Published in final edited form as:

Anal Chem. 2013 December 17; 85(24): 11765–11770. doi:10.1021/ac401986j.

Improved Synthesis of Carbon Clad Silica Stationary Phases

Imad A. Haidar Ahmad and Peter W. Carr*

Department of Chemistry, Smith and Kolthoff Halls, University of Minnesota, 207 Pleasant Street SE, Minneapolis, Minnesota 55455, United States

Abstract

Previously we described a novel method for cladding elemental carbon onto the surface of catalytically activated silica by a chemical vapor deposition (CVD) method using hexane as the carbon source and its use as a substitute for carbon clad zirconia.^{1,2} In that method, we showed that very close to exactly one uniform monolayer of Al (III) was deposited on the silica by a process analogous to precipitation from homogeneous solution in order to preclude pore blockage. The purpose of the Al(III) monolayer layer is to activate the surface for subsequent CVD of carbon. In this work, we present an improved procedure for preparing the carbon clad silica (denoted CCSi) phases along with a new column packing process. The new method yields CCSi phases having better efficiency, peak symmetry and higher retentivity compared to carbon clad zirconia.

The enhancements were achieved by modifying the original procedure in three ways: first, the kinetics of the deposition of Al(III) were more stringently controlled, second the CVD chamber was flushed with a mixture of hydrogen and nitrogen gas during the carbon cladding process to minimize generation of polar sites by oxygen incorporation, and third the fine particles generated during the CVD process were exhaustively removed by flotation in an appropriate solvent.

1. Introduction

Carbon clad stationary phases are useful phases for high pressure liquid chromatography. They are made by the vapor phase decomposition of saturated hydrocarbons leading to the deposition of elemental carbon and highly unsaturated hydrocarbons on the surface of appropriately activated silica. These phases have unique reversed phase selectivity,^{3,4} high stability in acidic media, high mechanical strength, and high retentivity.^{1–3} These properties make CCSi stationary phases good candidates for use in ultrafast liquid chromatography and two-dimensional liquid chromatography.^{5,6} CCSi phases are prepared in two steps: First, an Al(III) CVD catalyst is homogeneously precipitated on deprotonated silanol groups using the slow hydrolysis of urea in an acid solution of Al(III). Subsequently, carbon readily deposits from various common sources onto these activated silicas via CVD at temperatures of about 700 °C.

Three challenges must be overcome to prepare a useful material for liquid chromatography:

1) the hydrolytic generation of base must be quenched at a pH ~ 5 to preclude the bulk

*Corresponding author. Tel.: + 1 612 624 0253; fax: + 1 612 626 7541, petecarr@umn.edu.

precipitation of aluminum oxides and hydroxides in both solution and in the body of the pores of the silica, 2) the elimination or reduction of the number of highly reactive oxygen radicals resulting from oxygen leakage into the CVD chamber, and 3) the mechanical stabilization of the bed structure over long time scales.

In previous work,¹⁻³ the hydrolysis of urea was carried out at 100 °C. At this temperature, the pH rise becomes very fast especially when the reaction is close to the end point. Accordingly, quenching the reaction at the appropriate pH (~5) was not highly reproducible and resulting in not very reproducible surface properties of the particles from one run to the next. This problem was solved by adding urea in two steps, a limited amount of urea sufficient to nearly reach the end point was added at 100 °C, the temperature was subsequently dropped to 85 °C and more urea was added. Using this method, the procedure is very reproducible and the final pH can be controlled to better than ± 0.05 . The second challenge, the minimization of oxygen free radical formation due to the leakage of oxygen into the CVD chamber through the tubing used to connect the N₂ source to the oscillating CVD reactor, is accomplished by flushing the reactor with a mixture of hydrogen and nitrogen gas (95% N₂, 5% H₂). Hydrogen gas will rapidly scavenge the oxygen-based radicals thus minimizing their formation of oxygenated sites on the carbon surface. Finally, the packing instability of the column bed was solved by a special process which removed fines. The net result is a significant increase in plate count, peak symmetry, and bed stability.

2. Experimental

In this section the detailed procedure of the new method of making and packing the particles is presented.

Sample preparation

Thiourea was used to determine the dead time of the chromatographic system and to correct for extra column broadening. The test solutes were dissolved in pure water at concentrations of: 2.47 (thiourea), 1.70 (nitropropane), 1.81 (nitrobutane), 2.09 (nitropentane), 2.20 (nitrohexane), all concentrations are in micrograms per gram of water.

Another series of test solutes were used in this study as follows: N-benzylformamide, benzylalcohol, phenol, 3-phenylpropanol, benzonitrile, nitrobenzene, methylbenzoate, anisole, benzene, toluene, bromobenzene, acetophenone, ethylbenzene, p-xylene, p-dichlorobenzene, propylbenzene, butylbenzene, p-chlorotoluene, p-nitrobenzyl chloride, p-nitrotoluene, benzophenone, p-chlorophenol, and naphthalene.^{7, 8} The test solutes were dissolved in 40% ACN:60% H₂O at a concentration of 0.08 µg/mL and the injection volume was varied between 2-3 µL. The source of all chemicals was given in a prior work.^{5,6,9-11}

HPLC instrumentation, columns, and silica

An Agilent 1100 liquid chromatograph controlled by version B.04.02 Chemstation software (Agilent Technologies, Palo Alto, CA) was used. The Agilent 1100 was equipped with standard low-pressure mixing chamber, quaternary pump, auto sampler, Thermostatted Column Compartment (TCC) Model G13168, variable single wavelength UV detector and a

1 μL , 5 mm path flow cell with a sampling rate of 13.6 Hz. Data were acquired at wavelength of 210 nm. All the tubing connecting the parts of the system is PEEK tubing of internal diameter 0.005" and total length 18" (total volume equal to 2.3 μL). Experiments were run on two 2.1×33 mm columns packed with CCSi particles. The runs were performed at a flow rate of 0.5 mL/min at 40 °C to obtain the retention factor and plate count corrected for extra-column volume and dispersion (according to the equations shown in section on isocratic RPLC measurements). The mobile phase in channel A was water and in channel B pure acetonitrile. Peak standard deviations were computed from the peak half-widths given by the data system and the assumption of Gaussian conversion factors. The Agilent Poroshell 120 particles have a solid core (1.7 μm in diameter) and a porous silica layer (0.5 μm thick) surrounding it. The specific surface area of the silica is 126 m^2/g its nominal pore size is 120 Å. The material used was the same product as used in our prior work².

Improved procedure for metal adsorption

Previously we defined the exact amount of metal chloride needed to be added to silica.¹ In the earlier work, the reaction mixture was heated under reflux for two hours until the pH reached 4 - 4.3 at 100 °C and then it was quenched by cooling the solution to room temperature. In the current procedure, we ran the reaction first at 100 °C and then at 85 °C. The urea was added in two steps as shown in Figure 1. First, half the amount of urea needed to neutralize the acid was added and the reaction was refluxed for two hours, then the temperature was dropped to 85 °C and the other half of the urea was added when the temperature reached 85 °C. Subsequently the pH increases slowly until the desired pH is reached when the reaction is quenched (see Figure 1). The pH meter was initially calibrated at room temperature using two commercial standard buffers (4.0 and 7.0). During the reaction, the pH was measured at the reaction temperature 85 °C (Figure 1). The pH was measured as 4.5 when the reaction was quenched at 85 °C. After the reaction mixture cooled to room temperature the pH was measured again and was never above 6.

In this method one never sees any sign of cloudiness indicative of inhomogeneous precipitation of aluminum hydroxides or oxides. Thus no pores are plugged during the deposition of the Al(III). We proved previously¹ that virtually all Al(III) was removed from solution by titrating the solution with EDTA. The same silica particles were used in this work and the ratio of silica area to aluminum was kept the same. It is important to note that when the reaction was run in the absence of silica, at no time was any turbidity noted indicating that aluminum hydroxides never precipitate during the deposition process while not exceeding pH 6.0.

Improved method for carbon cladding

Chemical vapor deposition is used for cladding carbon on aluminum treated silica. The apparatus and the procedure is described elsewhere.³ There are two modifications to our older procedure: first, the carrier gas was changed to a mixture of 95 % nitrogen and 5% hydrogen instead of pure nitrogen. Second, the silica particles are heated in the flowing gas for one hour at 700 °C before the flow of the nitrogen / hydrogen mixture is passed through the hexane filled wash bottle.

Improved particle sizing

In this section a method of removing fines generated during the CVD process is presented along with a modified packing procedure.^{1,2} The fines are removed by flotation. The slurry of 5 g of CCSi particles in 250 mL of THF are sonicated for 5 minutes, followed by gentle shaking and then left to settle slowly. After the bulk of the particles have settled to near the bottom of the cylinder, The supernatant was carefully removed by pouring off the first ~ 80 mL of supernatant and then using a pipette to carefully remove the rest. We intentionally left about 20 mL of THF on top of the sedimented particles to prevent remixing of the particles and the supernatant. This process was repeated repeated four times. We note that fewer fine particles and less color appeared in the supernatant in each cycle.⁶

Column packing

The slurry is prepared by adding 0.5 grams of CCSi particles in 22 mL of NMP and it is packed using a pneumatic driven liquid pump at a pressure of 9000 psi where THF is the pushing solvent. The particles are initially pumped into the chromatography column at a pressure of 1000 psi for few seconds and then the pressure is gradually increased to 9000 psi and held at that pressure until 80 mL of solvent are collected from the column. Columns packed with CCSi particles have shown excellent stability at extreme chromatography conditions (temperatures higher than 100 °C and flow rates ~ 3mL/min). At such temperature and flow rate, CCSi columns packed by the new procedure were better than those packed with the old procedure and that is why we consider them to be better packed. Columns packed using the new procedure were stable for longer than 24 hours whereas bonded phase columns do not last more than ten hours under the aforementioned conditions.

Isocratic RPLC Measurements

The detailed equations for determining retention factor and plate count corrected for extra-column volume and dispersion are shown in this section. The k' values were calculated according to the following equation

$$k' = \frac{(t_R - t_o)}{(t_o - t_{ex})} \quad (1)$$

where t_o is the dead time of the column determined using thiourea as an unretained solute and t_{ex} is the retention time correction due to extra-column volume determined by removing the column and replacing the column with a zero dead volume connector. At a flow rate of 0.4 mL/min the extra-column delay was 0.066 min corresponding to a volume of 26 μ L. With the column in place the dead time at a flow of 0.4 mL/min was 0.262 min as determined with thiourea. Correcting for the extra-column volume gave a column dead volume of 78 μ L. Assuming a total porosity of 0.6 we estimate a column dead volume of 69 μ L.

Each of the retention times, variances, plate counts, and retention factors of the individual solutes were corrected for extra-column issues. The equations for correcting retention time ($t_{R,corr}$), variance (σ_{corr}^2), and plate count ($N_{iso,corr}$) are:

$$t_{R,corr} = t_{R,obs} - t_{ex} \quad (2)$$

$$\sigma_{corr}^2 = \sigma_{obs}^2 - \sigma_{ex}^2 \quad (3)$$

$$N_{iso,corr} = \frac{(t_{R,corr})^2}{\sigma_{corr}^2} \quad (4)$$

Where σ_{ex}^2 is the variance due to band broadening that take place in the extra-column components. $t_{corr,ex}$, σ_{corr}^2 , and $N_{iso,corr}$ are the corrected retention times, variance, and plate count due to extra-column band broadening, respectively. The variance due to band broadening (σ_{ex}^2) is determined by injecting thiourea after disconnecting the column from the system and replacing it with a zero dead volume connector.

2. Results and discussion

Metal addition

The change in pH vs. the reaction time for the new Al(III) deposition method is shown in Figure 1. The initial pH of the reaction is ~ 1.2 and increases slowly to ~ 2.1 after refluxing for two hours. Next, when the temperature of the reaction is dropped to 85 °C and another aliquot of urea is added, the pH starts to slowly approach the end point due to the substantial decrease in the decomposition rate of urea.¹² The main advantage of this modified procedure is that the reaction slows down as it reaches the end point. As a result, the reaction can be easily quenched at any desired pH with an precision of ± 0.05 pH units resulting in a much more reproducible batch to batch coating with Al(III).

LSER analysis of hydrogen treated versus untreated particles

As mentioned above, we expect that polar sites are formed due to the leakage of O₂ into the reactor^{13–15} along with the formation of free radicals due to crushing silica by the rocking action of the reactor.¹⁶ Previous reports have shown that hot H₂ gas is capable of reducing oxidized carbon surfaces.¹⁷ The hydrogen treatment reduces the number of polar groups on the carbon surface and improves peak shape by decreasing the degree of peak tailing (see Figure 2).

To determine whether the hydrogen-treated material has different selectivities from the untreated material, a set of judiciously selected analytes previously used to probe surface interactions via linear solvation energy relationships (LSER)^{7,8} was used to study both materials. The plot of Figure 3A shows that there is a very good correlation between the logarithmic retention factors on the two phases.

The data fit the line:

$$\ln k'_{H_s} = 0.974(0.005) \ln k' + 0.218(0.01); r^2 = 0.99938, se = 0.032 \quad (5)$$

This must be considered an excellent fit in terms of the r^2 value. However, the small intercept is not statistically zero. Note the quantities in parenthesis are the standard deviations of the intercept and slope. Further the slope is not unity. Based on the work of

Horvath¹⁹ this means that the retention mechanisms are chemically very similar, which is termed homeoenergetic, but definitely not identical, i.e. homoenergetic. The small intercept and proximity of the slope to unity suggests a slight difference in phase ratios of the two materials.

The data were further analyzed in Figure 3B. The solid line is the ratio of the retention factors computed according to Eq 6. On this scale it is evident that there is considerable scatter about the regression line. Moreover certain classes of compounds notably the simple polars, hydroxyl and halogenated solutes appear to fit the overall regression better than the alkylbenzenes and especially the nitro compounds. We note that all the alkylbenzenes lie above the regression line and the three nitro compounds lie well below the regression line. The fact that the hydroxylated and simple polar compounds seem to fit the overall regression suggests that the hydrogen bond donating and accepting properties of the two phases were not significantly altered by the hydrogen treatment. This is contrary to our expectations as to the role of hydrogen during carbon deposition. However, this result was confirmed by doing a more detailed analysis by using the LSER parameters of the probe solutes (see Eq 6).

We first regressed the logarithm of the retention on the treated phase against that of the untreated phase using the full set of solvatochromic explanatory parameters (π_2^* , $\Sigma\alpha_2^H$, $\Sigma\beta_2^H$, and R_2). We found that the coefficients of the hydrogen bond donor and hydrogen bond acceptor terms ($\Sigma\alpha_2^H$ and $\Sigma\beta_2^H$ respectively) were statistically zero. These terms were then dropped from the regression. The final result was:

$$\ln k'_{H_s} = 0.216(0.024) = 0.967(0.006) \ln k' - 0.081(0.02) \pi_2^* + 0.105(0.05) R_2; r^2 = 0.99958, se = 0.028 \quad (6)$$

We see that the coefficient of the solute dipolarity/polarizability parameter (π_2^*) indicates a lower value on the H₂ treated phase whereas the coefficient of the excess molar polarizability term (R_2) indicates an increase. This is in agreement with the displacement of the alkyl benzenes and the nitro compounds from the overall regression line. We believe that the H₂ treated phase is slightly less polar but slightly more polarizable than the untreated phase. Note that the overall standard error (se) of the fit in Eq 6 is only very slightly smaller than in Eq 5 so our conclusions must be considered rather tentative.

Effect of hydrogen treatment on plate count

Figure 4 shows the plate count for the untreated and hydrogen treated phases. The solute order is from least to most retained on the untreated phase. There was only one minor change in elution order between the two phases. Both were packed by the same procedure so the changes reflect differences in the particles and not the packing conditions. Note that in all cases the plate count for the H₂ treated phase is higher and in some instances almost two fold higher. The average and median plate count ratio on the hydrogen treated to the untreated phases are 1.8 and 1.7 respectively. The maximum plate count ratio was 3.4 for bromobenzene ($N_{H_2} = 838$) and the minimum ratio was 1.3 for naphthalene ($N_{H_2} = 279$).

What is truly remarkable about both carbon materials is the very wide range in the plate counts observed. There is a wide range of plate count seen in a narrow range in k' and over

the whole range in k' (see Fig. 5). For example, butylbenzene and p-dichlorobenzene both have retention factors of about 25 to 26 yet their plate counts are radically different on both phases. The lowest values are seen with the well retained nitroaromatic compounds. For example, nitrobenzene and nitrotoluene have k' values of 10 and 28 on the untreated phase respectively. On the other hand some of the highest plate counts are observed for the well retained alkyl benzenes as well as for weakly retained polar solutes such as phenol and benzyl alcohol. The plate count does appear to go through a maximum at intermediate values of the retention factor of about 5–15. The H_2 treatment really did not simplify or clarify our understanding of the solute dependence of peak broadening on carbon clad phases although the treatment improved the plate count and peak tailing.

Conclusions

A more reproducible (batch-to-batch) synthesis and column packing procedure for CCSi phases was developed. A uniform monolayer of Al (III) catalyst was deposited on the surface of silica by stringently controlling the rate of the urea hydrolysis by adjusting the hydrolysis temperature and the time and amount of urea added. Removing the fine particles from the slurry before packing increased the mechanical stability of the column bed. Also, flushing the particles with a hydrogen-nitrogen gas mixture (95% N_2 , 5% H_2) during the CVD process improved the chromatographic properties of the CCSi stationary phase without significantly changing their chromatographic selectivity (i.e. the band spacing was very similar to that of the untreated material). The improved chromatographic properties of the treated phase is evidenced by the decreased tailing of peaks and concomitantly in the 1.3 to 3.4 fold increase in plate counts of all 22 probe solutes. We believe that the improvement in peak shape results from a reduction in population of some pernicious polar sites which cause a great deal of peak tailing but which do not otherwise significantly contribute to retention.

Acknowledgements

The authors would like acknowledge financial support from National Institutes of Health and Agilent Technologies for the donation of the silica used in this study.

References

1. Paek C, McCormick AV, Carr PW. J. Chromatogr., A. 2011; 1218:1359–1366. [PubMed: 21295308]
2. Paek C, Huang Y, Filgueira MR, McCormick AV, Carr PW. J. Chromatogr., A. 2012; 1229:129–139. [PubMed: 22321953]
3. Paek C, McCormick AV, Carr PW. J. Chromatogr., A. 2010; 1217:6475–6483. [PubMed: 20850126]
4. Gu H, Huang Y, Filgueira M, Carr PW. J. Chromatogr., A. 2011; 1218:6675–6687. [PubMed: 21840009]
5. Stoll DR, Li X, Wang X, Carr PW, Porter SEG, Rutan SC. J. Chromatogr., A. 2007; 1168:3–43. [PubMed: 17888443]
6. Stoll DR, Cohen JD, Carr PW. J. Chromatogr. 2006; 1122:123–137.
7. Vitha M, Carr PW. J. Chromatogr., A. 2006; 1126:143–194. [PubMed: 16889784]
8. Jackson PT, Schure MR, Weber TP, Carr PW. Anal. Chem. 1997; 69:416–425. [PubMed: 9030054]
9. Soliven A, Haidar Ahmad IA, Filgueira MR, Carr PW. J. Chromatogr., A. 2013; 1273:57–65. [PubMed: 23261314]

10. Haidar Ahmad IA, Soliven A, Carr PW. submitted.
11. Stoll DR, Paek C, Carr PW. *J. Chromatogr., A*. 2006; 1137:153–162. [PubMed: 17078962]
12. Shaw WHR, Bordeaux JJ. *J. Am. Chem. Soc.* 1955; 77:4729–4733.
13. Giacobbe FW. *J. Appl. Polym. Sci.* 1990; 39:1121–1132.
14. Giacobbe F. *J. Appl. Polym. Sci.* 1991; 42:2361–2364.
15. Giacobbe FW. *J. Appl. Polym. Sci.* 1992; 46:1113–1116.
16. Vallyathan V, Castranova V, Pack D, Leonard S, Shumaker J, Hubbs A, Shoemaker D, Ramsey D, Pretty J, McLaurin. *Am. J. Respir. Crit. Care Med.* 1995; 152:1003–1009. [PubMed: 7663775]
17. Yang L, Vail MA, Dadson A, Lee ML, Asplund MC, Linford MR. *Chem. Mater.* 2009; 21:4359–4365.
18. Foley JP, Dorsey JG. *Anal. Chem.* 1983; 55:730–737.
19. Melander W, Stoveken J, Horvath C. *J. Chromatogr., A*. 1980; 199:35–56.

Figure 1.
Modified procedure showing the change in pH during the deposition reaction of Al (III) on silica.

Figure 2.

Mixture of nitrobutane, nitropentane, and nitrohexane on the hydrogen-treated phase (—) versus the untreated phase (—). $T=40\text{ }^{\circ}\text{C}$, $F=0.4\text{ mL/min}$, $80\%\text{H}_2\text{O}:20\%\text{ACN}$. Both columns are packed using the same procedure given in the experimental section. The ratio of B/A tailing factor (at 10% of peak height) for the peaks on the hydrogen- treated phase versus untreated phase are shown in parenthesis respectively for each peak: nitrobutane (1.92, 2.16), nitropentane (1.85, 2.36), nitrohexane (2.59, 3.46).¹⁸ Plate counts on the peaks on the hydrogen-treated phase versus untreated phase are shown in parenthesis respectively for each peak: nitrobutane (3600,2800), nitropentane (5100,3700), nitrohexane (4500,2670)

B

Figure 3.

A. Plot of logarithmic retention of the new (H₂ treated) phase vs. the logarithmic retention on the previously described untreated phase. The 22 LSER probes were used. See legend of Figure 3B and Eq. 6 for details. Chromatographic conditions: F=0.4 mL/min, T= 40 °C, 40% ACN:60% H₂O.

B-Plot of the ratio of the normalized retention factors on the two phases vs. logarithmic retention factor on the untreated phase: ● alkyl benzenes (benzene, toluene, ethylbenzene, p-xylene, n-propylbenzene, n-butylbenzene, naphthalene), ▲ nitro compounds (nitrobenzene, p-nitrotoluene, p-nitrobenzyl chloride), ◆ simple polar (N-benzylformamide, anisole, methylbenzoate, acetophenone, benzonitrile, benzophenone), ◇ alcohols (benzylalcohol, phenol, 3-phenylpropanol, p-chlorophenol), □ halogenated (bromobenzene, p-chlorotoluene, p-dichlorobenzene). Chromatographic conditions as in Figure 3A. The solid line is computed from Eq. 5.

Figure 4.

The plate count ratio of hydrogen-treated carbon-silica particles and non- hydrogen treated carbon-silica particles of the LSER descriptors. $F=0.4$ mL/min, $T= 40$ °C, 30 °C, and 40% ACN: 60% H_2O . Plate count values are corrected for extra-column dispersion based on Eq 4. Both columns were packed using the same procedure given in the experimental section. The solute order is from least to most retained on the untreated phase.

Figure 5.

The plate count ratio of hydrogen-treated carbon-silica particles and non-hydrogen treated carbon as a function of the retention factor on the hydrogen treated phase. Chromatographic conditions as in Figure 4.