



# Reducing residual silanol interactions in reversed-phase liquid chromatography

## Thermal treatment of silica before derivatization

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### Abstract

This study describes the thermal pretreatment of a silica gel between 150 and 800 °C before derivatization with dimethyloctadecylchlorosilane as a means of reducing residual silanol activity in HPLC bonded stationary phases. A time study was done from 12 to 48 h to find the optimum time needed for dehydroxylation. With increasing pretreatment temperatures, the number of reactive silanols is reduced from 8  $\mu\text{mol}/\text{m}^2$  to essentially zero at 1000 °C (where sintering occurs). The effects of the thermal pretreatments were observed with diffuse reflectance infrared Fourier transform spectroscopy (DRIFTS) and solid state cross polarization magic angle spinning (CP-MAS)  $^{29}\text{Si}$  NMR. Following derivatization, residual silanol activity and pH stability were tested by packing columns with the derivatized silica and carrying out a series of reversed-phase liquid chromatography (RPLC) experiments. Residual silanol activity was greatly reduced when the silica was pretreated at 800 °C, leading to less peak tailing for basic solutes. In a strongly basic mobile phase (pH 11.5) the pretreated silica was surprisingly stable, although bond cleavage of  $\text{C}_{18}$  groups from the surface was observed.

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### 1. Introduction

Silica gel has been the most widely used stationary phase support for reversed-phase liquid chromatography (RPLC) since the 1970s. The silica surface directly interacts with a solute, and indirectly, solutes interact with a bonded phase on the silica support [1]. Silica gel has many advantages as a support for

RPLC bonded phases. It can be fabricated into small, spherical particles (3 or 5  $\mu\text{m}$  in diameter) with narrow distributions of controlled pore sizes. The large surface area increases retention and loadability, and the surface is easily derivatized [2]. Silica gel is rigid and exhibits better performance and higher efficiency than other solid supports [3]. However, silica gel still has many disadvantages. Its useful pH range (3–8) for separations is small, peak tailing often occurs with basic solutes, and a complete understanding of the separation mechanism of hydrophobic phases bonded to it is not yet available [4].

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The surface of silica gel consists of two primary surface-active sites; silanols and siloxanes. Siloxane groups are hydrophobic and do not play an important role in surface derivatization or retention in RPLC. There are three distinct types of silanol groups on the silica surface, and these silanols are the dominant adsorption and derivatization sites (Fig. 1) [5]. The surface silanol concentration is approximately  $8 \mu\text{mol}/\text{m}^2$  on fully hydroxylated silica. However, less than one-half are typically reacted, due to steric hindrance of the large aliphatic (e.g.  $\text{C}_8$ ,  $\text{C}_{18}$ ) chains.

A common goal of treatment of the silica surface is reduction of the number of residual silanols left after the bonded phase has been attached. Residual silanols can be reduced after the main ligand has been bound to the surface by “endcapping”. Endcapping involves the bonding of small organosilanes to further reduce the residual silanol concentration after the primary alkyl ligand has been attached [6]. Alternatively, Pesek et al. used a silica hydride intermediate [7], while Sudo used high temperature silylation to endcap [8]. The silanols can also be reduced or blocked before derivatization or during chromatography. For example, Reta and Carr used divalent metals and amines as silanol blocking agents [9]. Hill and Vervoort and coworkers used triethylamine and longer hydrocarbon tertiary amines to block the silanols [10,11]. Halfpenny et al. used ultraviolet laser radiation to dehydroxylate the surface by controlling irradiance to get the desired degree of dehydroxylation [12].

Temperature dehydroxylation uses silanols to form hydrophobic siloxanes [13] and is also used before rehydroxylation for a more homogeneous bonded phase. Kirkland and Kohler selectively rehydroxylated the silica surface for better homogeneity [14].

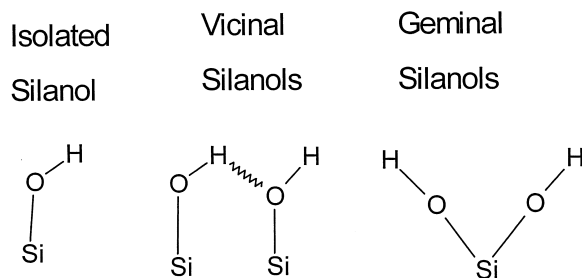


Fig. 1. Three types of silanols on silica surfaces.

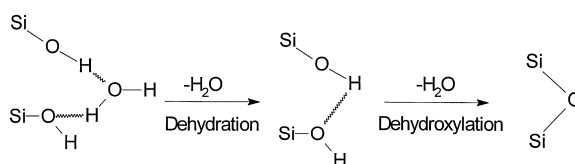


Fig. 2. Dehydration and dehydroxylation of the silica surface.

Kumar et al. refluxed in HCl for 12 h to form a more homogeneous surface [15]. Grossman et al. used calcination at high temperatures to dehydroxylate but followed with rehydroxylation [16]. Boudreau and Cooper showed one of the first dehydroxylations with no rehydroxylation [17]. The dehydroxylation was performed in the range of 180–840 °C (Fig. 2). There are no significant changes up to 400 °C. At lower temperatures, water is removed to form unstable siloxane bonds, and the siloxanes reform silanols after exposure to water. However, above 400 °C, the siloxane bonds are very stable, even after 2500 column volumes rehydroxylation did not occur. Sintering occurred as temperatures surpassed 800 °C and caused the surface area to decrease. When sintering occurs, the pores of the silica close, and the surface fuses together to form a solid network, resulting in chromatographically unusable silica.

The goal of the work described here is to reduce silanols on the silica surface by thermal dehydroxylation before derivatization in such a way that rehydroxylation will not occur. While this study only involved one specific type B silica, the results should provide general insights into the effects of thermal pretreatments on reversed-phase LC column materials.

## 2. Experimental

### 2.1. Dehydroxylation

The native silica used was Astrosil™ (Stellar Phases, Yardley, PA, USA), 14  $\mu\text{m}$  diameter with a pore size of 106 Å and surface area of 325  $\text{m}^2/\text{g}$ . The native starting material is manufactured using high purity organosilanes, and it is thermally stabilized to give narrow pore sizes. The metal contamination given by the manufacturer is sodium 12 ppm,

magnesium <9 ppm, calcium 14 ppm, aluminum 11 ppm, and iron <9 ppm. Dehydroxylation was carried out using a Lindberg tube furnace (Watertown, WI, USA) with constant nitrogen flow to prevent air from entering the system. Four different 0.5-g silica samples were placed into separate combustion boats (Fisher Scientific, Pittsburgh, PA, USA) and put into the tube furnace for 12, 24, 36 and 48 h at 400 °C. This was repeated at 500, 600, 650, 700, 750, 800 and 1000 °C to find the optimum time and temperature for dehydroxylation.

## 2.2. Derivatization reaction

The derivatization reaction was as described by Sentell et al. [18]. All thirty-two of the silica samples and a control sample treated at 150 °C were derivatized using octadecyldimethylchlorosilane (United Chemical Technologies, Bristol, PA, USA) in dichloromethane (Sigma–Aldrich, Milwaukee, WI, USA) with 4-dimethylaminopyridine (Sigma–Aldrich) as the acid acceptor, the reaction run under reflux for 48 h. At the end of 48 h, the silica was washed according to the same sequence described by Sentell et al. [18]. Each sample was analyzed for percent carbon with a CE Instruments NC 2500 elemental analyzer (Thermoquest, Rodano, Italy).

## 2.3. Column packing

Samples were suspended in chloroform and then packed into 15 cm×4.6 mm stainless steel columns (Sci-Con, Winter Haven, FL, USA) by the traditional slurry method using an air driven pump (Haskel Engineering and Supply, Burbank, CA, USA) at 7320 p.s.i. (1 p.s.i.=6894.76 Pa). The packing process used 200 ml of three solvents: chloroform, methanol, (Sigma–Aldrich), and methanol–water (50:50, v/v) (Barnstead, Dubuque, IA, USA).

## 2.4. Characterization of the silica surface

CP-MAS solid state <sup>29</sup>Si NMR spectra were obtained using a 200 MHz WPSY solid state NMR spectrometer (Bruker, Billerica, MA, USA). The Si frequency is one-fifth of the proton frequency. The rotor was spun at approximately 4.5 kHz, and the contact time was 4 ms. A delay time of 3 s was used.

Diffuse reflectance infrared Fourier transform spectroscopy (DRIFTS) spectra were obtained using an Avatar 360 FT-IR (Nicolet, Madison, WI, USA). Each spectrum was the result of 64 scans over the entire 400–4000 cm<sup>-1</sup> range. All silica samples were characterized before and after derivatization.

## 2.5. RPLC studies

Chromatograms were generated using two model 510 HPLC pumps (Waters, Milford, MA, USA) controlled by a model 680 automated gradient controller (Waters). Solutes were detected using a Spectroflow 757 absorbance detector (Kratos Analytical, Chestnut Ridge, NY, USA). Reagents used for RPLC studies were HPLC grade methanol, pyrrolidine (Sigma–Aldrich) and purified water (Barnstead).

A modified Engelhardt test was used to measure silanol activity [19]. A methanol–water (60:40, v/v) mobile phase solution was used at 2 ml/min. Column temperature was maintained at 40 °C, and detection at 254 nm. The interactions of aniline and phenol with the residual silanols were used to probe the amount of silanol activity. If aniline elutes before phenol there is no significant activity, while significant activity is indicated by a reversal in the retention order. Also, the asymmetry of *p*-ethylaniline peaks at 10% peak height was taken as another measure of silanol activity [19].

The retention factor (*k'*) of benzene and ethylbenzene was measured to study pH stability of the columns in a pH~11.5 pyrrolidine buffer [20]. The pyrrolidine buffer was chosen because it attacks residual silanols instead of siloxanes. The pH stability of the native bare silica was also studied by flushing with the pyrrolidine buffer. The pump pressure (p.s.i.) was recorded at column volume intervals, and the amount of dissolved silica was measured using the silicomolybdate complex method [21]. The column was also weighed before and after column flushing as a further measure of silica loss.

# 3. Results and discussion

## 3.1. Characterization before derivatization

The surface of silica can be characterized by a

variety of different methods. In this study, CP-MAS  $^{29}\text{Si}$  NMR and DRIFTS are used. Solid state NMR is advantageous because no solvent effects are present as in solution NMR. Cross polarization allows for greater  $S/N$  ratios by transferring magnetization from abundant protons to the diffuse  $^{29}\text{Si}$  nuclei. Magnetic relaxation is enhanced as well, allowing for faster analysis time and more signal averaging [22]. The three chemical shifts observed with  $^{29}\text{Si}$  NMR are  $-111$ ,  $-101$ , and  $-91$  ppm and are assigned to siloxanes, free and vicinal silanols, and geminal silanols, respectively [23]. In DRIFTS spectra, siloxanes give bands at  $810$  and  $1110\text{ cm}^{-1}$ , and silanols at  $3700\text{ cm}^{-1}$  (free silanols) and  $3600\text{ cm}^{-1}$  (geminal and vicinal silanols) [24].  $^{29}\text{Si}$  NMR and DRIFTS are thus complimentary, in that geminal silanols can be observed directly by NMR while free silanols are resolved by DRIFTS.

DRIFTS spectra were obtained after each time and temperature study. After 24 h there was no significant additional dehydroxylation at any temperature, so a time of 24 h was used for all dehydroxylation studies. Fig. 3 shows silica spectra at different pretreatment temperatures of  $150$ ,  $400$  and  $800\text{ }^\circ\text{C}$  for 24 h. As the temperature increases, the broad water ( $\sim 3600\text{ cm}^{-1}$ ) signal decreases tremendously. Also, the vicinal silanol ( $\sim 3650\text{ cm}^{-1}$ ) signal decreases. For the silica treated at  $800\text{ }^\circ\text{C}$ , the free and geminal silanol ( $\sim 3750\text{ cm}^{-1}$ ) signal is the only signal in the spectrum, showing there are no more than two silanol species on the surface. With the concentration

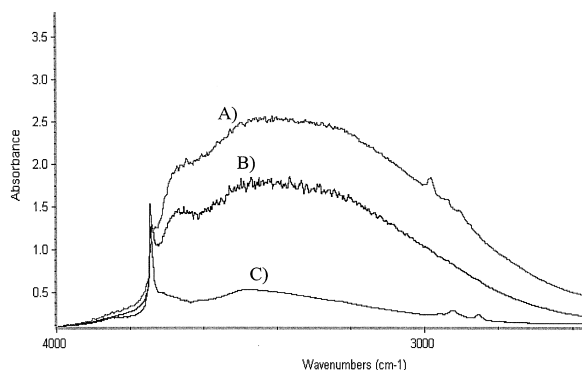


Fig. 3. DRIFTS spectra of Astrosil silica gel after pretreatment at (A)  $150\text{ }^\circ\text{C}$ , (B)  $400\text{ }^\circ\text{C}$  and (C)  $800\text{ }^\circ\text{C}$ . Peak assignments: free silanols,  $3750\text{ cm}^{-1}$ ; geminal and vicinal silanols,  $3650\text{ cm}^{-1}$ ; bound water, broad peak extending from  $3200$  to  $3600\text{ cm}^{-1}$ .

of silanols decreasing, the surface likely becomes more homogeneous with silanols spread more homogeneously across the surface, consistent with previous observations of thermal effects on silanol activity [17]. Since the vicinal silanols are removed first, there should be no silanols close enough to each other to hydrogen bond. The DRIFTS spectra indicate that the  $800\text{ }^\circ\text{C}$  dehydroxylation temperature is optimal for further studies, since temperatures above  $800\text{ }^\circ\text{C}$  resulted in sintering and chromatographically useless silica.

Solid state CP-MAS  $^{29}\text{Si}$  NMR spectra were also taken after dehydroxylation to compliment the DRIFTS spectra. Fig. 4(a) and (b) show silica dehydrated at  $150\text{ }^\circ\text{C}$  and silica dehydroxylated at  $800\text{ }^\circ\text{C}$ , respectively. After heating at  $800\text{ }^\circ\text{C}$  there is a significant reduction in the siloxane ( $-111\text{ ppm}$ ) and free + vicinal peaks ( $-101\text{ ppm}$ ), while the geminal peak remains fairly constant. It should be noted that the reduction in the siloxane peak is not due to loss of siloxane groups, but rather a decrease in sensitivity resulting from the loss of adjacent silanol groups that provide protons for cross polarization of  $^{29}\text{Si}$  atoms. When compared with the DRIFTS data, these NMR spectra indicate that the primary surface active groups removed are vicinal and free silanols. By using the dehydroxylated and bare silica NMR spectra, the total number of reactive silanols is approximated to be  $3.5 \pm 1\text{ }\mu\text{mol/m}^2$  by taking the ratio of the relative intensities of the silanol signals.

### 3.2. Characterization after derivatization

After derivatization of the dehydroxylated silica, a carbon analysis showed  $16.4\%$  carbon, which corresponds to a bonding density of  $2.54\text{ }\mu\text{mol/m}^2$ . DRIFTS spectra revealed that after derivatization there are fewer silanols on the surface after dehydroxylation at  $800\text{ }^\circ\text{C}$  compared to the silica treated at  $150$  and  $400\text{ }^\circ\text{C}$  (Fig. 5). DRIFTS spectra also show a signal from carbon stretching (peaks at  $<3000\text{ cm}^{-1}$ ) that assures the  $\text{C}_{18}$  ligand is attached to the surface. Fig. 6 shows solid state CP-MAS  $^{29}\text{Si}$  NMR spectra of the native silica dehydroxylated at  $800\text{ }^\circ\text{C}$  and derivatized with ODS. The peak at  $\sim 20\text{ ppm}$  is the Si–C bond, again showing  $\text{C}_{18}$  ligand attachment. The geminal silanol peak at  $-91\text{ ppm}$  is

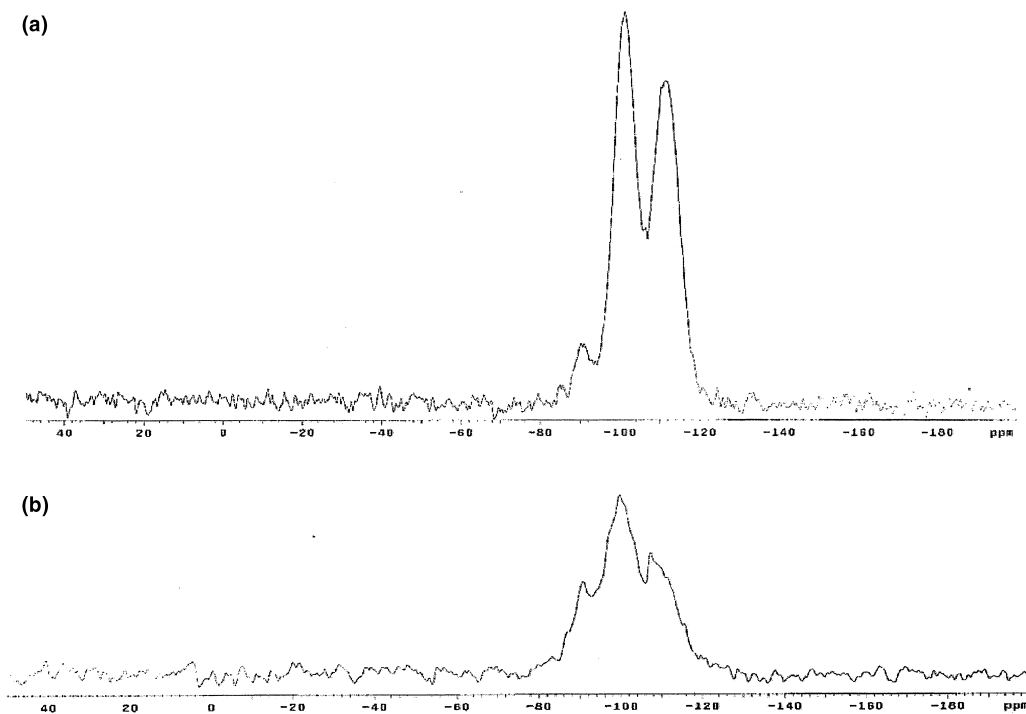


Fig. 4. (a) CP-MAS  $^{29}\text{Si}$  solid state NMR spectrum of Astrosil silica gel dehydrated at 150 °C. Peak assignments: siloxanes, -111 ppm; free and vicinal silanols, -101 ppm; geminal silanols, -91 ppm. (b) CP-MAS  $^{29}\text{Si}$  solid state NMR spectrum of Astrosil silica gel dehydroxylated at 800 °C.

completely gone, while the free + vicinal peak at -101 ppm is greatly reduced. These data suggest that the geminal silanols are the most reactive silanols on the surface. The siloxane peak at -111

ppm is now more intense than the remaining free and vicinal silanol peak, further suggesting that silanols are no longer the dominant surface active group after dehydroxylation at 800 °C and derivatization.

### 3.3. RPLC studies

Silica samples that had been derivatized after thermal treatment at 150 and 800 °C were each packed into a standard stainless steel LC column to

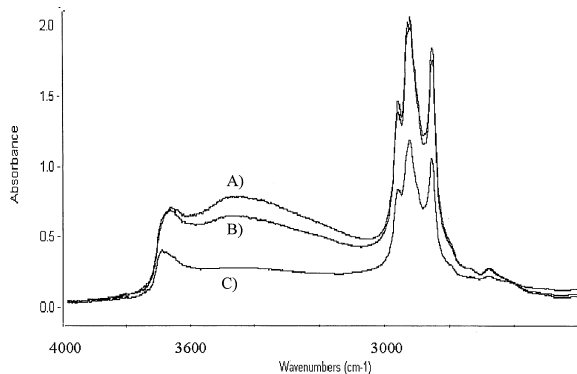


Fig. 5. DRIFTS spectra of  $\text{C}_{18}$  derivatized silica at different pretreatment temperatures of (A) 150 °C, (B) 400 °C, and (C) 800 °C.

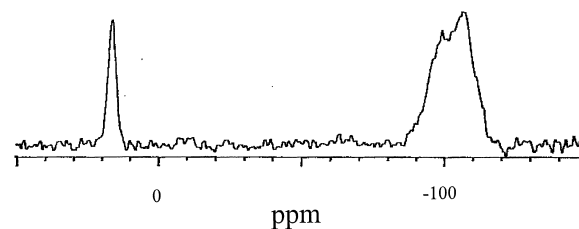


Fig. 6. CP-MAS  $^{29}\text{Si}$  solid state NMR spectrum of  $\text{C}_{18}$  derivatized silica after dehydroxylation at 800 °C. Peak at 20 ppm is due to Si-C bond in alkyl chain.

test the silica for stability and for silanol activity. The Engelhardt test on the silica dehydrated at 150 °C before derivatization resulted in phenol ( $k' = 1.9$ ) eluting before aniline ( $k' = 2.5$ ), indicating high silanol activity. The asymmetry factor was 3.0 at 10% peak height for *p*-ethylalanine after 10 000 column volumes for the 150 °C dehydrated silica. The same two tests were run on the 800 °C dehydroxylated and derivatized column, and phenol and aniline elute simultaneously with  $k'$  of 1.7. The coelution signifies silanol activity has been reduced and this is verified by an asymmetry for *p*-ethylalanine of 1.6, greatly reduced from 3.0 on dehydrated silica. Although the efficiency would of course be higher with smaller silica particles, the Engelhardt comparison was used here only to characterize silanol activity, not to determine column efficiency.

Lastly, a new column was packed to test for pH stability at pH 11.5 for the 800 °C dehydroxylated and derivatized silica. Fig. 7 shows decreasing retention of benzene and ethylbenzene as a function of volume of mobile phase pumped through the column. At high pH, dissolution of silica has been reported [20]. In this study, however, there was minimal dissolution of silica as measured by the silicomolybdate complex method. On the other hand, bond cleavage of the alkyl chains was indicated by a carbon analysis after the pH test that yielded 5.98% carbon, which corresponds to 0.79  $\mu\text{mol}/\text{m}^2$ . These results are in contrast to the results of Kirkland et al. [20], who observed significant silica dissolution but minimal alkyl bond cleavage in strongly basic mo-

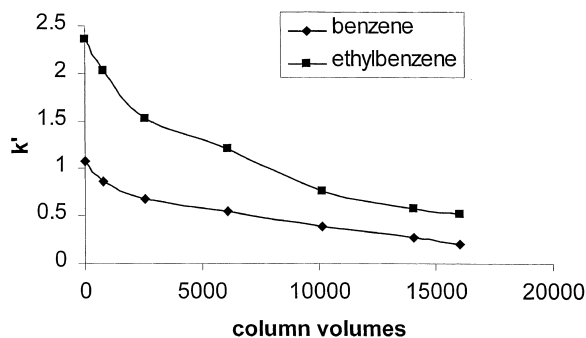


Fig. 7. Column longevity study of ODS derivatized silica after dehydroxylation pretreatment at 800 °C; mobile phase buffered with pyrrolidine to pH 11.5.

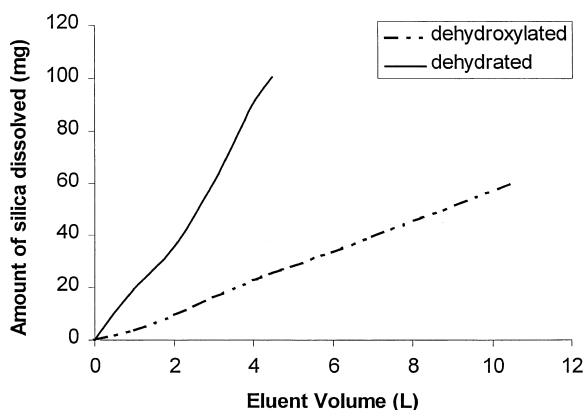


Fig. 8. Silica dissolution of dehydroxylated and dehydrated bare silica as a function of volume of basic mobile phase pumped through column.

bile phases. These contradictory results may well be due to the different silicas used in the studies cited.

Since there was minimal dissolution of silica at high pH, it was important to check the stability of the native silica before derivatization under normal pretreatment (150 °C) and dehydroxylated (800 °C) conditions. Using the 150 °C dehydrated silica, 100 mg of silica dissolved before the column completely blocked at 12 400 column volumes. The 800 °C dehydroxylated silica never clogged the column frit, however, and only 60 mg of silica dissolved after 29 400 column volumes (Fig. 8). These data confirm, for the silica used in this study, that the dehydroxylated form is more stable than the dehydrated form.

#### 4. Conclusion

Thermal dehydroxylation can be used to reduce the number of silanols on the silica surface before derivatization, yielding a bonded phase with significantly decreased residual silanol activity for Astrosil silica. Although different silicas have different properties, we believe these results suggest that a reduction of silanols will occur for all silicas. However, optimal dehydroxylation times and temperatures will have to be established for each particular silica. The dehydroxylated Astrosil silica is also more stable in basic mobile phases than when prepared by traditional way. No silica dissolution is

seen at high pH, although, bond cleavage of the alkyl chain was observed.

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