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Margaret Michelle Oliva
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DOI: <https://doi.org/10.31979/etd.yssa-7ykp>
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HYDROSILATION OF ACETYLENE COMPOUNDS FOR THE PREPARATION OF
A BONDED PHASE IN HPLC

A Thesis

Presented to

The Faculty of the Department of Chemistry

San Jose State University

In Partial Fulfillment

of the Requirements for the Degree

Master of Science

by

Margaret Michelle Oliva

May 1998

UMI Number: 1389671

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ABSTRACT

HYDROSILATION OF ACETYLENE COMPOUNDS FOR THE PREPARATION OF A BONDED PHASE IN HPLC

by Michelle Oliva

The goal of this thesis project was to prepare a bonded phase through hydrosilation of acetylenes that could be used as a stationary phase for high performance liquid chromatography (HPLC). HPLC is an important separation tool that is commonly used in many chemical analyses. The compounds selected were propiolic acid, 3,3-dimethyl-1-butyne, phenylacetylene, and 1-phenyl-2-propyn-1-ol. The latter two were packed into HPLC columns. These two were chosen for their different functional groups as well as their relatively short carbon chains.

One of the main focuses of this research was to determine if it is possible to attach two silicon atoms to the terminal carbon atom of each acetylene compound instead of just one silicon atom. This would allow for increased stability of the column as well as the possibility of giving better separations than the columns currently available. Cross Polarization-Magic Angle Spinning Nuclear Magnetic Resonance spectroscopy (CP-MAS NMR) and Diffuse Reflectance Infrared Fourier Transform spectroscopy (DRIFT) were used to verify the success of bonding.

Three classes of compounds were used to examine the viabilities of the phenylacetylene and 1-phenyl-2-propyn-1-ol columns: aromatic compounds, steroids, and benzodiazepines. These were selected in order to compare data from the new stationary phases with that in the literature. All HPLC testing was done in the normal phase mode.

ACKNOWLEDGMENTS

I would like to show my greatest appreciation to my advisor, Dr. Joseph Pesek, and his project scientist, Dr. Maria Matyska. Their support, dedication, and insight have helped me tremendously in completing this thesis. Because of their guidance and ability to teach, I have gained a greater understanding of chemistry.

I would also like to thank my committee members, Dr. Maureen Scharberg and Dr. Brad Stone, for their continued encouragement. Both helped me grow in countless ways during my time at San Jose State University. I also appreciate their critiquing of my manuscript.

Finally, I want to show my gratitude to my family and boyfriend for their never-ending love and faith in me. At times I felt it would be impossible to complete this project; however, their belief and confidence in me have helped me to conquer the task and to fulfill my goals.

DEDICATION

This thesis is dedicated to Brian...

Your love and support guided me through the long nights.

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Chapter I

INTRODUCTION

A. Silica

Silica has been used as a solid support in HPLC for many years because of its numerous advantages. It is very porous which gives it a high internal surface area and allows for good analyte-stationary phase interaction. It also has a known pore and particle size (both of which can be varied depending upon the desired parameters). Silica is easily modifiable and has high mechanical strength which allows it to remain stable in numerous solvents and hence give reproducible HPLC separations (1). The bond it forms with oxygen is short and stable. Using chemically modified silicas as part of the stationary phase have the benefit of increased versatility and selectivity when developing or choosing a column.

The one main drawback of silica is its pH limitation. Below pH 2 there is an increased chance of hydrolysis of the siloxane backbone ($\equiv\text{Si-O-Si}\equiv$) which is partially influenced by the impurities within the solid silica. Silica will rapidly dissolve in any solution with a pH of 8 or above because of the formation of silicate ions (2).

1. Modification Schemes Using Silica

There are many different silica modification routes that can be used to produce bonded phases depending upon the type of silica linkage that is preferred. In the past, organosilanization and chlorination followed by an organometallic (Grignard or alkyl-lithium) were commonly used to attach different ligands to the silica (3). However, both of these methods have particular shortcomings. Organosilanization produces a Si-O-Si-C linkage which is hydrolytically unstable towards certain mobile phase conditions; this in turn allows for chemical deterioration of the bonded phase which could lead to non-reproducible retention of analytes. While it does ultimately produce a Si-C linkage, the chlorination reaction is extremely sensitive to moisture: the chlorinated surface can be hydrolyzed back to silanols (3).

Besides the chlorination/organosilanization reactions, triethoxysilane (TES) silanization followed by hydrosilation is another reaction scheme that produces the desirable Si-C connection. First introduced by Chu *et al.* (4), TES silanization was used to reduce the number of unreacted silanols on the surface and to produce a Si-H monolayer.

(X = Si or H). This layer can then react with an olefin compound, for example, to produce the bonded material (5).

A catalyst is necessary for the above reaction to proceed; the most common one used today was first discovered by Speier *et al.* in 1957. Speier *et al.* studied numerous hydrosilation reactions involving the combination of silanes and terminal olefins in the presence of different platinum catalysts (4).

They found that the strongest catalyst was hexachloroplatinic acid dissolved in isopropanol which is now known as “Speier’s Catalyst” (6).

B. High Performance Liquid Chromatography

Through the early 1960s, liquid chromatography separations took place on large columns (5 cm wide by one meter long) that were packed with silica gel or alumina; this absorbent was then coated with a fitting solvent (7). A mixture to be analyzed would be poured into the top of the column and would travel through the column with the liquid, also referred to as the mobile phase, under the force of gravity (8). A single separation

used large quantities of solvent and absorbent; it also took a long time to collect and analyze the data. By the late 1960s, improvements to the separation technique were made and implemented; the particle size of the stationary phase was reduced from 50 μ m to 3-10 μ m and the instrumentation was refined. This enhanced method was called high-performance liquid chromatography (HPLC) (8).

Normal phase HPLC is made up of a nonpolar mobile phase (like n-heptane or chloroform) and a more polar stationary phase. This stationary phase is typically composed of polar hydrocarbons which can be physically or chemically bonded to an inert solid support, like silica, the latter method being preferred. Other solid supports like titania and alumina have also been used; however, silica is widely utilized because of the numerous desirable qualities outlined in Section A. Both the stationary and mobile phases contribute to the separation process. The mobile phase, along with the analytes, exerts some force as it travels through and interacts with the stationary phase. The force at which the mobile phase is pumped through the column is at relatively high pressures (10-50 bar) in order to achieve a good flow rate (7). While normal phase HPLC is not used as frequently as reversed-phase (polar mobile phase and nonpolar stationary phase), it can be used to successfully separate drugs, steroids, and aromatic hydrocarbons (9). Some of the materials synthesized in this project can be used in this separation mode.

C. Acetylene Compounds

Four different acetylene compounds were chosen for this project to determine their viabilities as HPLC bonded phases. They are listed in Table 1.

Table 1. Acetylene Compounds

Compound		Molecular Weight
Phenylacetylene	$C_6H_5C\equiv CH$	102.14
1-phenyl-2-propyn-1-ol	$HC\equiv CCH(OH)C_6H_5$	132.16
Propiolic acid	$HC\equiv CCOOH$	70.05
3,3-dimethyl-1-butyne	$(CH_3)_2CC\equiv CH$	82.15

These were selected because of their short carbon chains in order to facilitate the spectroscopic analysis of the products. The goal was to prove that these triple bond carbon compounds could exhibit an increased stability due to the possibility of two silicon linkages to the carbon atom. One possible hydrosilation reaction is given below:

The chromatographic properties of acetylene bonded stationary phases have not been studied in great detail. Much work to date has been done on terminal olefin groups ($R-C=CH_2$) (5,10). It was the hope that this project would uncover some advantages that exist by using terminal $C\equiv C$ compounds as part of a bonded phase. Another objective was

to determine if acetylene-bonded stationary phases are more versatile and stronger than olefin bonded phases as well as whether or not they offer any other chromatographic advantages over other available phases. Initial studies have shown that it is possible to bond acetylene compounds to silica hydride via hydrosilation. ^{13}C -CP-MAS NMR and DRIFT spectra have shown, though, that there is a mixture of both single and double Si-C linkages between the C-8 moiety and the surface (11). Other possible hydrosilation reactions are shown below:

Phenylacetylene:

1-phenyl-2-propyn-1-ol:

Propiolic acid:

3,3-dimethyl-1-butyne:

D. Goals

The first step was to synthesize the hydride intermediate on the silica surface and identify the Si-H bonds that were present. To confirm that the acetylene compounds successfully underwent hydrosilation, different characterization methods could be used; namely Diffuse Reflectance Infrared Fourier Transform Spectroscopy (DRIFT) and Cross-Polarization Magic Angle Spinning Nuclear Magnetic Resonance Spectroscopy (CP-MAS NMR). The final goal of the project was to show the potential of the new bonded phases by comparing them to other HPLC stationary phases. It was anticipated that these new phases could improve existing separations, help to increase column durability, and allow for separations that have not been done before.

E. Cross-Polarization Magic Angle Spinning Nuclear Magnetic Resonance Spectroscopy (CP-MAS NMR)

Nuclear Magnetic Resonance (NMR) spectroscopy is a tool that utilizes the electromagnetic properties of nuclei to give “fingerprints” of many chemical compounds. Information regarding the structural, thermodynamic, and kinetic features of a compound can be obtained from this highly sensitive and reproducible method (12). It is generally used in conjunction with other spectroscopic methods such as infrared (IR) and ultraviolet (UV).

Cross-Polarization Magic Angle Spinning NMR (CP-MAS NMR) is typically used for solid samples (since solids have limited molecular movement). Cross-polarization (CP) transfers magnetization from abundant, highly sensitive nuclei, like protons, to less abundant, low sensitivity nuclei like ^{13}C and ^{29}Si by forcing an overlap of proton and carbon (or proton and silicon) energies (13). When the proton and carbon (or proton and silicon) rotating frame energy levels match, magnetization from protons is transferred to the ^{13}C (and ^{29}Si) nuclei. Cross polarization gives the chemical shift tensor that is used to study the molecular motion of solid samples. The structure of molecules can also be determined from the heteronuclear dipole-dipole interaction (14).

Magic angle spinning (MAS) is a technique that rotates a solid sample around an axis (at around 5000 Hz) making an angle θ of 54.7° with respect to the external magnetic field ($3\cos^2\theta = 1$). At this angle, all nuclei tumble as if they were free entities in solution. In magic angle spinning, the time-average value of all the angles θ of all the different orientation vectors of the molecules in the solid becomes equal (15). This allows only the isotropic shift to be retained and the shielding anisotropies to be averaged to zero. Because of this, a broad NMR line breaks down into a narrow isotropic line and spinning sidebands in the frequency domain (14,15). With MAS, it has been shown that peaks become sharper and narrower and the overall resolution is very good (16). The combination of CP and MAS allows for the collection of better solid sample NMR spectra.

Chapter II

EXPERIMENTAL

A. Materials

1. Chemicals

The Chemical Abstracts Service (CAS) registry numbers of all of the chemicals used in this project are listed in Table 2.

2. Materials for Preparing the Bonded Phases

Phenylacetylene, 1-phenyl-2-propyn-1-ol, propiolic acid, and 3,3-dimethyl-1-butyne (Farchan Laboratories, Inc., Gainesville, FL) were used as received. A 5mM hexachloroplatinic acid (Strem Chemicals, Newburyport, MS) solution in 2-propanol was used as the catalyst in the hydrosilation reactions involving the above compounds. Vydac TP 106 Batch #7 [particle diameter: 6.5 μm ; pore diameter: 380 $\text{\AA}$; specific surface area: 106.5 m^2/g] (The Separations Group, Hesperia, CA) and Kromasil [particle diameter: 6.2 μm ; pore diameter: 100 $\text{\AA}$; specific surface area: 349 m^2/g] were used as the silica materials. Toluene (EM Science, Gibbstown, NJ), methylene chloride (J.T. Baker Inc., Phillipsburg, NJ), and diethyl ether (Fisher Scientific, Fair Lawn, NJ) were obtained in reagent grades.

Table 2. The CAS Registry Numbers of Chemicals Used

Chemical Name	CAS Registry Numbers
1-methylnaphthalene	[90-12-0]
1-phenyl-2-propyn-1-ol	[4187-87-5]
2,2-azobisisobutyronitrile	[78-67-1]
2-methylnaphthalene	[91-57-6]
2-propanol	[67-63-0]
3,3-dimethyl-1-butyne	[917-92-0]
4-androstene-3,17-dione	[63-05-8]
Acetonitrile	[75-05-8]
Ammonium hydroxide	[1336-21-6]
Andrenosterone	[53-41-8]
Anthracene	[120-12-7]
Benzene	[71-43-2]
Benzo[k]fluoranthene	[207-08-9]
Biphenyl	[92-54-4]
Chlorobenzene	[108-90-7]
Chlorodiazepoxide	[58-25-3]
Chloroform	[67-66-3]
Chrysene	[218-01-9]
Clonazepam	[1622-61-3]
Corticosterone	[50-22-6]
Diazepam	[439-14-5]
Dichloromethane (methylene chloride)	[75-09-2]
Diethyl ether	[60-29-7]
Ethyl acetate	[141-78-6]
Fluoranthene	[206-44-0]
Fluorene	[86-73-7]
Hexachloroplatinic acid (38-40% Pt)	[16941-12-1]
Hexane	[110-54-3]
Hydrochloric acid	[7647-01-0]
Isooctane (2,2,4-trimethyl pentane)	[540-81-1]
Methanol	[67-56-1]
n-heptane	[142-82-5]
Naphthalene	[91-20-3]
Nitrazepam	[146-22-5]
Nitric acid (conc.)	[7697-37-2]
Nitrobenzene	[98-95-3]
Oxazepam	[604-75-1]
p-dibromobenzene	[106-37-6]
Perylene	[198-55-0]

Phenanthrene	[85-01-8]
Phenylacetylene	[536-74-3]
Potassium bromide	[7758-02-3]
Prednisone	[53-03-2]
Propiolic acid	[471-25-0]
Pyrene	[129-00-0]
Sodium hydroxide	[1310-73-2]
Temazepam	[846-50-4]
Toluene	[108-88-3]

B. Synthetic Procedures

1. Silica Derivatization

In a typical procedure, 2.3 g of silica hydride was placed in a vacuum oven (VWR Scientific Model 1410) at 110 °C for twelve hours, and all relevant glassware was placed in an oven (Precision Scientific Company Model 17) at 110 °C overnight. 161 mL toluene, 23 mL of an acetylene compound, and 2.3 mL 5mM hexachloroplatinic acid in 2-propanol were mixed for one hour at 60-70 °C in a 250 mL round bottom flask equipped with a condenser tube, drying tube, heating mantle, magnetic stirrer, and an electric heater. The silica hydride was then added slowly through a funnel to the solution in the flask and was heated at 100 °C for 96 hours. The mixture was centrifuged (IEC HN-S Centrifuge, Needham Heights, MS) for ten minutes at 1500 rpm and the solid was washed with 15-20 mL portions of toluene, methylene chloride, and diethyl ether (three times with each solvent). The product was allowed to dry overnight in the hood at room temperature and

was dried further under vacuum at 110 °C for an additional 24 hours. The products were then characterized by DRIFT, CP-MAS NMR, EA (Elemental Analysis), and HPLC.

2. Azobisisobutyronitrile (AIBN) Free Radical Addition

A similar procedure was used to prepare the propiolic acid - bonded silica. 1.25 mL of Propiolic acid and 10.6 mL toluene were added to a round bottom flask. 0.0537 g of 2,2-Azobisisobutyronitrile (AIBN), as the catalyst, was added and the resulting solution was mixed for one hour at 60-70 °C. 0.5 g of silica hydride was then added, and the reaction was allowed to proceed for 100 hours at 100 °C. Toluene, methylene chloride, and diethyl ether were used to wash the mixture. After being dried under vacuum at 110 °C for 24 hours, the product was characterized using DRIFT, EA, and CP-MAS NMR.

3. Column Packing

Phenylacetylene and 1-phenyl-2-propyn-1-ol bonded silicas were packed into stainless steel columns (15 cm x 4.6 mm internal diameter, Alltech, Deerfield, IL) using a Haskell Pump (Burbank, CA) column packer. Filtered HPLC-grade methanol (Fisher Scientific) was used as the packing solvent. About 2.0 g of the bonded silica was stirred with a 90:10 carbon tetrachloride:methanol mixture before being poured into the reservoir

of the instrument. The solution was then packed into the column by nitrogen under about 6000 psi. The column stayed on the instrument for thirty minutes.

C. Instrumentation/Operating Procedures

1. Diffuse Reflectance Infrared Fourier Transform Spectroscopy (DRIFT)

The infrared spectra of the different silica samples were taken with a Perkin Elmer Model 1800 FT-IR Spectrophotometer (Norwalk, CT). A diffuse reflectance accessory (Spectra Tech., Stamford, CT) was used to analyze these solid samples. One part of a silica sample was mixed with one part of ground IR-grade potassium bromide and was placed in a sample cup (2 mm diameter and 2 mm depth). The top of the mixture was smoothed down with a glass plate. A pure potassium bromide sample was scanned and used as a reference each time a silica sample was analyzed. Each spectrum was scanned 200 times at a resolution of 2 cm^{-1} over a spectral range of $4000\text{--}450\text{ cm}^{-1}$. All spectra were normalized to 100% transmittance. A Perkin Elmer Series 7500 Professional Computer collected the data and a Hewlett Packard 7475A Plotter (Palo Alto, CA) was used to print the spectra.

2. Cross-Polarization Magic Angle Spinning Nuclear Magnetic Resonance Spectroscopy (CP-MAS NMR)

The CP MAS ^{13}C and ^{29}Si NMR spectra were recorded on a Bruker (Billerica, MS) MSL 300 Mhz spectrometer. This system incorporates the cross-polarization and magic angle spinning techniques mentioned in the introduction (Section I. E.). Solid samples (200-300 mg) were placed into a ZrO_2 double bearing rotor which spun the samples between 4700-5200 Hz. The following parameters were used during the analyses:

	^{13}C	^{29}Si
Pulse width:	6.5 μsec	5.0 μsec
Contact time:	5 msec	5 msec
Repetition rate:	5 sec	5 sec
External reference:	glycine	poly(hydrido)siloxane

The temperature of the probe was 20 ± 2 °C.

3. High Performance Liquid Chromatography (HPLC)

The Perkin Elmer (Norwalk, CT) HPLC system consisted of a Series 200 LC Pump, a UV-VIS multi-wavelength absorbance detector, and a solvent tray. A Hewlett Packard Model 3395 Integrator was interfaced with the system to print the chromatograms. Compressed helium at 50 mL/min sparged the various solvents used in

each analysis. It was usually left on during the entire analysis (at 5 mL/min) to prevent air bubbles from forming in the lines.

N-heptane, isooctane (Fisher Scientific, Fair Lawn, NJ), hexane - methylene chloride (J.T. Baker Inc., Phillipsburg, NJ), and ethyl acetate - chloroform (EM Science, Gibbstown, NJ) were used as the mobile phases for the various compounds analyzed. Hexane and ethyl acetate (Fisher Scientific) were HPLC grade. All other solvents were filtered through a 0.20 μ m nylon membrane (Alltech, Deerfield, IL) prior to being used on the column. The aromatic hydrocarbon samples were from the National Institute of Standards and Technology (Gaithersburg, MD), the benzodiazepines were from Hoffman-La Roche, Switzerland, and the steroids came from Aldrich (Milwaukee, WI). All injection samples were kept in capped sample vials (Thermo Separation Products, San Jose, CA). A sonicator (Bransonic 220, Branson Cleaning Equipment, Shelton, CT) was used to break up particles in some samples.

4. Elemental Analysis (EA)

The various bonded silicas were sent to Desert Analytics (Tucson, AZ) where the percent carbon in each sample was determined. The method used was combustion analysis. The elemental analysis results are used in the calculation of the surface coverage of the bonded groups and can be found in the Results section.

D. Surface Coverage

Using the carbon percentages determined by elemental analysis, the surface coverage, α_R , of the bonded silicas was calculated. The equation is as follows (17):

$$\alpha_R (\mu\text{mol}/\text{m}^2) = \frac{10^6 P_c}{(100 M_c n_c - P_c M_R) S_{\text{BET}}} \quad (5)$$

P_c is the carbon percentage by weight of the modified silica after subtracting any carbon that was present before bonding, M_c is the atomic weight of carbon, n_c is the number of carbon atoms in the bonded organic group (acetylene), M_R is the molecular weight of the acetylene compound, and S_{BET} is the Brunauer, Emmet, and Teller (BET) specific surface area (m^2/g) of the silica/hydride substrate (not the native silica but the hydride intermediate). BET refers to the nitrogen adsorption method developed by Brunauer, Emmet, and Teller (17) which is used in the specific surface area determination. The maximum surface coverage value that can be calculated is $8 \mu\text{mol}/\text{m}^2$.

E. Hydrolytic Stability Tests

100 mg of the modified silica was placed into a centrifuge tube (1.25 by 4 inches). About 3 mL of 10 mM hydrochloric acid ($\text{pH} = 2.02$) was added, and the solution was magnetically stirred for 24 hours at room temperature. The mixture was then centrifuged and the liquid was discarded. The solid material was washed with 5 mL portions of

diethyl ether three times, with centrifuging between washings. The product was allowed to dry in the hood overnight and was then dried at 100 °C under vacuum for 24 hours.

A second hydrolysis was done on the sample using the same procedure above. For the third hydrolysis, 0.3 mL of 10% methanol was added to the 3 mL of 10 mM HCl after the pH was adjusted to 2.

For the base hydrolysis, 10 mM sodium hydroxide was used (pH = 9). Only one base hydrolysis was performed. The IR spectra of the solid was taken to determine if carbon-hydrogen stretching was still present after being submitted to these washings (both acid and base). NMR spectra were then taken and compared to the original spectrum to determine if any changes in molecular structure of the bonded organic moiety occurred.

Chapter III

RESULTS AND DISCUSSION

A. Elemental Analysis Results

Table 3 gives the surface coverage (α) and percent carbon results for the four acetylene compounds that were bonded to silica.

Table 3. Surface Coverage and Percent Carbon Data Table

Compound	Surface Coverage, α ($\mu\text{mol}/\text{m}^2$)	Percent Carbon	After HCl Hydrolysis (α , %C)	After NaOH Hydrolysis (α , %C)
Propiolic acid - AIBN	4.61	1.71		
1-phenyl-2-propyn-1-ol	5.47	5.85	4.55, 4.92	4.02, 4.38
Phenylacetylene	4.23	4.14	5.35, 5.17	4.11, 4.03
3,3-dimethyl-1-butyne	0.81	2.00		

B. Confirmation of Phenylacetylene-Bonded Silica

1. DRIFT Spectra

a. Hydrosilation Reaction

Diffuse Reflectance Infrared Fourier Transform (DRIFT) spectroscopy was used to characterize the success of bonding silica hydride to the various acetylene compounds. Figure 1a shows the formation of the silica hydride intermediate by silanization of silica with TES. Note the silica-hydride band at $\sim 2250\text{ cm}^{-1}$. Figure 1b shows the DRIFT

Figure 1. (a) The DRIFT Spectrum of Vydac Silica Hydride

Figure 1 (b) The DRIFT Spectrum of Phenylacetylene-Bonded Silica.

spectrum of the phenylacetylene-modified silica with the Si-H stretching band at $\sim 2250\text{ cm}^{-1}$. When compared to the spectrum for the Vydac silica hydride, Figure 1a, the decrease of the Si-H band (at $\sim 2250\text{ cm}^{-1}$) confirms the bonding process. The appearance of carbon-hydrogen stretching bands between $\sim 2850 - 3100\text{ cm}^{-1}$ represents the bonding of phenylacetylene to the hydride intermediate (11). The aliphatic C-H stretching is between $2850 - 3000\text{ cm}^{-1}$ and the aromatic C-H stretching is between $3150 - 3000\text{ cm}^{-1}$. The Si-OH peak at $\sim 3430\text{ cm}^{-1}$ still appears in Figure 1b. This is probably due to the silanols that didn't react with the triethoxysilane (TES) because of steric hindrance considerations. The peak at $\sim 1610\text{ cm}^{-1}$ is representative of water absorbed on the surface.

b. Acid and Base Hydrolysis

Figures 2, 3, and 4 show the DRIFT spectra for phenylacetylene after being subjected to repeated HCl washings three times, respectively. Hydrolysis reactions were performed according to the procedure described in Section II E. It is evident that the Si-H and C-H stretching peaks are still present when these three spectra are compared to the original phenylacetylene-bonded silica spectrum (Figure 1b). However, the Si-H peak appears to have decreased in intensity in these figures.

Figure 5 shows the DRIFT spectrum after the bonded silica had been mixed with NaOH. This spectrum also reveals that the base hydrolysis did not really have an effect on

Figure 2. The DRIFT Spectrum of Phenylacetylene-Bonded Silica After the First HCl Hydrolysis.

Figure 3. The DRIFT Spectrum of Phenylacetylene-Bonded Silica After the Second HCl Hydrolysis.

Figure 4. The DRIFT Spectrum of Phenylacetylene-Bonded Silica After the Third HCl Hydrolysis.

Figure 5. The DRIFT Spectrum of Phenylacetylene-Bonded Silica After the First NaOH Hydrolysis.

the phenylacetylene-bonded silica: the Si-H peak at 2250 cm^{-1} and the C-H stretching between $2800\text{--}3100\text{ cm}^{-1}$ are still present. Overall, the phenylacetylene-bonded silica has proven its strength and durability.

2. CP-MAS ^{29}Si NMR Spectra

^{29}Si CP-MAS NMR was also used to characterize the new bonded phases. Figure 6a is the ^{29}Si CP-MAS NMR spectrum of Vydac silica hydride. Figure 6b shows the spectrum of phenylacetylene-bonded silica. When compared to Figure 6a, the decrease of the Si-H peak (A) at -83 ppm in Figure 6b confirms the bonding of phenylacetylene to the Vydac silica hydride. Peaks B and C have also decreased in intensity. Peak B represents silicon atoms with residual hydroxide groups attached, and peak C represents silicon atoms in the siloxane backbone (3), both of which are still on the surface even after hydrosilation. The silanols that are still present in Figure 6b were probably not able to react with the triethoxysilane (TES) due to the many pores inherent in the silica matrix (3). Typically, a peak representing the silicon-carbon linkage should appear around -65 ppm (18); however, it is possible that the peak could have shifted down to $\sim 76\text{ ppm}$ (19). While this bond can be very rigid and may inhibit detection by NMR, a small 'bump' does seem to appear around -91 ppm . This bump could be the silicon-carbon linkage.

The CP-MAS ^{29}Si NMR spectrum of phenylacetylene bonded silica after undergoing hydrolysis is shown in Figure 7. The same three peaks (A, B, and C)

Figure 6. (a) The CP-MAS ^{29}Si NMR Spectrum of Vydac Silica Hydride

Figure 6 (b) The CP-MAS ^{29}Si NMR Spectrum of Phenylacetylene-Bonded Silica.

Figure 7. The CP-MAS ^{29}Si NMR Spectrum of Phenylacetylene-Bonded Silica After Base Hydrolysis.

mentioned in Figure 6 are still present. Figure 7 corresponds well with the DRIFT spectrum analysis for this compound; that is, base hydrolysis did not seem to have an effect on the phenylacetylene bonded silica.

3. CP-MAS ^{13}C NMR Spectra

Figure 8a is the CP-MAS ^{13}C NMR spectrum of Vydac silica hydride. Figures 8b, 9a, and 9b show the CP-MAS ^{13}C NMR spectra for phenylacetylene bonded silica before (8b) and after base (9a) and acid (9b) hydrolysis, respectively. When compared to Figure 8a, the residual ethoxy peaks at 16 and 56 ppm are still present in the phenylacetylene bonded silica spectrum. These species are very persistent on the surface of the Vydac silica hydride and hence the significant intensity of their peaks in Figure 8a. In Figure 8b, the peak at 126 ppm denotes the phenyl carbons and the four small peaks between 118 - 147 ppm represent olefinic carbons. The appearance of the latter peaks indicates that some, if not all, of the bonded material has only one Si-C linkage. The small peak at 24 ppm is most likely a CH_2 resonance. Figure 9a almost mirrors Figure 8b and so it is concluded that the base hydrolysis had little or no effect on the bonded silica. Figure 9b shows roughly the same outcome. The NMR signal to noise ratio is very low and may be a result of an instrument malfunction. Actually, all NMR data collected on that day exhibit a noisy baseline. Overall, the phenylacetylene bonded silica appears to be quite rugged and resistant to acid and base hydrolysis. The carbon analysis results also show that the

Figure 8. (a) The CP-MAS ^{13}C NMR Spectrum of Vydac Silica Hydride

Figure 8 (b) The CP-MAS ^{13}C NMR Spectrum of Phenylacetylene-Bonded Silica.

Figure 9. (a)The CP-MAS ^{13}C NMR Spectrum of Phenylacetylene-Bonded Silica After Base Hydrolysis

Figure 9 (b) The CP-MAS ^{13}C NMR Spectrum of Phenylacetylene-Bonded Silica After Acid Hydrolysis.

phenylacetylene bonded silica has a high amount of bonded material on the surface; the surface coverage, α , was calculated to be $4.23 \mu\text{mol}/\text{m}^2$ (recall that the maximum value is $8 \mu\text{mol}/\text{m}^2$). This number is comparable to the values after the bonded silica was exposed to acid ($5.35 \mu\text{mol}/\text{m}^2$) and base ($5.17 \mu\text{mol}/\text{m}^2$) hydrolysis.

C. Confirmation of Bonding of 1-phenyl-2-propyn-1-ol to Silica Hydride

1. DRIFT Spectra

a. Hydrosilation Reaction

Figure 10 shows the DRIFT spectrum of the 1-phenyl-2-propyn-1-ol bonded to silica hydride. It is very similar to Figure 1b. A decrease in the Si-H band at $\sim 2250 \text{ cm}^{-1}$ and the appearance of C-H stretching, both aliphatic ($3000 - 2850 \text{ cm}^{-1}$) and aromatic ($3150 - 3000 \text{ cm}^{-1}$), between $2800 - 3100 \text{ cm}^{-1}$ indicate that the hydrosilation reaction was successful. Other important bands are noted on the spectrum.

b. Acid and Base Hydrolysis

Figures 11, 12, and 13 show the DRIFT spectra for the 1-phenyl-2-propyn-1-ol bonded silica after being subjected to the acid hydrolysis test, and Figure 14 is the spectra after the base hydrolysis. These spectra follow the same trend that the phenylacetylene

Figure 10. The DRIFT Spectrum of 1-phenyl-2-propyn-1-ol-Bonded Silica.

Figure 11. The DRIFT Spectrum of 1-phenyl-2-propyn-1-ol-Bonded Silica After the First HCl Hydrolysis.

Figure 12. The DRIFT Spectrum of 1-phenyl-2-propyn-1-ol-Bonded Silica After the Second HCl Hydrolysis.

Figure 13. The DRIFT Spectrum of 1-phenyl-2-propyn-1-ol-Bonded Silica After the Third HCl Hydrolysis.

Figure 14. The DRIFT Spectrum of 1-phenyl-2-propyn-1-ol-Bonded Silica After the First NaOH Hydrolysis.

bonded silica did: C-H and Si-H stretching bands are still very evident. This compound appears to be quite stable and resistant to hydrolysis.

2. CP-MAS ^{29}Si NMR Spectra

Figure 15 shows the CP-MAS ^{29}Si NMR spectrum of 1-phenyl-2-propyn-1-ol modified silica containing a Si-H peak (A) around -82 ppm; the silanol peak (B) at -100 ppm and siloxane peak (C) near -110 ppm are also shown. When comparing this spectrum to Figure 6a, it is evident that the Si-H peak has decreased in intensity, as is expected. The Si-C peak, while not apparent at first, may be hidden under the Si-H peak or may be around -90 ppm where a small resonance peak seems to emerge.

Figure 16 is the spectrum of the modified silica after undergoing base hydrolysis. The Si-H peak has increased in intensity and the siloxane peak (C) has decreased in intensity when compared to Figure 15. This implies that some of the siloxane backbone could have been destroyed in the hydrolysis. The silicon bonds in the 1-phenyl-2-propyn-1-ol bonded silica seem to have been slightly affected by base hydrolysis based on the decrease of Peak C in Figure 16 compared to Figure 15.

Figure 15. The CP-MAS ^{29}Si NMR Spectrum of 1-phenyl-2-propyn-1-ol-Bonded Silica.

Figure 16. The CP-MAS ^{29}Si NMR Spectrum of 1-phenyl-2-propyn-1-ol-Bonded Silica After Base Hydrolysis.

3. CP-MAS ^{13}C NMR Spectra

Figure 17 is the CP-MAS ^{13}C NMR spectrum of 1-phenyl-2-propyn-1-ol bonded silica. The peaks at 127 and 142 ppm can be assigned to the phenyl carbons, and the peaks from the Vydac hydride (17 and 60 ppm) are still present (see Figure 8a). The resonance at 75 ppm is probably due to a carbon attached to the phenyl ring, a hydrogen, and a hydroxide group. Three important pieces in this spectrum allude to a possible double Si-C linkage. First, the appearance of a $-\text{CH}_2-$ group resonance at 30 ppm indicates that the carbon triple bond could have broken completely and the terminal carbon atom be attached to two silicon atoms. Second, the suggestion of a very small peak at 11 ppm can be designated as a Si-C bond. The fact that it is almost non-existent suggests a double Si-C linkage since such a bond would be extremely rigid and may not be mobile enough for the NMR to detect. Finally, the absence of any olefinic peaks around 120 to 140 ppm supports the double Si-C linkage.

The NMR spectra of the modified silica after undergoing base and acid hydrolyses are shown in Figures 18a and 18b. All of the relevant peaks mentioned in Figure 17 are still very obvious in these two figures. Based on these spectra, it can be established that the 1-phenyl-2-propyn-1-ol bonded silica is sturdy and not easily degraded by hydrolysis. The carbon analysis of this bonded phase allowed for the surface coverage of the sample to be calculated; the result was $5.47 \mu\text{mol}/\text{m}^2$. A surface coverage value greater than $4 \mu\text{mol}/\text{m}^2$ is very promising because it means that more than 50% of the surface is covered

Figure 17. The CP-MAS ^{13}C NMR Spectrum of 1-phenyl-2-propyn-1-ol-Bonded Silica.

Figure 18. (a)The CP-MAS ^{13}C NMR Spectrum of 1-phenyl-2-propyn-1-ol-Bonded Silica After Base Hydrolysis

Figure 18 (b) The CP-MAS ^{13}C NMR Spectrum of 1-phenyl-2-propyn-1-ol-Bonded Silica After Acid Hydrolysis.

by the bonded moiety (assuming each organic component is bonded to a single silanol on the surface) (3).

D. Propiolic acid Analysis

1. Hydrosilation Reaction

a. DRIFT Spectra

Figure 19 is the spectrum for propiolic acid-bonded silica. The peak at 2250 cm^{-1} represents the Si-H bond and the one at 2175 cm^{-1} could be due to an acid carbonyl stretch. The C-H band which normally appears between $2800\text{--}3100\text{ cm}^{-1}$ is almost non-existent. The absence of the peak could be due to the carboxylic acid group “protecting” the carbon triple bond from reacting. Also, the concentration of the Pt catalyst could be too high causing a possible deposition of platinum metal on the hydride surface (20). A CP-MAS ^{13}C NMR analysis was done to investigate these possibilities.

b. CP-MAS ^{13}C NMR Spectra

Figure 20 is the CP-MAS ^{13}C NMR spectrum for Propiolic acid bonded silica. The enormous olefin resonance at 137 ppm attests to a single point of attachment to the surface. The small shoulder peak at 13 ppm indicates only a small amount of double

Figure 19. The DRIFT Spectrum of Propiolic Acid-Bonded Silica (Pt Catalyst).

Figure 20. The CP-MAS ^{13}C NMR Spectrum of Propiolic Acid-Bonded Silica (Pt Catalyst).

attachment. The Vydac hydride ethoxy peaks are also present (60 and 17 ppm). The beta carbon resonance appears at 33 ppm and a peak at 168 ppm is a -C=O resonance.

2. AIBN Free Radical Addition Reaction

The 2,2'-Azobisisobutyronitrile (AIBN) free radical addition reaction (see Ch. II, part B.2) was attempted because the hydrosilation reaction gave poor results based on the carbon analysis data. The surface coverage for propiolic acid was greater than 15 $\mu\text{mol}/\text{m}^2$ which does not agree with the theoretical maximum value of 8 $\mu\text{mol}/\text{m}^2$.

a. DRIFT Spectra

Figure 21 is the spectrum of propiolic acid after undergoing the AIBN free radical addition reaction. The usual Si-H peak at $\sim 2255\text{ cm}^{-1}$ is present. Another peak at $\sim 2175\text{ cm}^{-1}$ also appears; this probably represents an acid carbonyl stretch. While this reaction appears to be more successful than the silanization reaction, the carbon-hydrogen stretching between $2850\text{-}3000\text{ cm}^{-1}$ is not as intense as the phenylacetylene or 1-phenyl-2-propyn-1-ol spectra. Because of this, propiolic acid was not selected for further evaluation by HPLC.

Figure 21. The DRIFT Spectrum of Propiolic Acid-Bonded Silica (AIBN Free Radical Addition).

b. CP-MAS ^{29}Si NMR Spectra

The CP-MAS ^{29}Si NMR spectrum of Propiolic acid bonded silica is shown in Figure 22. The Si-H peak (A) is evident at -85 ppm. Both the silanol and siloxane peaks (B and C) are also labeled in the spectrum.

c. CP-MAS ^{13}C NMR Spectra

Figure 23 is the CP-MAS ^{13}C NMR spectrum for the AIBN free radical addition reaction of Propiolic acid with silica hydride. The Vydac hydride chemical shifts at 17 and 60 ppm correspond to the methyl and methylene resonances of the ethoxy moiety. A peak around 13 ppm on the shoulder of the Vydac hydride (17 ppm) may represent the Si-C linkage. At 127 ppm a large, broad peak is indicative of an olefin group. This same peak was also observed in Figure 20 when the hydrosilation reaction method was used. This result indicates that a single Si-C linkage at the surface is most probable (see Propiolic acid reactions in Section I C).

E. Evaluation of 3,3-dimethyl-1-butyne-Bonded Silica

1. DRIFT Spectra

The DRIFT spectra of Kromasil silica hydride and 3,3-dimethyl-1-butyne are shown in Figures 24a and 24b. When compared to the phenylacetylene and 1-phenyl-2-

Figure 22. The CP-MAS ^{29}Si NMR Spectrum of Propionic Acid-Bonded Silica by AIBN Free Radical Addition.

Figure 23. The CP-MAS ^{13}C NMR Spectrum of Propiolic Acid-Bonded Silica by AIBN Free Radical Addition.

Figure 24. (a) The DRIFT Spectrum of Kromasil Silica Hydride

Figure 24 (b) The DRIFT Spectrum of 3,3-dimethyl-1-butyne-Bonded Silica.

propyn-1-ol bonded silicas which were synthesized with Vydac TP 107 silica hydride, a stronger Si-H band at $\sim 2265\text{ cm}^{-1}$ appears (Figure 24b); this more intense band implies a greater silane coverage for the silanization product (4). The strong C-H stretching between $2800\text{--}3000\text{ cm}^{-1}$ also validates the success of the hydrosilation reaction.

2. CP-MAS ^{29}Si NMR Spectra

Figures 25a and 25b are the spectra for the Kromasil silica hydride and the 3,3-dimethyl-1-butyne bonded silica, respectively. In Figure 25a, Peak A is the Si-H peak, peak B represents silicons with residual hydroxide groups attached, peak C is the siloxane backbone of the Kromasil silica hydride, and peak D is the resonance for silicon atoms with OH and H atoms attached. When compared to this spectrum, Figure 25b shows a decrease of the Si-H resonance at -85 ppm . This clearly verifies the success of the hydrosilation bonding reaction.

3. CP-MAS ^{13}C NMR Spectra

Figure 26 shows the spectrum of the 3,3-dimethyl-1-butyne bonded silica. The methyl resonances are at 23, 26, and 34 ppm, and the gamma carbon resonance is at 65 ppm. Olefin peaks can be seen at 112 and 163 ppm which would imply that there is a single Si-C linkage. The Si-C bond should appear around 12 ppm but its intensity may be

Figure 25. (a) The CP-MAS ^{29}Si NMR Spectrum of Kromasil Silica Hydride

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Figure 25 (b) The CP-MAS ^{29}Si NMR Spectrum of 3,3-dimethyl-1-butyne.

Figure 26. The CP-MAS ^{13}C NMR Spectrum of 3,3-dimethyl-1-butyne-Bonded Silica.

too small to detect. Kromasil silica hydride does not show any resonances in the ^{13}C NMR.

F. Chromatographic Studies

1. Phenylacetylene Column

a. Aromatic Compound Analysis

Sixteen aromatic hydrocarbon samples dissolved in n-heptane were injected into the phenylacetylene column separately. N-heptane (Sigma-Aldrich, St. Louis, MO) was used as the mobile phase at first; isooctane (Sigma-Aldrich, St. Louis, MO) was then used as the mobile phase for evaluating the HPLC column by these sixteen compounds again. The retention values of these aromatic compounds were calculated and compared with two or three other sources. These results are shown in Tables 4 and 5.

Table 4. Retention Data of Aromatic Compounds on Phenyl Column with n-heptane as the Mobile Phase.

Number	Solutes	k' 1	k' 2	k' 3	k' 4
1	Benzene	0.04	0.14	0.13	0.09
2	Chlorobenzene	0.05	0.15	0.12	
3	p-Dibromobenzene	0.04	0.15	0.12	
4	1-Methylnaphthalene	0.06	0.22	0.12	
5	2-Methylnaphthalene	0.05	0.22	0.12	
6	Naphthalene	0.07	0.23	0.11	0.24
7	Nitrobenzene	1.07	1.34	0.30	
8	Anthracene	0.13	0.40	0.15	
9	Benzo[k]fluoranthene	0.26	0.59	0.32	

10	Biphenyl	0.08	0.24	0.11	0.24
11	Fluoranthene	0.16	0.34	0.20	0.67
12	Fluorene	0.10	0.32	0.12	0.33
13	Phenanthrene	0.13	0.39	0.15	0.47
14	Pyrene	0.15	0.49	0.16	0.64
15	Chrysene	0.23	0.76	0.27	
16	Perylene	0.33	0.66	0.37	

1. Phenyl column (Phenylacetylene) from the laboratory.
2. AGE column from the laboratory (AGE via allyl glycidal ether).
3. 7-OD column from the laboratory (7-OD diol via 7-octene-1,2-diol).
4. Diol cartridges, Lichrosorb 100 from Merck (data from Siouffi, J. Chromatogr. 368, 189 (1986)).

Table 5. Retention Data of Aromatic Compounds on Phenyl Column with Isooctane as the Mobile Phase.

Number	Solutes	k' 1	k' 2	k' 3
1	Benzene	0.24	0.15	0.20
2	Chlorobenzene	0.27	0.24	
3	p-Dibromobenzene	0.31	0.40	
4	1-Methylnaphthalene	0.32	0.42	
5	2-Methylnaphthalene	0.33	0.42	
6	Naphthalene	0.34	0.35	0.46
7	Nitrobenzene	1.84	0.94	
8	Anthracene	0.58		0.99
9	Benzo[k]fluoranthene	1.04		2.62
10	Biphenyl	0.37		0.46
11	Fluoranthene	0.60	0.95	1.52
12	Fluorene	0.44	0.49	0.68
13	Phenanthrene	0.53	0.67	1.00
14	Pyrene	0.57	0.91	1.44
15	Chrysene	0.89	1.24	2.20
16	Perylene	0.22	1.56	2.81

1. Phenyl column (Phenylacetylene) from the laboratory.
2. Diol LiChrosorb column (data from Siouffi, J. Chromatogr. 368, 189 (1986)).
3. LiChrospher 100 from Merck (data from Siouffi, J. Chromatogr. 368, 189 (1986)).

The retention factor, k' , is a measure of the relative retention of each peak on the column (8). $k' = (t_R - t_0) / t_0$ where t_R and t_0 are the retention times of a retained and non-retained sample, respectively. An optimal value would be between 1-10. Small k' values (0-1) limit peak resolution while large k' values improve resolution but can increase analysis times (7). It can also be observed that for nonpolar compounds, the retention increases with the number of hydrocarbon units so that k' of benzo[k]fluoranthene > pyrene > phenanthrene > biphenyl > benzene (21).

When comparing the k' results for the phenylacetylene column between the two mobile phases, it is evident that this aromatic bonded phase prefers a more nonpolar mobile phase like isooctane. While n-heptane and isooctane are both nonpolar solvents, isooctane can be considered slightly more nonpolar than n-heptane.

A few chromatograms for this column with the two mobile phases are shown in Figures 27a and 27b. Peak shape is good (except for nitrobenzene) for the compounds that were analyzed.

2. 1-Phenyl-2-propyn-1-ol Column

a. Aromatic Compound Analysis

The same two analyses done on the phenylacetylene column were also performed on the 1-phenyl-2-propyn-1-ol column. The results are shown in Tables 6 and 7.

Table 6. Retention Data of Aromatic Compounds on Mono-ol and Diol columns with n-heptane as the Mobile Phase.

Number	Solutes	k' 1	k' 2	k' 3	k' 4
1	Benzene	0.04	0.14	0.13	0.09
2	Chlorobenzene	0.04	0.15	0.12	
3	p-Dibromobenzene	0.02	0.15	0.12	
4	1-Methylnaphthalene	0.06	0.22	0.12	
5	2-Methylnaphthalene	0.06	0.22	0.12	
6	Naphthalene	0.08	0.23	0.11	0.24
7	Nitrobenzene	2.36	1.34	0.30	
8	Anthracene	0.19	0.40	0.15	
9	Benzo[k]fluoranthene	0.52	0.59	0.32	
10	Biphenyl	0.11	0.24	0.11	0.24
11	Fluoranthene	0.25	0.34	0.20	0.67
12	Fluorene	0.16	0.32	0.12	0.33
13	Phenanthrene	0.20	0.39	0.15	0.47
14	Pyrene	0.23	0.49	0.16	0.64
15	Chrysene	0.47	0.76	0.27	
16	Perylene	0.69	0.66	0.37	

1. Mono-ol column (1-phenyl-2-propyn-1-ol) from the laboratory.
2. AGE column from the laboratory (AGE via allyl glycidal ether).
3. 7-OD column from the laboratory (7-OD diol via 7-octene-1,2-diol).
4. Diol cartridges, Lichrosorb 100 from Merck (data from Siouffi, J. Chromatogr. 368, 189 (1986)).

Table 7. Retention Data of Aromatic Compounds on Mono-ol and Diol Columns with Isooctane as the Mobile Phase.

Number	Solutes	k' 1	k' 2	k' 3
1	Benzene	0.16	0.15	0.20
2	Chlorobenzene	0.19	0.24	
3	p-Dibromobenzene	0.19	0.40	
4	1-Methylnaphthalene	0.21	0.42	
5	2-Methylnaphthalene	0.19	0.42	
6	Naphthalene	0.21	0.35	0.46
7	Nitrobenzene	0.84	0.94	
8	Anthracene	0.54		0.99

9	Benzo[k]fluoranthene	0.51		2.62
10	Biphenyl	0.19		0.46
11	Fluoranthene	0.33	0.95	1.52
12	Fluorene	0.21	0.49	0.68
13	Phenanthrene	0.27	0.67	1.00
14	Pyrene	0.33	0.91	1.44
15	Chrysene	0.44	1.24	2.20
16	Perylene	0.61	1.56	2.81

1. Mono-ol column (1-phenyl-2-propyn-1-ol) from the laboratory.
2. Diol LiChrosorb column (data from Siouffi, J. Chromatogr. 368, 189 (1986)).
3. LiChrospher 100 from Merck (data from Siouffi, J. Chromatogr. 368, 189 (1986)).

When comparing the results for k' between the two mobile phases used, it appears that the 1-phenyl-2-propyn-1-ol column had larger k' values overall with isooctane. The k' values also increase for nonpolar compounds that have more hydrocarbon units in their structure. The order is the same as for the phenylacetylene column: benzo[k]fluoranthene > pyrene > phenanthrene > biphenyl > benzene. Overall, the retention values for both mobile phases are larger for the 1-phenyl-2-propyn-1-ol column because of its higher surface coverage: $5.47 \mu\text{mol}/\text{m}^2$ versus $4.23 \mu\text{mol}/\text{m}^2$ for the phenylacetylene bonded silica.

Figures 28a and 28b show some representative chromatograms for the bonded phase with n-heptane and isooctane as the mobile phases. The peak shape is good and is consistent with the phenylacetylene column results. Both the 1-phenyl-2-propyn-1-ol and phenylacetylene columns give comparable results (same elution order) to the diol columns in Tables 4-7. Lower retention values on these two phases are due to the hydride surface.

Figure 27(a). Chromatograms of Aromatic Compounds on the Phenylacetylene Column with n-heptane as the Mobile Phase. Flow rate: 1.0 mL/min. Compounds: A) p-dibromobenzene; B) Nitrobenzene; C) Chrysene; D) Fluorene.

Figure 27(b). Chromatograms of Aromatic Compounds on the Phenylacetylene Column with Isooctane as the Mobile Phase. Flow rate: 1.0 mL/min. Compounds: A) Chlorobenzene; B) p-dibromobenzene; C) Naphthalene; D) Chrysene.

Figure 28(a). Chromatograms of Aromatic Compounds on the 1-phenyl-2-propyn-1-ol Column with n-heptane as the Mobile Phase. Flow rate: 1.0 mL/min. Compounds: A) 1-methylnaphthalene; B) Anthracene; C) Pyrene; D) Chrysene.

Figure 28(b). Chromatograms of Aromatic Compounds on the 1-phenyl-2-propyn-1-ol Column with Isooctane as the Mobile Phase. Flow rate: 1.0 mL/min. Compounds: A) Anthracene; B) Chrysene; C) Benzo[k]fluoranthene; D) Nitrobenzene.

b. Steroid Analysis

Four common steroids were analyzed on the 1-phenyl-2-propyn-1-ol column: 4-androstene-3,17-dione, andrenosterone, corticosterone, and prednisone. Many attempts were made to study these compounds; however, multiple HPLC system problems interfered with most of this work. Two sample chromatograms are shown in Figure 29. The peak shape is good, and a separation would be possible with a gradient system. However, no HPLC separation was done with these compounds.

c. Benzodiazepine Separation

Benzodiazepines are drugs that have hypnotic and anesthetic properties. They are typically found in clinical and forensic medicine (22). Six benzodiazepines were selected to analyze: chlorodiazepoxide, clonazepam, diazepam, nitrazepam, oxazepam, and temazepam. Each compound was injected separately in numerous mobile phase compositions to determine their retention values. The goal was to find a suitable composition that would allow for a separation of two or more compounds. Figure 30 shows sample chromatograms of three benzodiazepines with different mobile phases. Based on the values obtained for oxazepam and nitrazepam, a mixture of the two was made and separated. This chromatogram is displayed in Figure 31.

Figure 29. Chromatograms of Steroids on the 1-phenyl-2-propyn-1-ol Column. Mobile phase: Methylene Chloride. Flow rate: 0.5 mL/min. Compounds: A) 4-androstene-3,17-dione; B) Prednisone.

Figure 30. Chromatograms of Benzodiazepines on the 1-phenyl-2-propyn-1-ol Column. Mobile phase: 50:50 Chloroform:Ethyl Acetate. Flow rate: 1.0 mL/min. Compound: A) Chlorodiazepoxide; Mobile Phase: 70:30 n-heptane:Chloroform. Flow rate: 1.0 mL/min. Compounds: B) Oxazepam; C) Nitrazepam.

Figure 31. Chromatogram of Benzodiazepine Separation on the 1-phenyl-2-propyn-1-ol Column. Mobile phase: 70:30 n-heptane:Chloroform. Flow rate: 1.0 mL/min. A) Oxazepam; B) Nitrazepam.

Chapter IV

CONCLUSION

Four bonded materials for high performance liquid chromatography were made via the TES silanization followed by hydrosilation reaction scheme and the capabilities of two as stationary phases were studied. Diffuse Reflectance Infrared Fourier Transform spectroscopy, Cross-Polarization Magic Angle Spinning Nuclear Magnetic Resonance spectroscopy, and HPLC were used to characterize the bonded phases synthesized in this project. This data showed that it is possible to bond acetylene compounds to silica hydride surfaces via hydrosilation. One compound in particular seemed to form a bond between two silicon atoms and one carbon atom. The CP-MAS ^{13}C NMR did not rule out the possibility of a double Si-C linkage between the acetylene moiety and the silica surface. The compound, 1-phenyl-2-propyn-1-ol, was packed into a column and tested chromatographically with aromatic compounds, steroids, and benzodiazepines to evaluate its performance as a new bonded phase. The surface coverage for this material was calculated to be $5.47 \mu\text{mol}/\text{m}^2$ which is exceptional when compared to the maximum value of $8 \mu\text{mol}/\text{m}^2$. Overall, this stationary phase was comparable to other stationary phases that have hydroxyl groups in their makeup.

Future work includes the following: 1) other acetylene compounds need to be tested for the possible presence of the double Si-C linkage to the surface; 2) the long term

stability of the bonded phases should be examined; and 3) the robustness and reproducibility of the new phases should be investigated.

Chapter V

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IMAGE EVALUATION TEST TARGET (QA-3)

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