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Synthesis and evaluation of a C_8 stationary phase on a silica hydride surface by hydrosilation of 1-octyne

Joseph J. Pesek*, Maria T. Matyska, Xiaofan Pan

Department of Chemistry, San Jose State University, One Washington Square, San Jose, CA 95192, USA

Abstract

The silanization/hydrosilation method is used to bond an alkyne (1-octyne) to a silica hydride surface. The new bonded material is characterized by elemental analysis and diffuse reflectance infrared Fourier transform spectroscopy. The hydrophobic behavior of this material was determined by the retention characteristics of aromatic solutes and the shape selectivity as well as phase classification (monomeric or polymeric) was measured by the polycyclic aromatic hydrocarbon mixture standard reference material 869. The presence of residual silanols on the bonded phase was probed by several basic solutes at pH 7. Long-term stability studies were conducted by measuring retention and peak symmetry of basic compounds over several thousand column volumes at pH 10.
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1. Introduction

Endcapping is one of the effective methods to minimize the number of unreacted accessible silanols. The residual silanols can be removed by secondary silanization with short-chain alkyl silanes such as hexamethyldisilazane or trimethylchlorsilane and it does not affect the remaining bonded groups significantly [1]. Kirkland et al. [2] described column packings with monomeric ''sterically protected'' bonded silanes containing bulky side groups such as diisopropyl and diisobutyl. Monomeric C_{18} packings with these sterically protecting side groups are more resistant to hydrolysis and are stable in high temperature environments. Also, polar analytes cannot interact with the non-blocked silanols, and in comparison with the conventional C_{18} phases, the retention reproducibility is substantially improved. However, these approaches do not increase the carbon coverage of the bonded phase to any great extent. In order to improve the hydrolysis stability and reduce the silanol activity, a chromatographic stationary phase was prepared by horizontal polymerization of mixed trifunctional silanes into dense monolayers [3], which was first suggested by Wirth and Fatunmbi [4]. These phases are produced by polymerization of a mixture of long-chain and short-chain alkyltrichlorosilanes onto porous silica particles under anhydrous conditions, except for a monolayer of water on silica.

*Corresponding author. Tel.: +1-408-924-4939; fax: +1-408-924-4945.

E-mail address: pesek@sjsu.edu (J.J. Pesek).

Another approach to simultaneously endcap the bonded material and improve its hydrolytic stability is the silanization/hydrosilation method for producing stationary phases [5–7]. The first step, silanization, results in a monolayer of hydride on the surface and removes most of the silanols. The high degree of cross-linking and very few residual

$$\equiv Si-OH + (OEt)_3Si-H \xrightarrow{H^+} \equiv Si-O-Si-H + nEtOH$$
with OY groups above and below
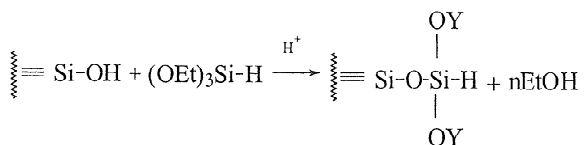
silanols are due to the fact that hydrogen is small compared to the organic groups used in conventional organosilanization. The second step, hydrosilation, attaches the organic moiety to the new hydride surface via a silicon–carbon bond

$$\equiv Si-H + R-CH=CH_2 \xrightarrow{cat.} \equiv Si-CH_2-CH_2-R$$
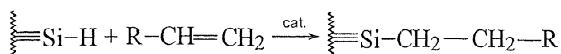
where cat.=catalyst, a metal complex such as hexachloroplatinic acid or free radical initiator.

More recently hydrosilation of alkynes has been introduced as a means of bonding organic moieties to hydride surfaces [8]. The advantage of this approach is that it could lead to more than a single attachment of the bonded group to the surface. Some of the possible structures for hydrosilation of an alkyne on silica hydride are shown below:

Structure I

Structure II

Structure III

Structure IV
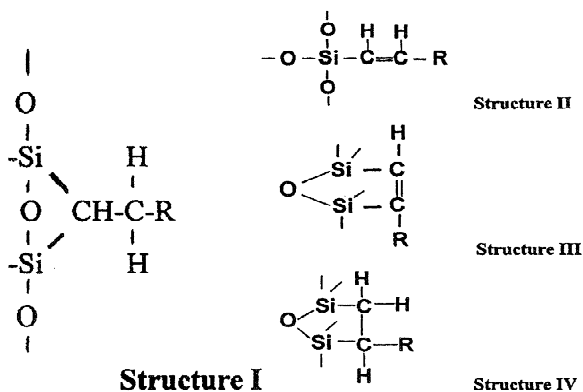
This study explores further the hydrosilation of alkynes on silica hydride. In particular, 1-octyne is used as the organic moiety to be bonded to the surface and the resultant material is then character-

ized both by both spectroscopic and chromatographic methods.

2. Experimental

2.1. Materials

The silica used for the stationary phases came from The Separations Group (Hesperia, CA, USA) (Vydac TP 106, surface area 106 $m^2 g^{-1}$, particle size 6.3 μm), Eka Nobel (Bohus, Sweden) (Kromasil, surface area 340 $m^2 g^{-1}$, particle size 5 μm) and Microclear/Stellar Phases (Langhorne, PA, USA) (Astrosil, 320 $m^2 g^{-1}$, particle size 4.5 μm). The triethoxysilane (TES) for the silanization step was purchased from Huls-Petrarch (Bristol, PA, USA). Hexachloroplatinic acid and the 1-octyne used in the hydrosilation reaction were purchased from Aldrich (Milwaukee, WI, USA). One chromatographic test mixture, standard reference material (SRM) 869, was obtained from the National Institute of Standards and Technology (Gaithersburg, MD, USA). The remainder of the materials, solutes for chromatographic testing and solvents for synthesis and the mobile phases, were purchased from commercial suppliers in the highest purity available. Water for chromatographic mobile phases was prepared on a Milli-Q system (Millipore, Bedford, MA, USA).

2.2. Instrumentation.

Diffuse reflectance infrared Fourier Transform (DRIFT) spectra were obtained on a Mattson (Madison, WI, USA) Infinity Series instrument interfaced to a Venturis FX-2 computer. Carbon-13 cross-polarization (CP), magic-angle spinning (MAS) nuclear magnetic resonance (NMR) spectra were recorded on a Varian (Palo Alto, CA, USA) INOVA 400 MHz spectrometer system using a pulse width of 7 μs, a contact time of 1.5 ms and delay time of 3 s. The HPLC system consisted of a Shimadzu LA-6 pump, a Shimadzu SPD-6A UV spectrophotometric detector operated at 254 nm (Shimadzu, Kyoto, Japan) and a Hewlett-Packard (HP) 3396A Integrator (Palo Alto, CA, USA).

2.3. Synthetic procedures

The synthetic procedure for preparation of the hydride intermediate has been reported previously [5,7]. Speier's catalyst used for the hydrosilation reaction was prepared as described above and stored in a freezer until needed. The hydrosilation bonding procedure used to modify the hydride intermediate has been described in detail elsewhere [6,7]. The bonded materials were packed into 150 mm×2.1 mm stainless steel tubes (Alltech, Deerfield, IL, USA) by a high-pressure slurry packing procedure with a Haskell (Burbank, CA, USA) pneumatic amplification pump. Approximately 1.9 g of the bonded material was placed in a carbon tetra-chloride–methanol (9:1, v/v) solution. The column was packed at ~500 atm using methanol as the driving solvent (1 atm=101 325 Pa).

2.4. Chromatographic procedures

Methanol was chosen as the organic eluent component in these studies. Columns were operated using a flow-rate of 0.7 ml min^{-1}. The basic compound test was carried out at several temperatures with the column thermostated in a column heater (Model CH-30, Fiatron Systems, Oconomowoc, WI, USA). At least 10 column volumes were purged through before use with each new mobile phase. Each solute was made up at a concentration of 100 mg l^{-1} in the relevant mobile phase. A 0.2-μg amount of the analyte was injected in each run to prevent significant overloading of the column. The determination of t_0 was carried out using KNO_3. The pH of the mobile phase was measured before adding methanol. Isocratic conditions were used. No buffer was used in the aqueous component, but new mobile phases were prepared daily to ensure constant pH.

3. Results and discussion

The treatment of silica with TES results in a stable Si–H bond. The FT-IR spectra of both the Kromasil and Vydac silica hydrides synthesized for this investigation reveal a significant decrease in the free Si–OH stretching band at ~3750 cm^{-1} and the appearance of an Si–H stretching peak near 2250 cm^{-1} (Fig. 1A). These results confirm the formation of the hydride intermediate and are in agreement with data obtained in earlier studies [5–7].

Silica hydride reacts via hydrosilation with the 1-octyne and a stable Si–C bond is formed. The carbon content of the two bonded phases (C_8 from octyne on Kromasil and on Vydac silicas) obtained from elemental analysis of the materials was 13.8 and 5.7% (w/w) carbon, respectively, after subtracting the amount on the hydride intermediate (0.5 and 0.2%, respectively). From the surface area values provided by the manufacturer, the bonding densities were calculated to be 4.72 and 5.82 μmol m^{-2}, respectively using a modified form of a standard equation [9]. These results suggest that a high coverage of bonded ligands was obtained. Therefore, after the silanization and hydrosilation reactions only a small amount of the starting surface silanols are likely to remain and be exposed so that with these stationary phases the influence of Si–OH groups on the retention of solutes should be very negligible. It is also possible that the high surface coverage could be due to some polymerization during the silanization reaction. However, conditions used in this study were identical those determined previously that lead to the formation of a hydride monolayer [5]. In addition, the results show that the wider pore silica resulted in a phase with higher coverage.

Fig. 1 shows the infrared spectra for silica hydride and the two C_8 phases synthesized in this study. The band for Si–H stretching at 2244 cm^{-1} is lower in intensity for the C_8 phase (Fig. 1B) when compared to the spectrum of the hydride intermediate (Fig. 1A). In addition, the absence of any significant free SiOH stretching at ~3750 cm^{-1} and the appearance of C–H stretching bands in the region of 3000–2800 cm^{-1} confirm bonding of the 1-octyne to the hydride intermediates and suggest that the surface below the C_8 hydrocarbon consists mainly of Si–H moieties. For both C_8 phases (Fig. 1B and C), small peaks above 3000 cm^{-1} indicate the presence of some olefin. These peaks are slightly more intense on the Kromasil silica (Fig. 1B). As shown in the structures above, both II and III could account for the presence of this band in the IR spectrum. It is possible that the smaller pores of the Kromasil silica could affect the bonding of the alkyne such that fewer double
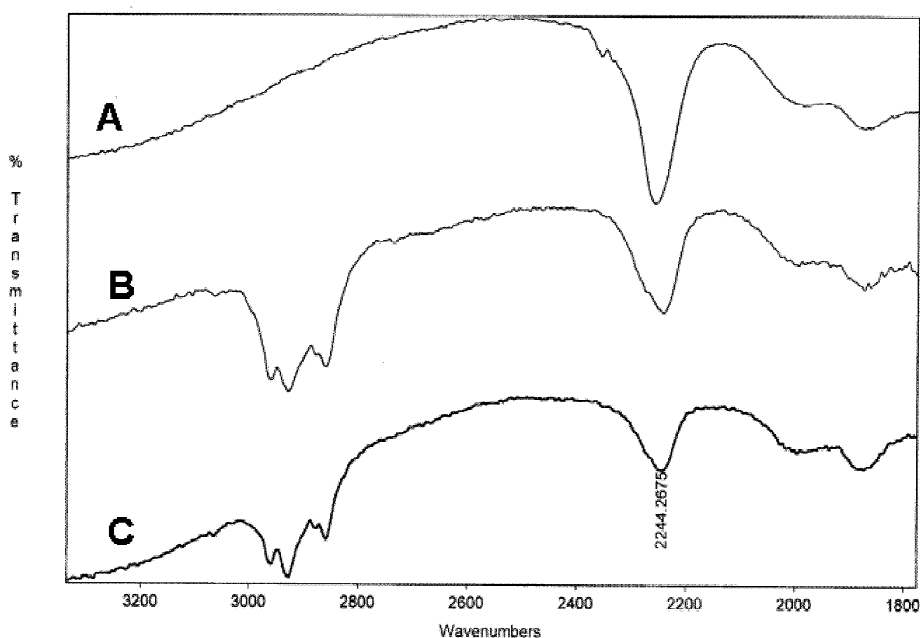
Fig. 1. DRIFT spectra of silica hydride and the C_8 stationary phases synthesized from 1-octyne: (A) silica hydride on Kromasil; (B) C_8 on Kromasil silica hydride; and (C) C_8 on Vydac silica hydride.

attachments to the surface occur (hence the presence of structure II) or that steric considerations could lead to a different bonding mechanism (hence the presence of structure III). It is not possible to distinguish from the IR data which of these two possibilities is more likely.

The presence of the octyl moiety on the silica surface was further characterized by the use of ^{13}C CP-MAS NMR spectroscopy. Fig. 2A is the spectrum of the material that results from the hydrosilation reaction of 1-octyne on silica hydride. The spectrum of this material is quite similar to hydrosilation products of 1-octyne on other silica hydrides since the formation of the hydride intermediate eliminates any differences that might exist between silicas from different sources. In the chemical shift range of 10 to 40 ppm, the peaks for the expected aliphatic carbons are observed. Between 140 and 160 ppm four peaks are observed that can be attributed to olefinic carbons (structures II and III). These results confirm those obtained in the DRIFT spectra that also indicate a bonded structure with olefinic carbons is present on the silica hydride surface. Fig. 2B illustrates that the C_8 moiety bonded to a silica

hydride made by hydrosilation of an alkyne (1-octyne) is different than the one produced by an alkene (1-octene). No olefinic peaks are observed and the peak pattern is different in the aliphatic carbon region.

The hydrophobic properties of the C_8 phases produced in this study were determined by measuring the retention of benzene, toluene, ethylbenzene, isopropylbenzene, *tert.*-butyl benzene and anthrancene in methanol–water (65:35). A typical chromatogram of the test solutes is shown in Fig. 3 for the Kromasil C_8 material. All the solutes eluted with symmetrical peaks and all the solutes are baseline resolved. The same result is obtained on the Vydac C_8 except benzene and toluene are not baseline resolved. Table 1 presents the retention data. With 65% methanol, the Kromasil C_8 phase had approximately four-times longer retention (as measured by k') than the Vydac C_8 phase for the hydrophobic solutes. However, the hydrophobic selectivity of the C_8 bonded on Kromasil silica with a larger surface area ($340 \text{ m}^2 \text{ g}^{-1}$) is identical to that of the C_8 bonded on Vydac silica with a smaller surface area ($106 \text{ m}^2 \text{ g}^{-1}$) and the bonded phases produced by
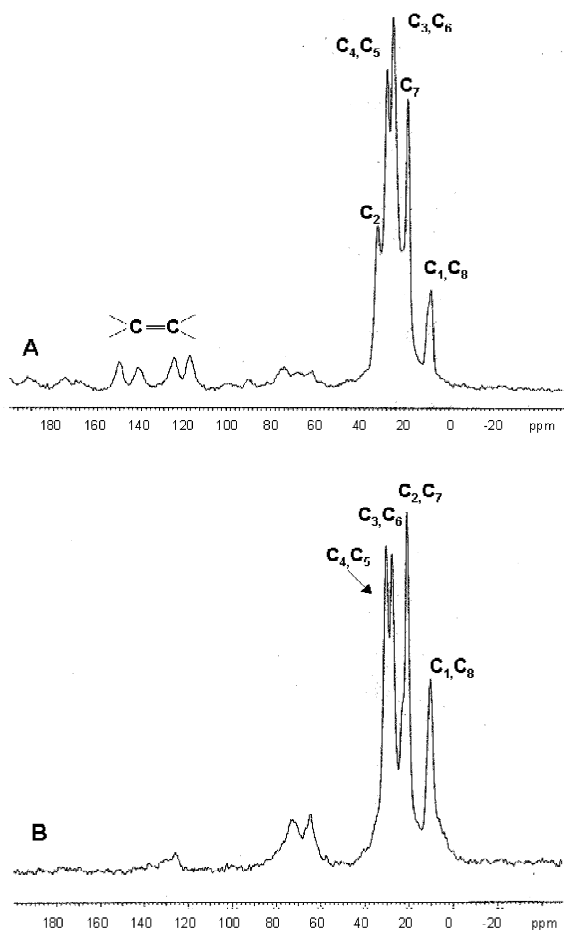
Fig. 2. ^{13}C CP-MAS NMR spectra of C_8 moieties bonded to silica hydride surfaces: (A) 1-octyne on Kromasil silica hydride and (B) 1-octene on Kromasil silica hydride.
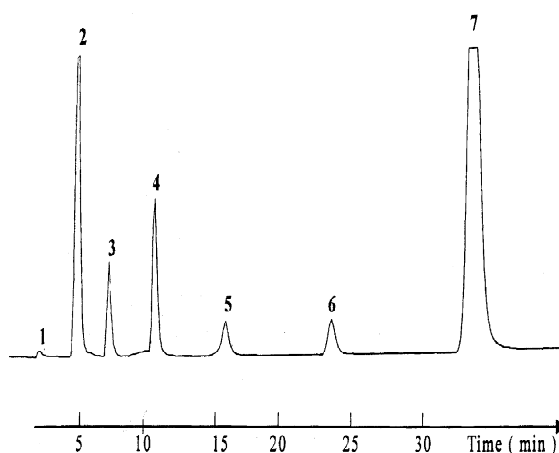

Fig. 3. Chromatogram of hydrophobic solute test mixture on the C_8 column using Kromasil silica hydride. Mobile phase: MeOH–water (65:35). Flow-rate = 0.7 ml min^{−1}. Detection at 254 nm. Solutes: 1 = sodium chloride; 2 = benzene; 3 = toluene; 4 = ethylbenzene; 5 = isopropylbenzene; 6 = tert.-butyl benzene, 7 = anthracene.

hydrosilation of an alkyne are typical reversed-phase in character. Absolute retentions are different due to differences in carbon content and the silica porosity. It is possible, therefore, to compensate for differences in retention due to variation of carbon content just by altering the methanol–water ratio. With C_8 bonded to Kromasil silica usually 10% more organic eluent component is required to achieve similar analysis times as on the C_8 bonded on the Vydac support. Also shown in Table 1 are the retention data for a C_8 column made by hydrosilation of 1-octene on Kromasil hydride. This material has a surface coverage of 3.63 μmol m^{−2}. Since the amount of carbon on this stationary phase is intermediate

between the two phases made from 1-octyne, the k' values are also in-between the octyne C_8 phases. However, the α values for adjacent peaks are measurably lower on the C_8 from octene than on either C_8 from octyne phase. Since the structure of the octyl moiety on the surface as shown by ^{13}C CP-MAS NMR spectra is different for octene and octyne hydrosilation, this result is not unexpected.

The shape selectivity of a stationary phase depends on the type of bonding chemistry and the alkyl chain length used. An important parameter affecting selectivity toward PAHs is phase type, i.e., whether a phase was prepared by monomeric or polymeric synthesis chemistry. SRM 869 provides a sensitive measure of the polymeric or monomeric character of the phase [10,11]. From Table 2 it can be seen that both of the phases showed an elution order BaP < PhPh < TBN with $\alpha_{\text{TBN/BaP}} > 1.7$. These phases are classified as having monomeric-like selectivity [10,11]. The shape selectivity behavior of the two bonded-phases was essentially unaffected by changes in pore size of the silica support. Shape selectivity depends on the type of bonding chemistry used in the preparation of the stationary phase. The silanization/hydrosilation approach results in monomeric-like attachment of the C_8 moiety, even for alkynes such as 1-octyne. In addition, C_8 is a short alkyl chain and

Table 1
Retention properties of hydrophobic mixture*

Column	Property	Peaks*					
		2	3	4	5	6	7
C_8 bonded on	t_R	5.22	7.58	11.04	16.40	24. 41	35.27
Kromasil	k'	1.09	2.04	3.42	5.56	8.77	13.11
	α		1.87	1.68	1.63	1.58	1.50
C_8 bonded on	t_R	3.60	4.35	5.35	6.70	8.83	11.63
Vydac	k'	0.27	0.55	0.90	1.38	2.14	3.14
	α		1.96	1.65	1.54	1.55	1.40
C_8 from 1-octene	t_R	4.84	5.92	7.28	8.89	11.72	14.04
on Kromasil	k'	1.10	1.56	2.17	2.87	4.10	5.09
	α		1.42	1.39	1.33	1.43	1.24

* Mobile phase=methanol–water (65:35, v/v) at 0.7 ml min^{-1}. Temperature~25 °C. Peaks: 2=benzene, 3=toluene, 4=ethylbenzene, 5=isopropylbenzene, 6=*tert.*-butyl benzene, 7=anthracene.

not as likely as C_{18} to have good shape selectivity as demonstrated by these results.

The analysis of basic compounds using reversed-phase high-performance liquid chromatography continues to receive much attention, due to problems of poor peak shape, which are generally attributed to detrimental interaction of these analytes with unreacted silanol groups. The considerable interest in this area is partially due to the large number of important pharmaceuticals and other clinically significant compounds which possess basic groups. The system pH in this study was between 7 and 8. Peak shapes are generally worst around pH 7 because support silanol groups and basic solutes are often partially ionized. At lower pH, peak shape may be improved due to reduced dissociation of silanols, whereas at higher pH, decreasing protonation of the base may improve peak symmetry. In each case, reduced ion-exchange interaction should result. The solutes chosen for this study were intended to exemplify the different types of basic compounds that may be expected to interact with silica surfaces. The compounds were *N,N*-dimethylaniline (a basic tertiary amine), $pK_a=5.15$, aniline (a primary

amine), $pK_a=4.63$, and diphenylamine $(C_6H_5)_2NH$ (a secondary amine), $pK_a=13.21$. The effect of changing the methanol concentration at room temperature, 40, 50 and 60 °C on the capacity factors and the column performance of the three solutes, was investigated. Typical results are shown in Fig. 4 where it can be seen that all three solutes eluted as symmetrical peaks and all of them were baseline resolved. This was the case for all temperatures and methanol concentrations on both columns. Plots of log k' vs. % MeOH are not linear at both high and low organic composition but exhibit a linear decrease in retention with increasing organic content in the mobile phase at intermediate compositions, typical
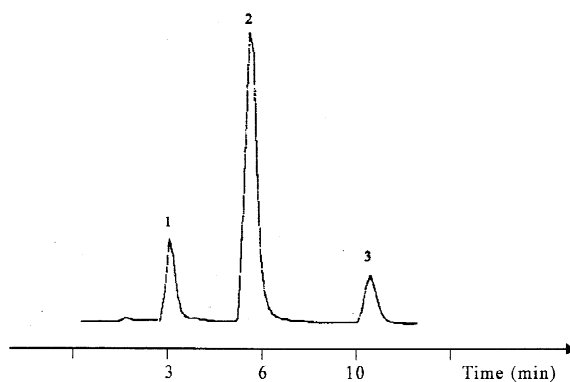
Table 2
Elution order and column selectivity for SRM 869

Bonded phase	Elution order	$\alpha_{TBN/BaP}$
Kromasil C_8	BaP<PhPh<TBN	1.751
Vydac C_8	BaP<PhPh<TBN	1.799

Fig. 4. Chromatograms of basic solutes on the C_8 Vydac hydride silica column. Mobile phase: MeOH–water (50:50). Flow-rate 0.7 ml min^{-1} and detection at 254 nm. Solutes: 1=aniline, 2=*N,N*-dimethylaniline, 3=diphenylamine.
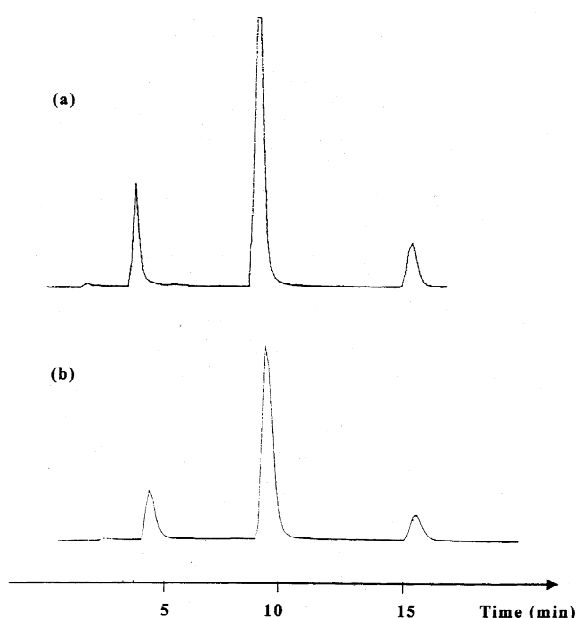
Fig. 5. Chromatograms of basic solutes on a Kromasil hydride C_8 column: (A) initial chromatogram on new column and (B) after purging with pH 10 mobile phase for 4000 column volumes. Conditions and solutes as in Fig. 4.

reversed-phase behavior. The plots of log k' vs. methanol concentration at different temperatures show decreased retention as the temperature increases, corresponding to the expected increase in mass transfer. Measurement of column asymmetry factors under all of the experimental conditions studies gave values that varied from 1.01 to 1.21. This result suggests that there were few residual silanols and any that were present were well protected by the bonded ligands or the hydride surface. The basic compounds are more retained by the C_8 bonded on Kromasil column than by C_8 bounded on Vydac column because of its higher carbon load. The column efficiency of C_8 on Kromasil column is higher than that of the C_8 on Vydac column. Because packing variations cannot be eliminated, it is not possible to draw any specific conclusions about the two phases from this data.

The stability of the bonded phases was investigated at high pH (9–10) by monitoring changes in the retention factor and theoretical plate number of pyrene. The k' was measured before exposure to high pH conditions and after periodically purging the
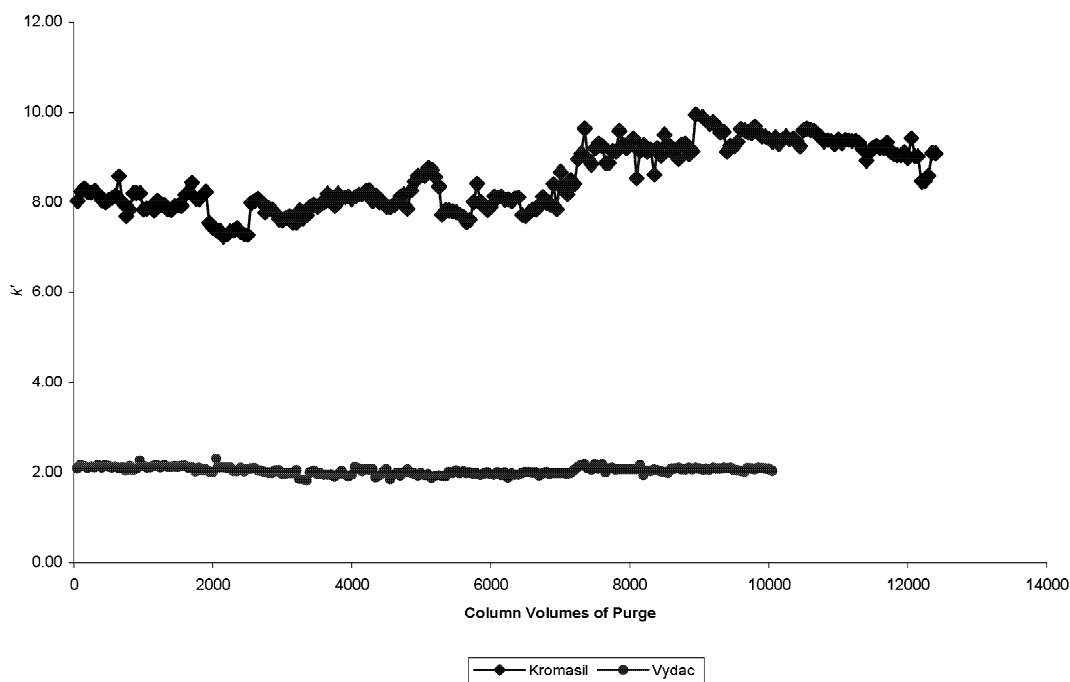

Fig. 6. Plot of the capacity factor (k') of pyrene vs. number of column volumes of pH 10 mobile phase passed through the two C_8 columns. Mobile phase: MeOH–water (70:30). Other conditions as in Fig. 3.

columns at room temperature with a measured number of column volumes of a mobile phase consisting of methanol–water (70:30, v/v) with unbuffered sodium hydroxide solution delivered at 0.7 ml min^{-1}. Any significant change in retention and theoretical plate number (N) of this compound suggests the loss of bonded stationary phase under this aggressive mobile phase condition. Whenever possible, the capacity factors measured were corrected during the hydrolysis experiments for any changes that occurred for a control column when it was not exposed to hydrolysis conditions. Those corrections compensated for small differences caused by mobile phase preparation, evaporative loss of methanol, temperature variation and manual injection. The capacity factor and N of pyrene are the accepted measures of hydrophobic retention and column efficiency and thus should be a sensitive indicator of silica matrix-bonded phase loss. In Fig. 5 the separation of the three basic compounds from another batch of C$_8$ on Kromasil hydride is shown

initially as well as after purging with 4000 column volumes at pH 10. As seen, the retention behavior the column exhibits toward basic compounds was hardly affected by the high pH mobile phase. The resistance of the two C$_8$ bonded silica-based columns to highly alkaline conditions is also illustrated in Figs. 6 and 7, which shows that after purging with 10 000 column volumes of a basic mobile phase, there is a little change in k' and plate numbers. The rate of column degradation appears to be very slow. The two columns exhibit resistance to basic hydrolysis through the silicon–carbon bond, and perhaps the double attachment of the organic moiety to the surface. The high ligand density and the presence of the hydride monolayer may offer additional protection of the underlying siloxane bonds that normally would be susceptible to hydrolysis at these high pH values. The excellent stability of the two C$_8$ bonded phases in basic solvent environments favors their use at high pH for increased separation capabilities.
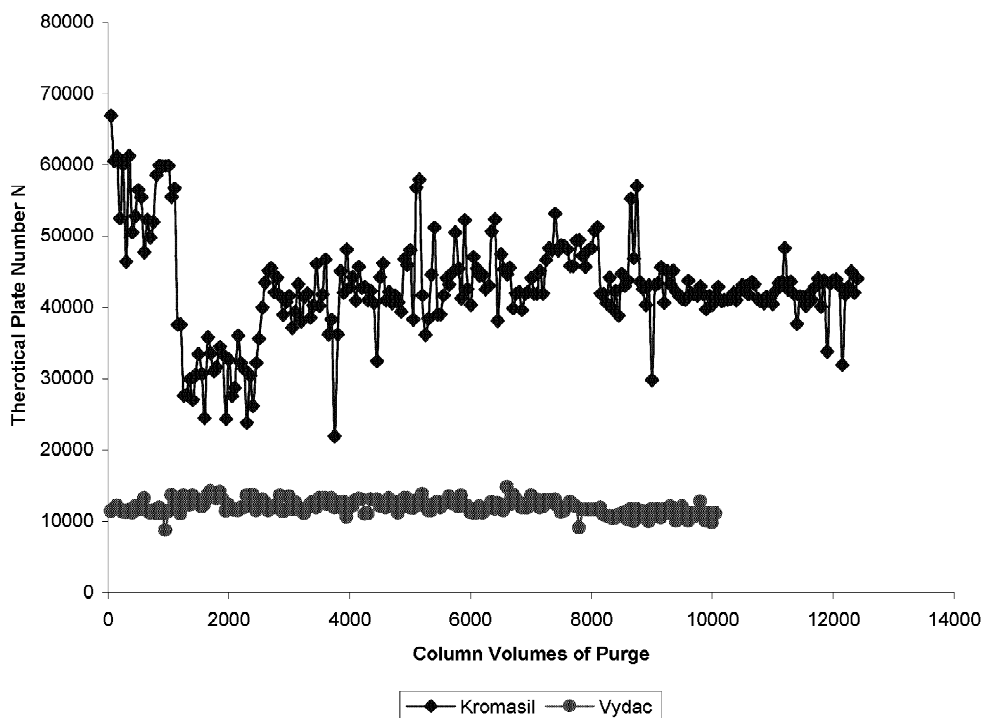

Fig. 7. Plot of the number of theoretical plates for pyrene vs. number of columns of pH 10 mobile phase passed through the two C$_8$ columns. Conditions as in Fig. 5.

4. Conclusions

C_8 stationary phases synthesized by the silanization/hydrosilation method possess high surface coverage and a direct Si–C linkage, possibly a double attachment at the surface. The two phases prepared in this study on two different silica supports possess the expected monomeric selectivity toward the SRM 869 test mixture. Mobile phase composition studies revealed typical reversed-phase character. Analyses of basic compounds reveal little or no interaction with residual silanols. These high-density C_8 bonded silica materials are remarkably stable in alkaline pH mobile phases, which makes them good candidates for biological and pharmaceutical applications.

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