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Synthesis and characterization of endcapped C_{18} stationary phases using a silica hydride intermediate

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Abstract

The effect of endcapping on an octadecyl bonded phase synthesized by the silanization/hydrosilation method is investigated. The endcapping reagent is a 1:1 molar ratio of trimethylchlorosilane (TMCS) and hexamethyldisilazane (HMDS). Two approaches for endcapping are possible for this synthetic method that produces a silica hydride intermediate: bonding of TMCS–HMDS after silanization (on the hydride intermediate) or after hydrosilation (on the C_{18} product stationary phase). The use of TMCS–HMDS is designed to eliminate the few remaining silanols on the silica hydride intermediate. The endcapping process is characterized spectroscopically by diffuse reflectance infrared Fourier transform (DRIFT), ^{29}Si cross polarization magic angle spinning nuclear magnetic resonance spectroscopy (CP-MAS-NMR) and ^{13}C -CP-MAS-NMR. The octadecyl bonded phases are characterized chromatographically by measuring the capacity factors of several hydrophobic and basic test solutes as well as the separation factors among various solute pairs. Finally, long-term stability tests are done on both products at high and low pH. © 2002 Elsevier Science B.V. All rights reserved.

Keywords: Stationary phases, LC; End-capping; Silica hydride

1. Introduction

Endcapping, the use of a small organosilane reagent, was proven some time ago to be an effective means of removing many of the unreacted and accessible silanol groups on silica as well as to increase the hydrophobicity of the surface [1–4]. The endcapping properties of the two reagents used in this study, trimethylchlorosilane (TMCS) and hexamethyldisilazane (HMDS), were characterized a number of years ago [1]. For most reversed-phase applications of high-performance liquid chromatog-

raphy (HPLC) a bulky organic moiety such as octadecyl (C_{18}) is attached to the silica surface. In fact most HPLC stationary phases consist of an organic group equivalent to C_4 or larger bonded to the surface. The net result is that no more than about 50% of the available silanols can react with the organosilane reagent leaving a considerable number of Si–OH groups that can potentially interact with analytes. It is these strongly adsorbing sites on the surface of silica that are the target of any endcapping scheme.

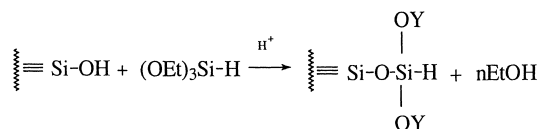
More than a decade ago, the silanization/hydrosilation process was developed to overcome some of the drawbacks associated with the use of organosilane reagents for the synthesis of HPLC stationary phases [5–7]. The first step involves

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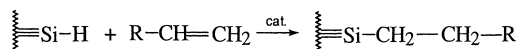
reaction of the silica, or other oxide, surface with triethoxysilane (TES) that under carefully controlled conditions results in the formation of a hydride monolayer.

SILANIZATION



The essential result is that virtually all {greater than 90% as determined by ^{29}Si cross polarization magic angle spinning nuclear magnetic resonance spectroscopy (CP-MAS-NMR) [6]} of the accessible silanols are replaced by hydrides. The second step in this process is the hydrosilation reaction where the desired organic moiety is attached to the hydride surface via catalytic addition of an unsaturated functional group (olefin, alkyne or cyano). Hydrosilation leads to the formation of a direct Si–C bond at the surface that has been shown to be more hydrolytically stable than the Si–O–Si–C linkage that is formed during an organosilanization reaction [7–9].

HYDROSILATION



cat = catalyst, typically hexachloroplatinic acid

Theoretically the silanization/hydrosilation process should require minimal endcapping since the most of the silanols are removed during the formation of the hydride monolayer. However, complete absence of silanols is not possible for two reasons. First, TES cannot react with 100% of the silanols so as is the case with any reaction on silica there must be some Si–OH groups left on the surface. Second, TES will not completely cross-link with 100% efficiency leading to the presence of silanols on the new hydride surface. While the number of Si–OH groups is not great, they could pose a problem for some analyses, particularly those involving basic compounds.

In this study two approaches to endcapping hydride based stationary phases are evaluated. In one

case the endcapping takes place before the hydrosilation bonding reaction (EBB) while in the other method the hydrosilation bonding reaction is done before endcapping (BBE). The availability of two endcapping options is unique to the silanization/hydrosilation synthetic method and may offer some advantages with respect to chromatographic performance. In addition, the stability of these stationary phases is also evaluated in determining their usefulness for practical applications.

2. Experimental

2.1. Materials

The endcapping reagents, TMCS and HMDS, were obtained from United Chemical Technologies (Bristol, PA, USA). The silanization reagent, TES, and the hydrosilation reagent, 1-octadecene, were both purchased from Sigma–Aldrich (Milwaukee, WI, USA) and used as received. The catalyst for the hydrosilation reaction, chloroplatinic acid hexahydrate (Strem, Newbury Port, MS, USA), was prepared in a nitrogen filled glove box by dissolving 0.4 g in 100 ml of isopropanol at 93 °C. The solvents used in the synthetic reactions, toluene (EM Science, Gibbstown, NJ, USA), diethyl ether (Fisher Scientific, Fair Lawn, NJ, USA), dioxane (Fisher Scientific), and tetrahydrofuran (J.T. Baker, Phillipsburg, NJ, USA) and the methanol (Fisher Scientific) for the chromatographic experiments were obtained in reagent grade. The silica used for the preparation for the stationary phases was Vydac TP 106 (The Separations Group, Hesperia, CA, USA) with a particle diameter of 6.5 μm , a pore size of 100 Å, and a surface area of 106.5 m^2/g .

2.2. Synthetic procedures

The synthetic procedure for preparation of the hydride intermediate has been reported previously [5]. The 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]. The endcapping process

utilized a 1:1 molar ratio of TMCS and HMDS in toluene and has also been described previously [10]. The combination of hydrosilation and endcapping were done in two sequences: hydrosilation of 1-octadecene on the hydride intermediate followed by endcapping (BBE) and endcapping the hydride intermediate followed by hydrosilation of this product with 1-octadecene (EBB).

2.3. Characterization of materials

The bonded phases were characterized by a number of methods. Elemental analysis was used to determine the surface coverage of the bonded moiety. The samples were sent to Desert Analytics (Tucson, AZ, USA) for carbon analysis and a standard equation modified for the hydrosilation procedure (based on the reacting molecule being an olefin) was used to determine the surface coverage expressed as $\mu\text{mol}/\text{m}^2$ [11]. The bonded materials were characterized spectroscopically first by diffuse reflectance infrared Fourier transform (DRIFT) using a Perkin-Elmer Model 1800 spectrometer (Norwalk, CT, USA). The diffuse reflectance accessory (Spectra Tech, Stamford, CT, USA) utilized a 2 mm sample cup. The sample was mixed with KBr (1:1, w/w), placed in the sample cup and then the surface was smoothed with a microscopic slide. After the cup was placed in the instrument, the sample compartment was purged with nitrogen for 25 min. The sample was scanned 100 times at a resolution of 2 cm^{-1} over the spectral range of 4000 to 450 cm^{-1} and ratioed against pure KBr as a reference. The second means of spectroscopic characterization was CP-MAS-NMR. All spectra were acquired on a Bruker (Billerica, MA, USA) MSL 300 spectrometer. The samples were placed in a zirconia double bearing rotor and spun at 4700 to 5200 rpm. Both ^{13}C and ^{29}Si spectra utilized a $5\text{ }\mu\text{s}$ contact time and a 5 s repetition rate while pulse widths were 6.5 and $5.0\text{ }\mu\text{s}$, respectively. External glycine and polyhydrosiloxane were used as references for ^{13}C and ^{29}Si , respectively.

2.4. Chromatographic methods

The bonded materials were packed into $15\text{ cm}\times 4.6\text{ mm}$ I.D. stainless steel columns (Alltech As-

sociates, Deerfield, IL, USA) using a pneumatic amplification pump (Haskell, Burbank, CA, USA). Approximately 1.9 g of the bonded material was placed in a carbon tetrachloride–methanol (9:1, v/v) solution. The column was packed at $\sim 500\text{ atm}$ using methanol as the driving solvent (1 atm = 101 325 Pa). The columns for the long-term stability tests ($50\text{ mm}\times 4.6\text{ mm}$ I.D.) were packed by Higgins Analytical (Mountain View, CA, USA).

The HPLC system consisted of a Waters (Milford, MA, USA) Model 515 pump, a Waters multiwavelength detector and Rheodyne (Cotati, CA, USA) injection valve. The mobile phases were filtered through a $0.2\text{ }\mu\text{m}$ nylon membrane, sonicated and purged with He. KNO_3 was used to measure the void volume.

3. Results and discussion

3.1. Elemental analysis and spectroscopic characterization

In order to determine the effect that the two procedures have on the final surface coverage of the C_{18} moiety, three samples were sent for elemental (carbon) analysis: the hydride intermediate that has been endcapped; the endcapped intermediate that was bonded with 1-octadecene; and the hydride intermediate that was directly bonded with 1-octadecene. The first sample is needed in combination with the second sample to determine by difference the final concentration of octadecyl moieties bonded to the surface. Based on the carbon analysis obtained and using the standard equation for calculating surface coverage [5,11] modified for the silanization/hydrosilation procedure, the two bonded phases had the following amounts of octadecyl moiety ($\mu\text{mol}/\text{m}^2$): BBE = 3.16 and EBB = 2.26. These values are corrected for the amount of carbon (0.3%) on the hydride intermediate. As might be expected, endcapping the readily accessible silanols on the hydride surface will shield some of the hydride sites that are primary sites for the subsequent hydrosilation reaction. However, only chromatographic tests can determine whether the silanols that exist on the hydride surface are most effectively eliminated before or after the hydrosilation reaction.

More definitive confirmation of the endcapping process on silica hydride can be obtained from the DRIFT and ^{13}C -CP-MAS-NMR spectra that are shown in Fig. 1. The DRIFT spectrum (Fig. 1A) contains the characteristic Si–H stretch near 2250 cm^{-1} that is present on the hydride intermediate after treatment of silica with TES. In addition, strong C–H stretching bands are observed in the spectral region between 2800 and 3000 cm^{-1} . The hydride intermediate can display weak bands in this region, particularly for the type of silica (Vydac) used in this study. The C–H stretch in this silica hydride is due to the presence of some residual ethoxy groups on the surface. It is not known at present why some types of silica retain ethoxy groups from the silanization reaction while others show no evidence of these species on the silica hydride. In any case, when

present the intensity of the C–H stretch is much lower than that observed in Fig. 1A. Further verification that the endcapping process occurs on silica hydride is shown in the ^{13}C -CP-MAS-NMR spectrum (Fig. 1B). The methyl groups from the endcapping reagent (TMCS–HMDS, 1:1) are clearly seen at the expected chemical shift ($\sim 0\text{ ppm}$). In addition, the residual ethoxy groups are clearly seen by the peaks near 17 and 60 ppm that correspond to the methyl and methylene carbons, respectively. Therefore, both DRIFT and solid state NMR spectra clearly demonstrate that some residual silanols are present and can be endcapped on the silica hydride surface.

Another interesting question is whether after bonding the octadecyl moiety to the hydride surface there are accessible silanols that can still be endcapped by the TMCS–HMDS reagent. Elemental analysis cannot be used to provide this information on the BBE phase because the amount of carbon added by endcapping is relatively small in comparison to the amount already on the surface in the form of the octadecyl moiety. A similar argument can be made for a comparison of the C_{18} phase and the endcapped C_{18} phase by DRIFT spectroscopy. The increase in the intensity of the C–H stretching bands between 2800 and 3000 cm^{-1} is negligible after the C_{18} phase is endcapped with the TMCS–HMDS reagent. However, CP-MAS-NMR has the capability to detect bonding of the endcapping reagent in the presence of the octadecyl moiety on the silica surface as shown in Fig. 2. In the ^{29}Si -CP-MAS-NMR (Fig. 2A), a distinct peak near 12 ppm is seen that is characteristic of a silicon atom surrounded by three alkyl carbons and an oxygen. The larger peaks at higher field have been identified previously [5–7] as representing the silica matrix (-111 and -101 ppm) and the hydride surface (-84 ppm). A similar conclusion can be made from the ^{13}C -CP-MAS-NMR spectrum (Fig. 2B). The peak near 0 ppm confirms that the surface can be endcapped even after the octadecyl moiety has been bonded to the hydride intermediate (BBE). Therefore, spectroscopic characterization confirms that the C_{18} group can be successfully bonded to the hydride surface after endcapping with TMCS–HMDS (EBB) and that some endcapping is possible even after hydrosilation with 1-octadecene on silica hydride (BBE).

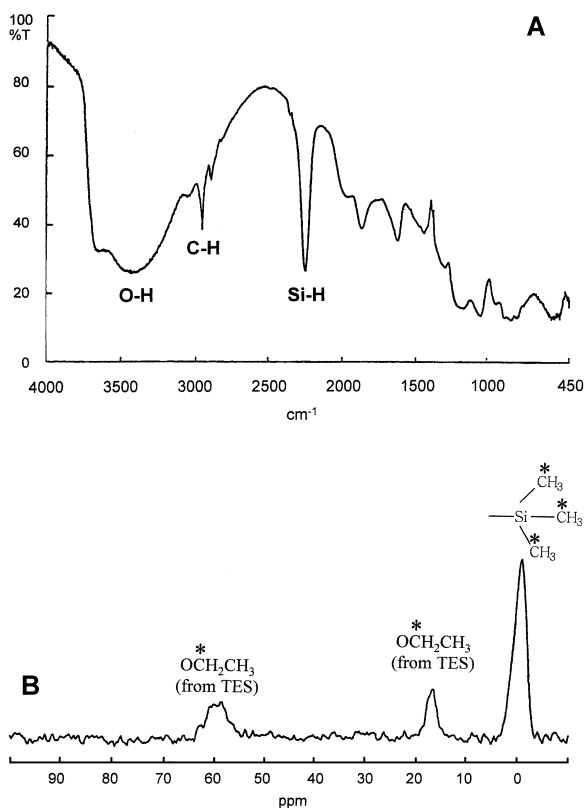


Fig. 1. Spectra of silica hydride endcapped by TMCS–HMDS: (A) DRIFT and (B) ^{13}C -CP-MAS-NMR.

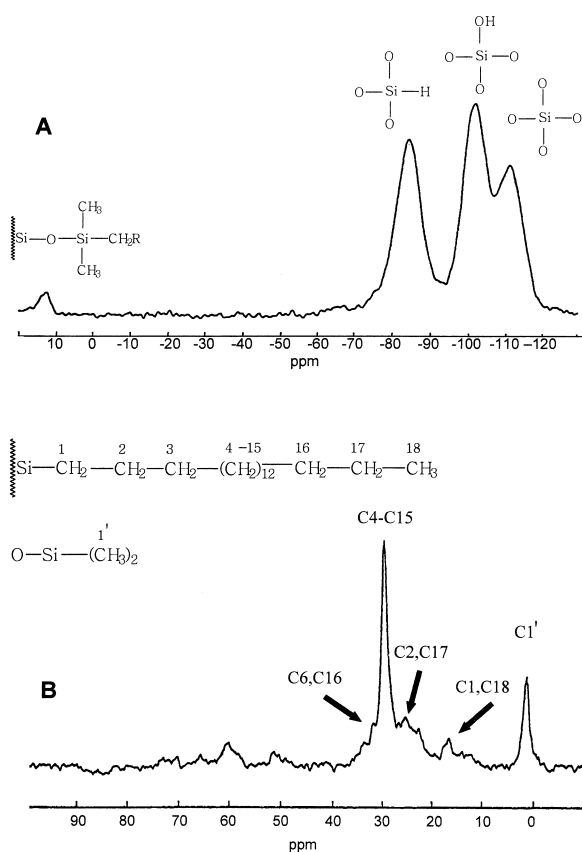


Fig. 2. CP-MAS-NMR spectra of C_{18} phase endcapped with TMCS-HMDS: (A) ^{29}Si and (B) ^{13}C .

3.2. Chromatographic characterization

While spectroscopic evaluation can confirm the success of the bonding and endcapping procedures, only chromatographic tests can determine the potential usefulness of these new materials. The first test applied to the two phases was a simple check of hydrophobic behavior. The mixture consisted of various aromatic hydrocarbons and sodium chloride as a marker for the void volume. Table 1 shows a comparison of the capacity factors (k') for several solutes on the two columns. Based on the results of the elemental analysis in particular as well as the spectroscopic characterization, the BBE phase should be more hydrophobic. This conclusion is confirmed by the results in Table 1 where greater retention is

Table 1
Capacity factors (k') for reversed-phase test mixture on the two endcapped columns

Column	Solute					
	A	B	C	D	E	F
BBE	0.32	0.58	1.08	1.74	2.66	5.14
EBB	0.14	0.34	0.64	1.02	1.75	2.23

Mobile phase: methanol–water 60:40; flow-rate=0.5 ml/min; detection at 254 nm. Solute: A=benzene; B=toluene; C=ethylbenzene; D=isopropylbenzene; E=*tert.*-butylbenzene; F=anthracene.

obtained on the BBE phase. In addition, the resolution of adjacent pairs of solutes is significantly improved on the BBE column with respect to the EBB phase. Both the absolute amount of carbon and the amount of C_{18} moiety bonded to surface in terms of $\mu\text{mol}/\text{m}^2$ is greater on the BBE material. A similar conclusion can be drawn for a comparison of the results on the two columns for SRM 869 that consists of three polycyclic aromatic hydrocarbons (Table 2, Fig. 3). The separation factor (α) between the last two components is significantly higher on the BBE column, which again confirms that it is more hydrophobic than the EBB phase. The α value of the first two components is used to classify the type of phase (monomeric vs. polymeric) [12,13]. Values near 1.5 indicate a typical moderate to high loaded monomeric phase. Such a value for the separation factor would be expected based on the bonding procedure and the elemental analysis obtained for the two materials. Similar to the results obtained for the aromatic hydrocarbons reported in Table 1, the retention of the SRM mixture is greater on the BBE column than the EBB phase as shown in Fig. 3. As a

Table 2
Separation factors (α) for SRM 869 on the two endcapped columns

Column	AB	BC
BBE	1.51	4.19
EBB	1.42	1.29

Mobile phase: acetonitrile–water (65:35); flow-rate=1 ml/min; detection at 211 nm. Solute: A=benzo[*a*]pyrene; B=phenanthro[3,4-*c*]phenanthrene; C=tetrabenzonaphthalene.

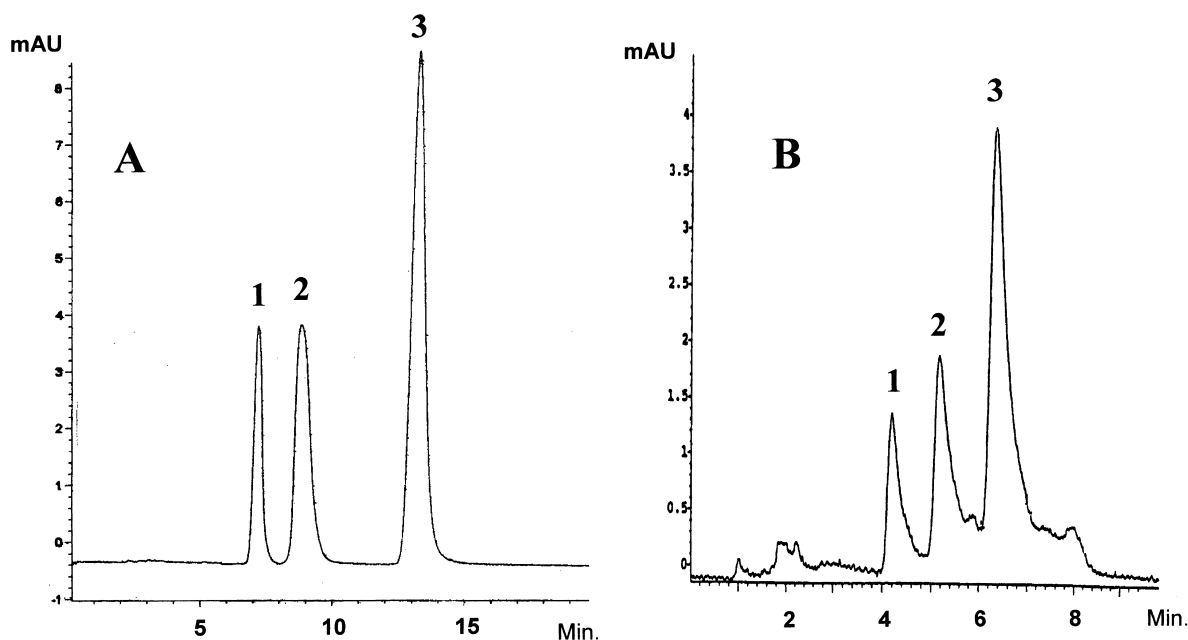


Fig. 3. Reversed-phase separation of SRM 869 on (A) BBE column and (B) EBB column. Mobile phase: acetonitrile–water (65:35); flow-rate = 1 ml/min; detection at 211 nm. Solutes: 1 = benzo[*a*]pyrene; 2 = phenanthro[3,4-*c*]phenanthrene; 3 = tetrabenzonaphthalene.

final chromatographic characterization test, the asymmetry factor (A_s) for pyridine was measured as a function of methanol in the mobile phase. Conditions were otherwise the same as those in Table 1 with the efficiency of a neutral marker being 30 000 plates/m. Over the range of 60 to 100% methanol, the value of A_s for pyridine on the BBE column was 1.2 ± 0.1 and 1.4 ± 0.2 on the EBB column (\pm values represent one standard deviation). These asymmetry factors are reasonable at neutral pH and indicate minimal interactions between the basic solute and residual silanols on the surface. It also appears that bonding of the C_{18} moiety first followed by endcapping offers slightly less access to the surface by the solute or results in fewer residual silanols on the surface. Either possibility would result in slightly better peak symmetry.

3.3. Stability studies

The presence of an Si–C bond between the surface and the attached C_{18} moiety should result in high stability of this material. Such a result has been observed when the silanization/hydrosilation process

has been used to attach a variety of organic groups to fused-silica surfaces for use in capillary electrophoresis and capillary electrochromatography [9,14–16]. The addition of the endcapping reagent may provide additional protection for the surface and hence enhanced stability of the bonded material. An example of a stability study at high pH (10) using pyridine as the test solute on the short (50 mm) column is shown in Fig. 4. At this pH pyridine interacts with the stationary phase through hydrophobic interactions and is not a selective probe for silanols. As can be seen a small change occurs in the value of the k'_{pyridine} on the BBE phase during the first 1000 column volumes ($\sim -1\%$) and then the overall percentage change in the capacity factor changes to only $\sim -2.5\%$ over the next 3000 column volumes. The BBE column was subjected to an additional 3000 column volumes of the mobile phase and the retention of pyridine was tested again with the resulting change in k' from its starting value being less than 3%. The results for the EBB column that are slightly different from the BBE column are also shown in Fig. 4. For this phase the decrease in k' from its initial value over the first 2000 column

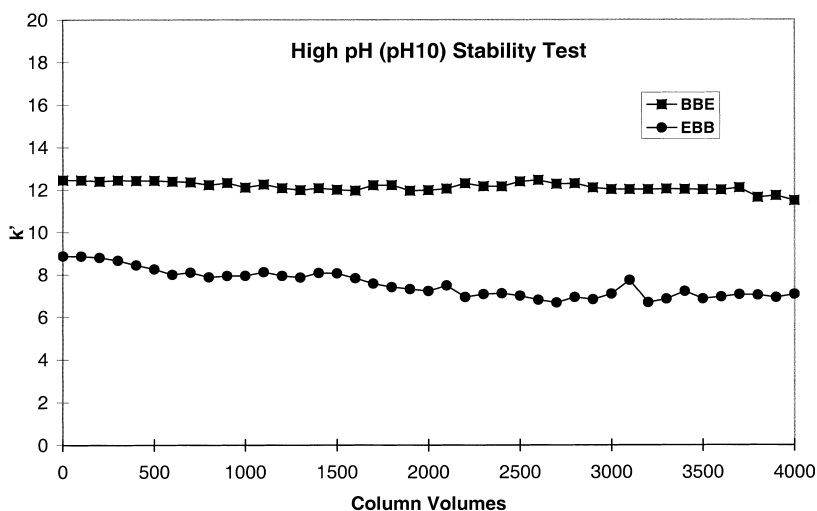


Fig. 4. Percent change in capacity factor (k') of pyridine as a function of column volumes of mobile phase at high pH on the two types of endcapped C_{18} phases. Conditions: mobile phase: methanol–water (60:40) at pH 10 (0.05 M borate buffer) at a flow-rate of 1 ml/min.

volumes was approximately 2.5% and then the loss increased to about 5% over the next 2000 column volumes. After an additional 3000 column volumes of the high pH mobile phase, k'_{pyridine} was measured again and the change from its starting value was less than 7%. In conjunction with the capacity factor measurements, the A_s for pyridine was also determined over the same 4000 column volume test sequence. The results obtained on the two columns are shown in Fig. 5. In neither case is there a

discernible trend toward poorer symmetry over the 4000 column volumes of mobile phase. The variations are somewhat greater on the EBB column and in general the peak symmetry is slightly poorer than on the BBE phase. The latter results are the same as those obtained on the longer column described above. The number of theoretical plates per meter of pyridine was also used as a measure of column stability over the 4000 column volume test. The data for this aspect of the test is shown in Fig. 6. In both

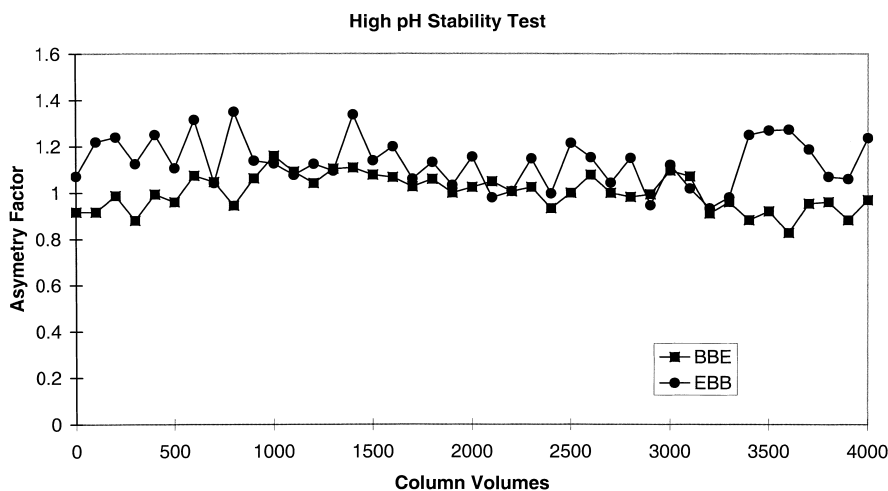


Fig. 5. Asymmetry factor (A_s) of pyridine as a function of column volumes of mobile phase at high pH on the two types of endcapped C_{18} phases. Conditions as in Fig. 3.

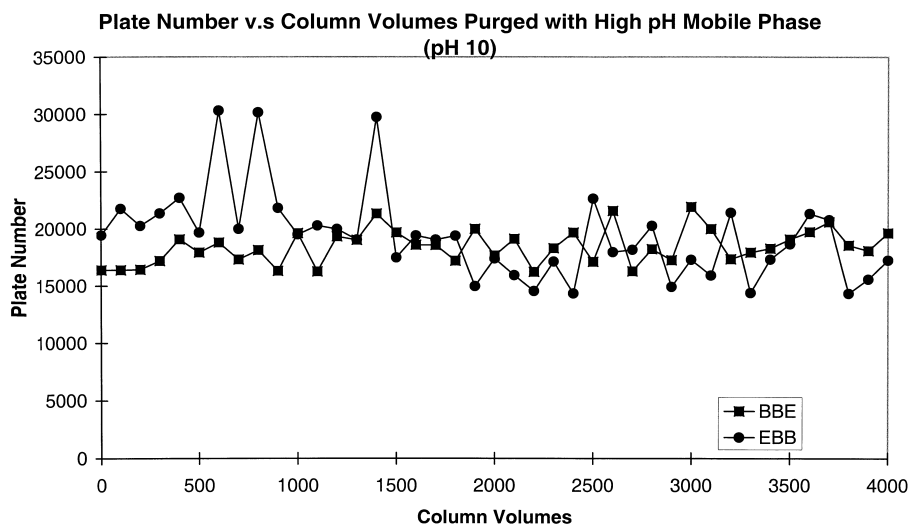


Fig. 6. Plate number (N) of pyridine as a function of column volumes of mobile phase at high pH on the two types of endcapped C_{18} phases. Conditions as in Fig. 3.

cases there is considerable scatter of the results but again no identifiable trend, particularly toward lower efficiency that would indicate significant deterioration of the columns.

While considerable interest exists in the stability of separation materials at high pH, significant applications especially in the area of biochemical applications exist at low pH. Therefore, similar stability studies were conducted using 1.9% trifluoroacetic

acid (TFA) at pH 2 in a methanol–water (60:40) mobile phase. An example of the data obtained on the BBE column for the variation in k'_{pyridine} over a 4000 column volume test is shown in Fig. 7. As can be seen, within experimental error there is no discernible trend in the capacity factor of pyridine over the course of the test although the variation between measurements increases particularly over the last 1000 column volumes. Similar results with

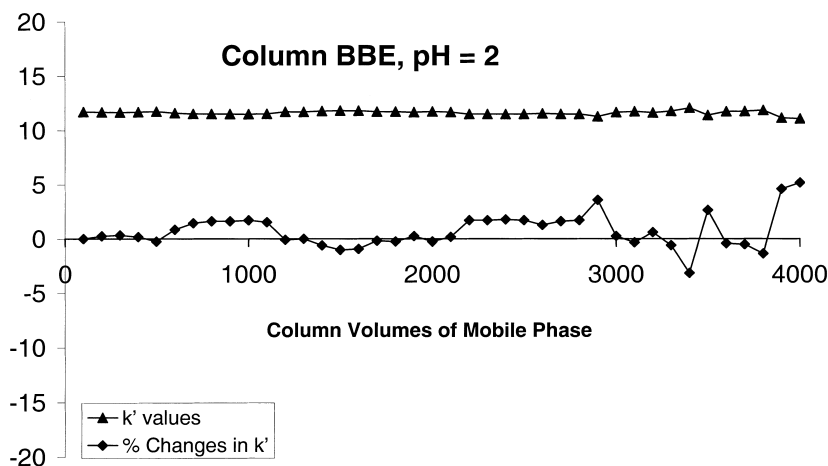


Fig. 7. Capacity factor (k') and percent change in capacity factor of pyridine as a function of column volumes of mobile phase at low pH on a C_{18} phase endcapped after hydrosilylation. Conditions: mobile phase: methanol–water at pH 1.9 (60:40) (2% TFA) at a flow-rate of 1 ml/min.

respect to the variation in k' are also obtained on the EBB column. For both bonded materials, asymmetry factors and column efficiencies do not show significant variations at low pH. These results indicate that both materials have good stability at low pH and could be suitable for applications under these conditions.

4. Conclusions

It has been demonstrated that hydride surfaces made by silanization with triethoxysilane have a few unreacted silanols. This result is confirmed by the fact that further reaction with the endcapping reagent, TMCS–HMDS, results in attachment of a trimethylsilyl moiety to the surface as determined by DRIFT and CP-MAS-NMR. These results support an earlier study that drew the same conclusions based on ^{29}Si -CP-MAS-NMR data of the silica hydride surface [5]. It has also been demonstrated that some of these remaining surface silanols can be endcapped even after an octadecyl moiety has been attached to the silica hydride by hydrosilation. The two phases produced in this study, EBB and BBE, both displayed typical reversed-phase behavior. However, both carbon analysis and the resulting chromatographic characterization show that the BBE phase is more hydrophobic resulting in greater retention for typical nonpolar organic solutes. Since both phases are endcapped, interaction of basic solutes with the surface is minimized and peak symmetry is very good. Under the conditions used in this study (solute and mobile phases) it appears that the most accessible silanols, the ones that are most likely to be disruptive to chromatographic processes, cannot be detected. Long-term stability studies indicate that both materials are resistant to degradation in both basic and acidic mobile phases. Therefore, the endcapping processes appears to result in a phase

with good protection of the surface and long lifetimes.

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References

- [1] D.B. Marshall, C.H. Lochmuller, *Anal. Chim. Acta* 142 (1982) 63.
- [2] D.B. Mashall, C.L. Cole, A.D. Norman, *J. Chromatogr. Sci.* 25 (1987) 262.
- [3] L.C. Sander, S. Wise, *CRC Crit. Rev. Anal. Chem.* 18 (1987) 319.
- [4] J. Nawrocki, *J. Chromatogr. A* 779 (1997) 29.
- [5] J.E. Sandoval, J.J. Pesek, M. Auvinen, E. Jonsson, C.-H. Chu, *Anal. Chem.* 65 (1993) 808.
- [6] J.E. Sandoval, J.J. Pesek, *Anal. Chem.* 63 (1991) 2634.
- [7] J.J. Pesek, M.T. Matyska, J.E. Sandoval, E.J. Williamsen, *J. Liq. Chromatogr. Rel. Technol.* 19 (1996) 2843.
- [8] E.F. Vansant, P. Van Der Voort, K.C. Vrancken, *Characterization and Chemical Modification of Silica*, Elsevier, Amsterdam, 1995.
- [9] M. Chiari, M. Nesi, J.E. Sandoval, J.J. Pesek, *J. Chromatogr. A* 717 (1995) 1.
- [10] J.J. Pesek, M.T. Matyska, J. Dimandja, S.O. Akapo, *Anal. Chem.* 68 (1996) 1954.
- [11] G.E. Berendsen, L. DeGalan, *J. Liq. Chromatogr.* 1 (1978) 561.
- [12] L.C. Sander, S.A. Wise, *Anal. Chem.* 56 (1984) 504.
- [13] L.C. Sander, S.A. Wise, *Anal. Chem.* 59 (1987) 2309.
- [14] M.T. Matyska, J.J. Pesek, J.E. Sandoval, U. Parkar, X. Liu, *J. Liq. Chromatogr. Rel. Technol.* 23 (2000) 97.
- [15] M.T. Matyska, J.J. Pesek, A. Katrekar, *Anal. Chem.* 71 (1999) 5508.
- [16] M.T. Matyska, J.J. Pesek, R.I. Boysen, M.T.W. Hearn, *Anal. Chem.* 73 (2001) 5116.