Communication

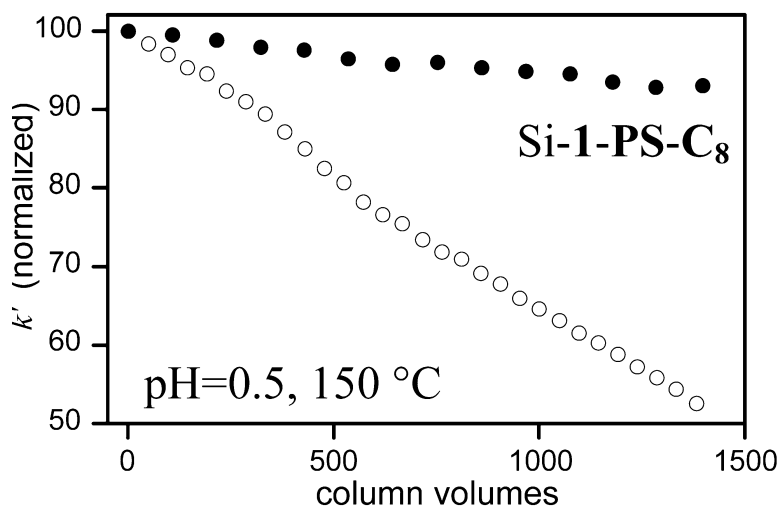# An Ultra Acid Stable Reversed Stationary Phase

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An Ultra Acid Stable Reversed Stationary Phase

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Considerable effort has been invested in the synthesis of stationary phases with superior acid stability for reversed phase liquid chromatography (RPLC).[1−11] Acid stable phases are critically important for peptide/protein separations (proteomics) and for liquid chromatography−mass spectrometric (LC−MS) analysis of basic compounds. Exceptional media stability is necessary to fully exploit selectivity differences and peak shape improvements for small, basic solutes using highly acidic mobile phases. To this end, the current "gold standard" for acid stable RPLC stationary media are the "sterically protected" C_{18} silica phases.[12] Two bulky (e.g., isopropyl, isobutyl) substituents on the silane reagent lead to "shielding" of the underlying silane−silica siloxane bonds. While these phases exhibit minimal acid-catalyzed hydrolysis and phase loss (1.0% trifluoroacetic acid (TFA), pH = 1.0, 1.0 mL/min, 90 °C, 25 000 column volumes),[12] we have shown that under accelerated acid aging conditions (5.0% TFA, pH = 0.5, 2.0 mL/min, 150 °C) the retention factor (k') for a neutral solute (dodecaphenone) is significantly diminished (losses up to 50%) after only 1400 column volumes (see below). There is a need for exceptionally high temperature acid stable phases for separation optimization (especially for hydrophobic peptides/proteins), enhanced column lifetime, temporal stability in solute retention, and ultrafast liquid chromatography for biomolecule separations.[13] We recently developed silica stationary phases that exhibited dramatically improved acid stability as compared to sterically protected C_{18} materials.[1] These highly cross-linked stationary phases were prepared by covalently linking a chloromethylated aromatic silane to the silica surface followed by $AlCl_3$-catalyzed cross-linking, typically in the presence of added aromatic molecules (e.g., oligomeric polystyrene). This methodology has several advantages including minimization of pore blockage by confining the cross-linking reaction to the silica surface through the use of orthogonally reactive reagents, activation of the aromatic groups after the initial cross-linking reaction, the ability to reactivate the cross-linked aromatic surface by subsequent chloromethylation, and the susceptibility of the cross-linked coating to a variety of further modifications providing a suite of stationary phases with unique chromatographic selectivities.

In our original approach,[1] we employed a self-assembled monolayer (SAM) of chloromethyl−phenylethyl−trichlorosilane $(Cl-CH_2-C_6H_4-C_2H_4-SiCl_3)$ on silica. While these SAM phases gave a high surface density of chloromethyl groups, this synthesis method leads to inevitable monolayer defects that produce deleterious silanols.[14] More importantly, we determined that these SAM phases tenaciously adsorb organic bases (specifically compounds possessing ammonium residues). This generally results in seriously diminished chromatographic efficiency for such analytes.[15] In an effort to remedy this problem, we have developed a procedure for the production of silica stationary phases with remarkable acid and thermal stability using conventional silanization with a related chlorosilane, **1** (Figure 1), followed by extensive cross-linking of this modified phase in the presence of added aromatic reagents. This straightforward methodology minimizes the introduction of
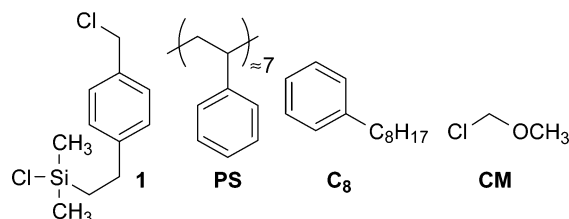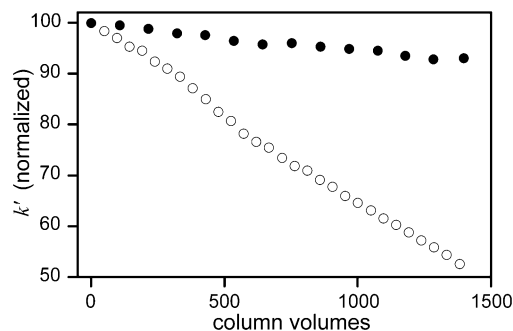
Figure 1. Reagents employed for the synthesis of Si-**1**-PS-**C₈**.

Figure 2. Normalized k' using Si-**1**-PS-**C₈** (●) (initial $k' = 5.15$) and **SBC₁₈** (○) (initial $k' = 7.60$) for dodecanophenone: 47.5/47.5/5.0 acetonitrile/water/TFA mobile phase (pH = 0.5) at 150 °C and 2.0 mL/min (columns: 5.0 × 0.46 cm). The plate count for benzene on the Si-**1**-PS-**C₈** is 98 000/m (reduced plate height = 2.0).

additional silanols to the surface and, to the best of our knowledge, results in the most acid stable silica-based stationary phases yet reported.

The reagents used for the modification of the silica (Type B Zorbax) particles are shown in Figure 1. The general protocol is as follows (see Supporting Information for details): after reaction of the silica with **1** using a conventional silanization process, a slurry of this phase (Si-**1**) and oligomeric polystyrene (**PS**, $M_n = 770$ g/mol) in nitrobenzene was treated with $AlCl_3$ to initiate a Friedel−Crafts (FC) coupling reaction.[16] The aromatic moieties in this new phase, Si-**1**-PS, were chloromethylated with chloromethyl methyl ether (**CM**), and a second $AlCl_3$-catalyzed FC reaction (i.e., self-condensation) was performed. The resultant "reactivated" and further cross-linked Si-**1**-PS was subsequently treated with octylbenzene (**C₈**) and benzene in a final FC alkylation, giving Si-**1**-PS-**C₈**. Elemental analysis of Si-**1** gave 6.29 wt % carbon and 1.41 wt % chlorine, consistent with the incorporation of 2.9 $\mu mol/m^2$ of **1**. Si-**1**-PS-**C₈** contained 13.2 wt % carbon and 0.5 wt % chlorine. These data are consistent with a highly cross-linked polymer coating the surface of the silica.

The stability of Si-**1**-PS-**C₈** was compared to a commercially available sterically protected C_{18} phase (**SBC₁₈**).[17] Before the stability test, Si-**1**-PS-**C₈** was pretreated using a set of four acetonitrile/water/TFA gradients at 150 °C.[18] Figure 2 shows the normalized k' for dodecanophenone versus the number of column volumes in highly acidic media at 150 °C. Si-**1**-PS-**C₈** is much
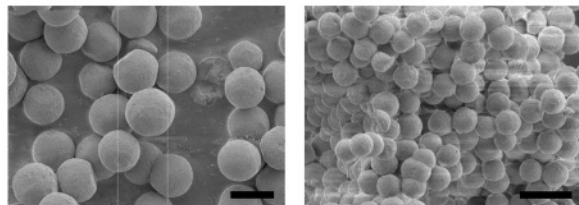
Figure 3. SEM images of Si-**1**-**PS**-**C$_8$** as synthesized (left) and post HF treatment (right). Both scale bars are 5 μm.

more stable than **SBC$_{18}$**, showing only 7% reduction in k' after 1400 column volumes. The rapid initial decrease in k' in Si-**1**-**PS**-**C$_8$** was attributed to hydrolysis of the silane−silica siloxane bonds. This was corroborated by comparison of the ^{29}Si SS−NMR data (Figure S1) of as-synthesized Si-**1**-**PS**-**C$_8$** and Si-**1**-**PS**-**C$_8$** after this acid stability test. Also, after the acid stability test, the elemental analysis of Si-**1**-**PS**-**C$_8$** showed very little loss in carbon and near complete loss of chlorine (13.00 wt % carbon and 0.02 wt % chlorine). Removal of the chlorine in Si-**1**-**PS**-**C$_8$** was confirmed by ^{13}C SS-NMR (Figure S2). These data are consistent with hydrolysis of residual −CH$_2$−Cl groups as a result of the pretreatment and acid aging test.

We explored the separation of a variety of basic solutes using Si-**1**-**PS**-**C$_8$** (after the gradient pretreatment described above) and compared the results with **SBC$_{18}$** by evaluating peak shapes. The U.S.P.[19] tailing factors for perphenazine, desipramine, nortriptyline, and amitriptyline in 15/85 0.1% TFA in acetonitrile/0.1% TFA in water (pH = 2.0) at 40 °C were nearly identical (Figure S3), and the peak shapes for both phases were highly symmetric. In fact, the U.S.P. tailing factors for all solutes were somewhat closer to 1.00 using Si-**1**-**PS**-**C$_8$**. This may be due to the presence of hydroxyl groups (i.e., from hydrolysis of the chloromethyl groups described above) in this phase; related "polar embedded phases" have been reported to show improved peak shapes for basic solutes.[20]

Critically important to the utility of any new stationary phase is pore accessibility because pore blockage can result in inferior chromatographic efficiency.[21] Using inverse size exclusion chromatography,[22] we calculated the fraction of pore volume accessible (K) to polystyrene probes of different molecular weights. Generally, the K values for **SBC$_{18}$** were within a few percent of those of Si-**1**-**PS**-**C$_8$**, although both were less than the K values for bare silica (Figure S4). A comparison of stationary phase volume as calculated from inverse size exclusion chromatography data and by the carbon content data for Si-**1**-**PS**-**C$_8$** showed that these two numbers were nearly the same (∼0.06 mL/column), further suggesting no significant pore blockage.[23] The high degree of cross-linking and formation of a cross-linked network on the surface of and within the silica pores was verified by scanning electron microscopy (SEM) images of the Si-**1**-**PS**-**C$_8$** phase before and after complete removal of all silica with HF (Figure 3). After complete removal of silica (verified gravimetrically), porous polymeric spheres, presumably a negative image of the interior surface of the initial Si-**1**-**PS**-**C$_8$** particles, remain. Some shrinkage was observed likely due to removal of the supporting silica skeletal framework and the drying of the polymer during the required processing to obtain the SEM image.

Si-**1**-**PS**-**C$_8$** is a new ultrastable RPLC phase that is readily synthesized using straightforward processes. This phase shows remarkable and unparalleled acid stability, has excellent separation ability (k'), and exhibits high chromatographic efficiency (minimal pore blockage). This combination of attributes is unprecedented and will be extremely useful for next generation RPLC phases.

Supporting Information Available: A detailed description of the synthesis of Si-**1**-**PS**-**C$_8$** and Figures S1−S4 (PDF). This material is available free of charge via the Internet at http://pubs.acs.org.

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JA036339D