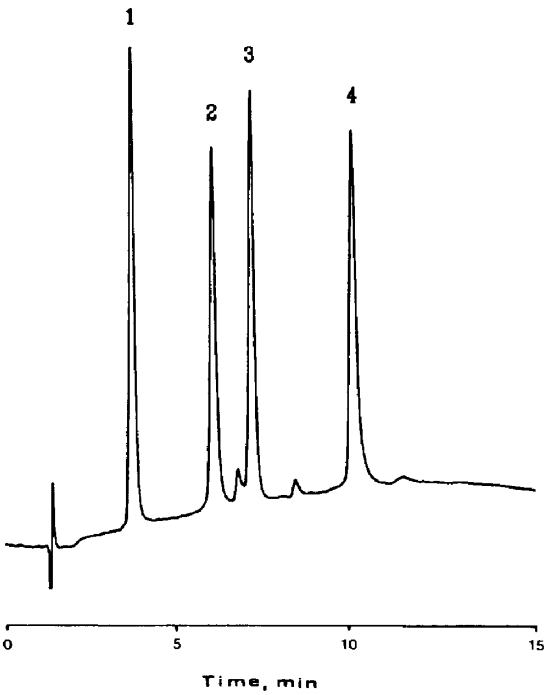


Fig. 8. Typical separation of a standard protein mixture on a C_8-Vydac TP (5.8 μm, 29.1 nm) prepared via hydride. Mobile phase: solvent A: 0.10% (v/v) TFA in acetonitrile–water (95:5), solvent B: 0.10% (v/v) TFA in water. Gradient: 25–100% A in 30 min at 1.5 ml/min. Sample injection: 5.0 μl of 0.4 mg/ml of each protein. Detection at 214 nm. Peaks: 1 = ribonuclease B; 2 = cytochrome *c*; 3 = lysozyme; 4 = myoglobin.

the hydrolytic advantage should reduce to a minimum the fraction contamination that might result from coelution of phase degradation by-products with the analyte(s) of interest.

The inherently deactivated surface underneath the anchored alkyl ligands provides for symmetric solute peaks. Nevertheless, darkening of bonded silicas during olefin silanization appears to have deleterious effect on the separation of organic amines, resulting in some cases in irreversible adsorption of certain solutes. Data presented here show that the irregular retention behavior of silanol-sensitive probes such as certain crown ethers cannot be described in the light of a dual-mechanism model.

Finally, the separating potential of C_8 and C_{18} phases prepared by the new method was demonstrated. Separation profiles of complex PAH mixtures indicate selectivity typical of monomeric stationary phases. Separation of alkaloids, polypeptides and proteins clearly confirmed the applicability of the new phases to the separation of a wide variety of samples.

Acknowledgements

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References

[1] L.C. Sander and S.A. Wise, *Adv. Chromatogr.*, 25 (1986) 139.
[2] D.C. Locke, J. T Schmermund and B. Banner, *Anal. Chem.*, 44 (1972) 90.
[3] K. Unger, W. Thomas and P. Adrian, *Kolloid Z. Z. Polym.*, 251 (1973) 45.
[4] J.J. Pesek and S.A. Swedberg, *J. Chromatgr.*, 361 (1986) 83.
[5] J.E. Sandoval and J.J. Pesek, *Anal. Chem.*, 61 (1989) 2067.
[6] J.E. Sandoval and J.J. Pesek, *Anal. Chem.*, 63 (1991) 2634.
[7] C. Chu, E. Jonsson, M. Auvinen, J.J. Pesek and J.E. Sandoval, *Anal. Chem.*, 65 (1993) 808.
[8] G.E. Berendsen, K.A. Pikaart and L. de Galan, *J. Liq. Chromatogr.*, 3 (1980) 1437.
[9] G.E. Berendsen and L. de Galan, *J. Liq. Chromatogr.*, 1 (1978) 561.
[10] J.L. Glajch, J.C. Gluckman, J.G. Charikofsky, J.M. Minor and J.J. Kirkland, *J. Chromatogr.*, 318 (1985) 23.
[11] J.L. Glajch, J.J. Kirkland and J. Köhler, *J. Chromatogr.*, 384 (1987) 81.
[12] J.N. Sagliano, T.R. Floyd, R.A. Hartwick, *J. Chromatogr.*, 443 (1988) 155.
[13] J.J. Kirkland, J.L. Glajch and R.D. Farlee, *Anal. Chem.*, 61 (1989) 2.
[14] J. Köhler, D.B. Chase, R.D. Farlee, A.J. Vega and J.J. Kirkland, *J. Chromatogr.*, 352 (1986) 275.
[15] L.C. Sander and S.A. Wise, *J. Chromatogr.*, 316 (1984) 163.
[16] L.C. Sander and S.A. Wise, *Anal. Chem.*, 56 (1984) 504.

[17] L.C. Sander and S.A. Wise, *Anal. Chem.*, 59 (1987) 2309.
[18] A. Nahum and Cs. Horváth, *J. Chromatogr.*, 203 (1981) 53.
[19] K.E. Bij, Cs. Horváth, W.R. Melander and A. Nahum, *J. Chromatogr.*, 203 (1981) 65.
[20] H. Engelhardt and H. Müller, *Chromatographia*, 19 (1984) 77.
[21] T. Hearn, in Cs. Horváth (Editor), *High Performance Liquid Chromatography —Advances and Perspectives*, Vol. 3, Academic Press, New York, 1983.
[22] H. Engelhardt and M. Jungheim, *Chromatographia*, 29 (1990) 59.
[23] H. Engelhardt, H. Löw and W. Götzinger, *J. Chromatogr.*, 544 (1991) 371.
[24] B.A. Bidlingmeyer and F.V. Warren, *Anal. Chem.*, 56 (1984) 1583A.
[25] J. Köhler and J.J. Kirkland, *J. Chromatogr.*, 385 (1987) 125.
[26] S.J. Schmitz, H. Zwanziger and H. Engelhardt, *J. Chromatogr.*, 544 (1991) 381.
[27] J. Chalk and J.F. Harrod, *J. Am. Chem. Soc.*, 87 (1965) 16.
[28] J.L. Speier, *Adv. Organomet. Chem.*, 17 (1979) 407.