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**THE SYNTHESIS AND CHARACTERIZATION OF C8 PHASE ENDCAPPED
WITH AMPS FOR 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

Sophie Marwiah James

May 2002

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ABSTRACT

SYNTHESIS AND CHARACTERIZATION OF C8 PHASE ENDCAPPED WITH AMPS FOR HPLC

by Sophie James

Two bonded phases were synthesized for use as stationary phases for high performance liquid chromatography (HPLC) using Vydac silica. The first bonded phase involved the attachment of 1-octene (C8) to Vydac through a Vydac hydride intermediate by a hydrosilation reaction. This was followed by the attachment of 2-acrylamido-2-methyl-1-propanesulfonic acid (AMPS) to endcap the C8-Vydac stationary phase by a second hydrosilation reaction. The second bonded phase involved the direct attachment of AMPS to Vydac through the Vydac hydride intermediate by hydrosilation.

The two synthesized bonded stationary phases were characterized using Diffuse Reflectance Infrared Fourier Transform Spectroscopy (DRIFT), Cross Polarization-Magic Angle Spinning Nuclear Magnetic Resonance Spectroscopy (CP-MAS NMR) and Elemental Analysis.

To determine column efficiency and to generate comparative data, chromatographic studies were conducted on the two synthesized bonded stationary phases, C8-Vydac endcapped with AMPS and AMPS on Vydac, and a third previously prepared C8-Vydac stationary phase.

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CHAPTER I

INTRODUCTION

A. High Performance Liquid Chromatography

1. An overview of HPLC

a. Theory of Operation

High Performance Liquid Chromatography (HPLC) is one of the most widely used separation techniques today, be it biochemical, analytical or pharmaceutical.

Liquid Chromatography was first discovered in 1903 by M.S. Tsweet, who used a chalk column to separate the pigments of green leaves. However, it was not until the 1960's that greater emphasis was placed on the development of liquid chromatography. The basic HPLC instrumentation includes a pump, injector, column, detector and a data collection system. The column is considered to be the heart of the system because separation occurs within this component [1,2].

The chromatographic process can be defined as a separation technique involving mass-transfer between the two major components of HPLC: the stationary phase and the mobile phase. The principle behind this technique is that certain compounds have different migration rates through a given column with a particular mobile phase. When a sample is introduced into the system, HPLC uses the liquid mobile phase to transport the sample through a column containing the stationary phase under high pressure. As the sample travels through the column, the components of the sample migrate according to their interactions with the mobile or stationary phase and these compounds are then separated according to their retention times. This interaction of the sample with the

stationary phase can occur through an adsorption or partitioning mechanism. In the absorption mechanism, some compounds when introduced into the column are more strongly absorbed than others. The least absorbed compounds will elute faster than the more strongly absorbed compounds thus resulting in a difference in their individual retention times. For the partitioning mechanism, separation occurs based on the differences in the partition coefficients of the compounds between the stationary phase and the mobile phase [1,2].

b. Modes of Separation

There are two major separation modes in HPLC. They are the normal phase mode and the reverse phase mode. In normal phase chromatography, the stationary phase is strongly polar in nature and the mobile phase is non-polar. Polar compounds are thus retained longer than less polar compounds resulting in longer retention times. Retention is governed by the adsorption mechanism in normal phase HPLC.

Reversed phase chromatography is the inverse of normal phase. In reverse phase HPLC, the stationary phase is non-polar in nature, while the mobile phase is polar thus resulting in greater retention of non-polar compounds. In the reverse phase mode, the partitioning process rather than adsorption govern HPLC retention of compounds by the column. The separation mode used for this research project is reverse phase HPLC [1,2].

c. Resolution and Column Efficiency

The success of the HPLC technique greatly depends on the degree of resolution. Resolution can be defined as the separation between two peaks. The greater the

separation between two adjacent peaks, the better the resolution. Resolution can be determined from the equation:

$$R=1.18\Delta t_R/W_1+W_2$$

Where Δt_R is the difference in retention times between two peaks and W_1 and W_2 are the widths of the two peaks.

The success of separation in HPLC depends on several key factors. They can be listed as, column selectivity (α), the sample capacity factor (k') and column efficiency (N). Column selectivity (α) can be defined by the equation:

$$\alpha= (t_{R2} - t_o)/(t_{R1} - t_o)$$

Separation is achievable for an $\alpha > 1$. The sample capacity factor (k') can be obtained from the equation:

$$k'= (t_R - t_o)/t_o$$

Where t_R is the retention time of a retained peak and t_o is the retention time of a reference peak. The column efficiency (N) is based on the number and height of the theoretical plates. For a packed column, the efficiency (N) can be determined by the equation:

$$N= 5.54 (t_R/W)^2$$

Where t_R is the retention time of a solute and W is the width of the peak.

Using the Van Deemter Equation below:

$$HETP = L/N$$

The height equivalent to a theoretical plate (HETP) can be calculated if the effective number of plates is known. Where L represents the length of the column and N is the

effective number of plates. A Low height equivalent to a theoretical plate value represents high column efficiency [1,2,3].

B. Silica

Some of the solid supports that can be used for surface modification are titania, alumina, zirconia, thoria and silica [4,5]. Silica is one of the most widely used solid supports in liquid chromatography. This is because there are several advantages to using silica. It is commercially available in almost any particle size and is reasonably priced. Silica resists swelling, is very rigid and has a strong mechanical stability. It provides very high efficiency and is superior to many other solid supports. Silica can also be obtained in different pore sizes and can be modified in various ways.

The silica surface is said to have several active groups present. These active groups are shown in **Figure 1** [6]. Some of the active groups present on silica's surface are siloxane, free silanols, geminol silanol, vicinol silanols and bound water. The presence of some of these active groups can actually be of an advantage or disadvantage depending on the type of analysis being conducted. In HPLC, these groups can sometimes enhance the separation of some compounds. On the other hand, the presence of these groups can sometimes act as interference in the separation of certain compounds. Of all the active surface groups, the group that is most widely used in the modification of the silica surface is the free silanol group. One of the goals of this research project was to modify the silica surface to create a silica hydride intermediate by triethoxysilane silanization. And then attaching at least two different organic moieties to silica by hydrosilation.

Figure 1. Active Groups on Silica Surface

1) Siloxane

2) Free Silanols

3) Geminol Silanol

4) Vicinol Silanol

5) Bound Water

1. Modification of Silica surface

There are several methods that can be use for the modification of the silica surface. **Figure 2** lists some modification reactions [7].

REACTION TYPE	REACTION	SURFACE LINKAGES
ESTERIFICATION	$\text{Si-OH} + \text{R-OH} \rightarrow \text{Si-OR} + \text{H}_2\text{O}$	Si-O-C
ORGANO-SILANIZATION	a). $\text{Si-OH} + \text{X-SiR}'_2\text{R} \rightarrow \text{Si-O-SiR}'_2\text{R} + \text{HX}$ OY b). $\text{Si-OH} + \text{X}_3\text{-Si-R} \rightarrow \text{Si-O-Si-R} + 3\text{HX}$ OY Y = Si or H	Si-O-Si-C
CHLORINATION FOLLOWED BY REACTION OF GRIGNARD REAGENTS AND ORGANOLITHIUM COMPOUNDS	$\text{Si-OH} + \text{SOCl}_2 \xrightarrow{\text{Toluene}} \text{Si-Cl} + \text{SO}_2 + \text{HCl}$ a). $\text{Si-Cl} + \text{BrMgR} \rightarrow \text{Si-R} + \text{MgClBr}$ or b). $\text{Si-Cl} + \text{Li-R} \rightarrow \text{Si-R} + \text{LiCl}$	Si-C
a). TES SILANIZATION	$ \begin{array}{ccc} \begin{array}{c} \\ \text{O} \\ \\ \text{-Si-OH} \end{array} & & \begin{array}{c} \\ \text{O} \\ \\ \text{-Si-O-Si-H} \end{array} \\ \begin{array}{c} \\ \text{O} \\ \\ \text{-Si-OH} \end{array} & \rightarrow & \begin{array}{c} \\ \text{O} \\ \\ \text{-Si-O-Si-H} \end{array} \\ \begin{array}{c} \\ \text{O} \\ \\ \text{-Si-OH} \end{array} & & \begin{array}{c} \\ \text{O} \\ \\ \text{-Si-O-Si-H} \end{array} \end{array} $	a). Si-H monolayer
b). HYDROSILATION	$ \begin{array}{ccc} \begin{array}{c} \\ \text{O} \\ \\ \text{-Si-OH} \end{array} & & \begin{array}{c} \\ \text{O} \\ \\ \text{-Si-O-Si-H} \end{array} \\ \begin{array}{c} \\ \text{O} \\ \\ \text{-Si-OH} \end{array} & \xrightarrow{\text{Catalyst}} & \begin{array}{c} \\ \text{O} \\ \\ \text{-Si-O-Si-H} \end{array} \\ \begin{array}{c} \\ \text{O} \\ \\ \text{-Si-OH} \end{array} & & \begin{array}{c} \\ \text{O} \\ \\ \text{-Si-O-Si-H} \end{array} \end{array} $ b). $\text{Si-H} + \text{CH}_2 = \text{CH-R} \rightarrow \text{Si-CH}_2\text{-CH}_2\text{-R}$	b). Si-C

Figure 2. Methods for the modification of a silica surface

One of the earliest known surface modification methods is by esterification. This method involves a reaction between the silanol group and an alcohol and results in the formation of a Si-O-C linkage. The linkage formed in this reaction was however found to be very unstable and not very useful. The next method involves an organosilanization reaction. In this method, an organosilane reagent with a halide, methoxy or ethoxy group reacts with “free” silanols to form a single attachment of an organic group to the silica surface. Using an organosilane with three reactive groups usually results in the formation of a multilayer polymeric stationary phase due to crosslinking between the adjacent organosilane groups. A disadvantage to this method is that an acid is formed as a by-product during the reaction and the Si-O-Si-C bonds can be hydrolyzed by the acid produced during the reaction [7]. Another method involves the chlorination of “free” silanols with thionyl chloride and attaching the organic moiety using a Grignard reagent or an organolithium compound. The disadvantage of this method is that the chlorinated intermediate is very unstable and a very dry working environment must be maintained in order to carry out this synthesis [7].

A more recent approach for the modification of a silica surface involves triethoxysilane (TES) silanization followed by a hydrosilation reaction [8]. The initial step of this reaction involves a reaction between the silanol groups and triethoxysilane resulting in the formation of a hydride monolayer. The hydride intermediate formed in this method is very stable in air and water and can be stored for long periods of time without degradation. After the formation of the hydride monolayer, an organic group

can then be attached via a hydrosilation reaction. Reaction sequence for silica surface modification by TES silanization and hydrosilation is provided below:

a. TES SILANIZATION

b. HYDROSILATION

In this reaction, a terminal olefin or an unsaturated organic compound can be attached in the presence of a catalyst by the reaction of the hydride intermediate with the unsaturated organic compound [8]. Another reaction for attaching an organic moiety to the silica surface is through a free radical addition reaction also involving a catalyst.

For this research project, Vydac silica was selected for surface modification. The silica surface was first modified by the triethoxysilane (TES) method to create the Vydac hydride intermediate. After creating the hydride intermediate, the organic moiety was then attached using the hydrosilation method.

C. 1-Octene and AMPS Bonded Silica

Three different columns were selected to conduct this research project: a previously prepared C8 column by Dr. Pesek's research group; a C8 endcapped with AMPS column; and an AMPS on silica column. The latter two columns were prepared as part of this research project.

Table 1: Organic Compounds

Compound	Molecular Formular	Molecular Weight
1-Octene	$C_8H_{14}C=CH_2$	112.208
2-acrylamido-2-methyl-1-propanesulfonic acid (AMPS)	$C_7H_{13}NO_4$	207.25

The preparation of the C8 column involved the attachment of 1-octene to silica through a Vydac hydride intermediate in a hydrosilation reaction. Dr. Pesek's research group as stated earlier previously prepared the C8 column. The second column involved the attachment of C8 to silica using Vydac hydride in a hydrosilation reaction and then endcapping with 2-acrylamido-2-methyl-1-propanesulfonic acid (AMPS) in a second hydrosilation reaction. The third column involved the direct attachment of AMPS to silica using the Vydac hydride intermediate. The reaction schemes are provided in **Figure 3**. For the C8 endcapped with AMPS column, after the creation of the Vydac hydride intermediate, the organic olefin, 1-octene (C8) was attached to silica through the silica hydride to form a monomeric C-8 bonded phase via hydrosilation and using hexachloroplatinic acid as the catalyst. After the attachment of C8 to Vydac, the column was endcapped with AMPS in a second hydrosilation reaction also using

hexachloroplatinic acid as the catalyst. For preparation of the AMPS on Vydac silica column, AMPS was directly attached to the silica surface through the Vydac hydride intermediate by hydrosilation. The reaction was carried out by the free radical addition mechanism using an azobisisobutyronitrile (AIBN) catalyst [9].

D. Characterization Methods

The characterization methods selected for this study were Diffuse Reflectance Fourier Transform Infrared Spectroscopy (DRIFT), Cross-polarization, Magic Angle Spinning NMR (CP-MAS NMR) and Elemental Analysis.

1. Diffuse Reflectance Fourier Transform Infrared Spectroscopy (DRIFT)

DRIFT is a spectroscopic surface analytical technique that can be used to determine the presence of organic moieties or functional groups on a silica surface. DRIFT is used in this project for the surface characterization study because diffuse reflectance increases the sensitivity of the IR technique for solid samples. In IR, certain types of bond provide vibrational frequencies in the absorption spectrum that can be used as fingerprints for structural information. For example, N-H, C-H, O-H, C=O, C-O, C=C and CN bonds provide absorption frequencies that occur within specific regions of the IR spectrum [13,14]. The DRIFT technique involves mixing a small amount of a solid sample with potassium bromide (KBr) and obtaining the spectrum. KBr is added to the solid sample to enhance the transmission of light through the sample. This method provides specific structural information that allows structural comparison of the starting material and the synthesized product. The spectrum can be used to confirm the success of a specific reaction.

Figure 3. Reaction schemes for the creation of bonded silica:

1. 1-Octene

Figure 3 (Continued)

2. AMPS on Vydac

Figure 3 (Continued)

3. C8 Endcapped with AMPS

Where R=(CH₂)₅-CH₃

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

CP-MAS NMR is another technique that is often used for solid state surface analysis because it enhances the signal level for these compounds [15]. NMR is one of the most powerful tools for generating chemical structure information. NMR spectroscopy is based on the measurement of absorption of electromagnetic radiation in the radio-frequency region of 4 to 900 MHz [13]. In NMR, the nuclei of atoms present in a sample placed under a strong magnetic field experience energy levels splitting. Electromagnetic radiation is absorbed by the nuclei due to the splitting of their energy levels and this is directly related to the molecular structure of the compound.

Using cross-polarization in NMR eliminates the problem caused by slow spin-lattice relaxation time. CP is a pulsed technique that causes the Larmor frequencies of proton nuclei and ^{13}C nuclei to become identical. This condition allows the magnetic fields of the precessing proton nuclei to interact with the fields of the ^{13}C nuclei [13]. This results in a faster relaxation and the transfer of magnetization from abundant, highly sensitive nuclei like protons to less abundant, low sensitivity nuclei like ^{13}C and ^{29}Si [16]. Magic angle spinning (MAS) utilizes the rotation of solid samples at an angle of 54.7° with respect to the external magnetic field, which increases the sensitivity of this technique for these solid samples by narrowing the absorption lines [17].

2a. CP-MAS Carbon-13 NMR

For surface analysis carbon-13 (^{13}C) NMR and silicon-29 (^{29}Si) NMR are some of the most widely used NMR techniques. Carbon-13 CP-MAS NMR can be used for

the determination of structures of organic and biochemical species in the solid state [13]. This technique provides a chemical shift for ^{13}C in various chemical environments of the organic moiety attached to the silica surface. Carbon-13 CP-MAS NMR can therefore be used to determine the success of a chemical bonding reaction.

2b. CP-MAS Silicon-29 NMR

Silicon-29 CP-MAS NMR on the other hand can be used to characterize the oxide surface. It provides information about the active groups present on the initial silica surface before modification and the bonded phase after modification. This information allows one to compare the amount of Si-OH on the original silica to that of the final product. Other Si peaks can be used to identify other surface features.

3. Elemental Analysis

Elemental analysis is a quantitative micro-combustion method that can be used for determining the amount of compound bonded to a silica surface. It is based upon a high-temperature oxidation of the organic compounds, converting the elements of interest to gaseous molecules [13]. A high percent carbon result can be interpreted as a high carbon loading on the surface indicating that a substantial amount of the organic moiety has been successfully attached to the silica surface.

E. Goals

The initial step in this project was to prepare the Vydac hydride intermediate using the TES silanization reaction. After preparing the intermediate, IR studies were conducted to confirm the success of the reaction. The next step in the project was to synthesize two other stationary phases. The first involved the attachment of C8 to

Vydac in a hydrosilation reaction followed by the attachment of AMPS in a second hydrosilation reaction to endcap the stationary phase. The second stationary phase involved directly attaching AMPS to silica.

After the synthesis of the C8, C8 endcapped with AMPS and the AMPS on Vydac stationary phases, several characterization methods were used to confirm the success of the hydrosilation reactions. It is well known that after the modification of a silica surface there are usually several residual silanol groups present on the surface. The presence of these residual silanol groups can sometimes create interference during the analysis of basic compounds [6]. Residual silanol groups on silica result in strong interactions with basic solutes during analysis and this can cause major peak tailing for these compounds and poor column performance. One of the goals of this project was to enhance column efficiency by reducing the amount of residual silanol and hydride groups present on the silica surface by endcapping using AMPS. Endcapping can be defined as a secondary derivatization reaction that can be used to shield and reduce residual silanol groups on a modified silica surface [10,11,12].

The final goal of this research project was to test the ability of the C8 column, the C8 endcapped with AMPS column and the AMPS on Vydac column for the separation of the Perkin Elmer Universal and Supelco Reverse Phase Test mixtures; and several basic organic compounds. The data generated from this study were compared to determine the separation capabilities of the three columns.

CHAPTER II. Experimental

A. Materials

1. Chemicals

For this research project, several chemicals and reagents were required for the successful completion of the different chemical reactions and analyses. Most of the chemicals for analysis in this project were purchased from Sigma-Aldrich, Perkin Elmer EM Science, J. T. Baker, Fisher and Supelco. Their Chemical Abstract Service (CAS) registry numbers are listed in **Table 2**.

Table 2. The CAS Registry Numbers of Chemical Used.

Chemical Name	CAS Registry Number
Trimethylchlorosilane	[75-77-4]
Hexamethyldisilazane	[999-97-3]
1-Octene	[111-66-0]
Toluene	[108-88-36]
Methanol	[67-56-1]
Ethanol	[64-17-5]
2-Propanol	[67-63-0]
Hydrochloric acid	[7647-01-0]
Triethoxysilane (TES)	[998-30-1]
Hexachloroplatinic acid (38-40%)	[16941-12-1]
Potassium Nitrate	[7757-79-1]
Potassium Bromide	[7758-02-3]
Pyridine	[7291-22-7]
P-toluidine	[106-49-0]
Aniline	[62-53-3]
Diphenylaniline	[603-34-9]
N,N-dimethylaniline	[121-69-7]
AMPS (99%)	[15214-89-8]
Azobisisobutyronitrile (AIBN)	[4187-87-5]
Tertiary butyl peroxide	[110-05-4]
Dichloromethane	[75-09-2]
Diethyl Ether	[60-29-7]
Dioxane	[123-91-1]

Tetrahydrofuran (THF)	[109-99-8]
Acetonitrile	[75-05-8]
Ethylbenzene	[100-41-4]
Isopropylbenzene	[98-82-8]
t-butylbenzene	[104-51-8]
Anthracene	[120-12-7]
Acetophenone	[98-86-2]
Uracil	[66-22-8]
Sodium perchlorate	[7601-89-0]
Tetracycline	[60-54-8]

2. Preparation Materials for the Bonded Stationary Phases

1-Octene and 2-acrylamido-2-methyl-1-propanesulfonic acid (AMPS) were purchased from Sigma Aldrich (Milwaukee, WI). Hexachloroplatinic acid hydrate was purchased from Strem Chemicals (Newbury Port, MS). Triethoxysilane (TES) was purchased from Huls Petriarch Systems (Huls, Bristol, PA). The sources of the solvents were as follow: Toluene (EM Science, Gibstown, NJ), diethyl ether and dioxane (Fisher Scientific, Fair Lawn, NJ), and tetrahydrofuran, (THF) (J.T. Baker Chemical Co. Phillipsburg, NJ). All chemicals were reagent grade and of high purity. The silica used for this project was Vydac TP 106 (The Separations Group, Hesperia, CA) with a particle diameter of 6.5 microns, a pore size of 300 Angstroms and a surface area of 106.5 m²/g.

B. Experimental Methods

1. Preparation of Speier's Catalyst

The Speier's catalyst was prepared in a nitrogen filled glove box. The procedure involved the addition of 0.4 g of chloroplatinic acid hexahydrate (Strem Chemicals, Newbury Port, MS) to a 100 mL isopropanol solution at 93°C. The prepared 10 mM

speier's catalyst was stored in a freezer.

2. Silica Hydride formation via (TES) Silanization

The TES silanization reaction for creating the silica hydride was done according to Chu et al. [8]. All glassware used to carry out the silanization of silica was dried at 110°C overnight before use. Silica was also dried overnight in a vacuum oven at 110°C prior to synthesis.

In a nitrogen filled glove box, 8.45 mL of 1 mM triethoxysilane (TES) was measured into a 50 mL volumetric flask. Distilled 1,4-dioxane was then added to the flask and filled to the 50 mL mark. A 3 necked 500 mL round bottom flask was equipped with a West condenser, addition funnel, stopper, thermometer and magnetic stirring bar. 10 g of Vydac silica was transferred to the round bottom 500 mL flask, followed by 245 mL of dioxane and then 10 mL of a 2.3 M hydrochloric acid (HCl) solution. All joints of the flask were sealed with parafilm to prevent leakage. The reaction mixture was gradually heated to 93°C and allowed to reflux at this temperature for about one hour. The TES solution was then added dropwise to the flask through the addition funnel for about 20 to 30 minutes. The reaction mixture was allowed to reflux for another hour after the addition of TES. After an hour, the apparatus was cooled to room temperature, the dioxane poured off and replaced with toluene.

Aliquots of the reaction mixture were placed in four different centrifuge tubes and centrifuged for 10 minutes. After centrifuging, the mixture was washed three times using a 1:1 (v/v) tetrahydrofuran (THF)/water mixture followed by two washings with THF and two washings with ether. The product was transferred to a watch glass and

placed in the hood to evaporate the solvent and dry overnight. The following day the synthesized silica hydride was placed in a vacuum oven (VWR Scientific Model 1410) and dried at 110°C for at least 24 hours. The silica hydride was then transferred to an airtight vial and sealed.

3. Attachment of C8 to Vydac via Hydrosilation

The attachment of 1-octene to silica via the Vydac hydride intermediate was conducted according to Sandoval et al. [10]. All glassware was dried at about 100°C overnight. Under a hood, a 100 ml three necked round bottom flask was equipped with a West condenser, a drying tube, thermometer, magnetic stirring bar and an electric heater. 2.4 g of the Vydac hydride intermediate was dried at 60 °C in a vacuum oven overnight. 36 mL of 1-octene was measured and added to the 100 mL round bottom flask, followed by the addition of 122 µL of the Speier's catalyst. The flask was placed on the electric heater. The reaction mixture was heated to 60-70 °C and allowed to reflux for 1 hour. The 2.4 g of Vydac hydride intermediate was then slowly added to the reaction mixture and heated to 100 ±2 °C. The reaction was allowed to continue at this temperature for about 90 hours.

After 90 hours, the mixture was allowed to cool to room temperature, separated into aliquots and centrifuged (IEC HN-S Centrifuge, Needham HTS, MS) for ten minutes at about 1500 rpm and the supernatant decanted. The product was then washed three times with a 40 mL aliquot of toluene followed by a 40 mL aliquot of dichloromethane and a 40 mL aliquot of diethyl ether. The product was transferred to a watch glass and allowed to dry overnight in a hood. Next, the product was dried in a

vacuum oven at 110 °C for 24 hours.

4. Endcapping C8 Stationary Phase with AMPS via Hydrosilation

In a second hydrosilation reaction, 2-acrylamido-2-methyl-1-propane sulfonic acid (AMPS) was attached to Vydac through the Vydac hydride intermediate to endcap the C8 stationary phase. In an effort to reduce the effect of residual hydride and silanol groups found on the silica surface after attaching C8 to Vydac through the Vydac hydride intermediate, the C8 stationary phase endcapped with AMPS. This involved reacting the residual hydride groups with AMPS in a second hydrosilation reaction thereby further reducing the amount of hydride groups on silica [11,12]. The reaction was conducted following Sandoval et al. [11].

All glassware was dried at about 100°C overnight in an oven. A 100 ml three necked round bottom flask was equipped with a West condenser, a drying tube, thermometer, magnetic stirring bar and an electric heater. 2.4 g of the synthesized C8 on Vydac product was dried at 60 °C in a vacuum oven overnight. 2.4 g of AMPS was weighed out and dissolved in 32 mL of ethanol. The solution was transferred to the 100 mL round bottom flask and heated to 60 °C. When the solution reached the desired temperature of 60 °C, 280 µL of the Speier's catalyst was added to the mixture and allowed to react for 1 hour. 2.4 g of the C8 on Vydac product was then added to the flask and heated to 68 °C. The reaction was allowed to continue for 90 hours at this temperature.

After 90 hours, the mixture was allowed to cool, centrifuged for ten minutes at about 1500 rpm and the supernatant decanted. The product was washed three times

with 40 mL aliquots of ethanol, transferred to a watch glass and allowed to dry overnight in a hood. The next day, the product was dried in a vacuum oven at 110 °C for 24 hours. To determine the success of the bonding reaction in creating the organic moiety, elemental analysis, DRIFT and CP-MAS NMR spectroscopy were utilized.

5. Free Radical Hydrosilation attachment of AMPS to Silica

A second bonded stationary phase was created via another hydrosilation reaction. In this reaction, 2-acrylamido-2-methyl-1-propane sulfonic acid (AMPS) was directly attached to silica via the Vydac hydride intermediate using AIBN as the catalyst. As in previous reactions, all glassware was dried at about 100°C overnight in an oven. The reaction was conducted following Sandoval et al. [16].

A 100 ml three necked round bottom flask was equipped with a West condenser, a drying tube, thermometer, magnetic stirring bar and an electric heater. 2.4 g of Vydac hydride was dried at 60 °C in a vacuum oven overnight. 2.4 g of AMPS was weighed out and dissolved in 32 mL of ethanol. The solution was transferred to the 100 mL round bottom flask and heated to 60 °C. When the solution reached the desired temperature of 60 °C, 280 µL of the azobisisobutyronitrile (AIBN) catalyst was added to the mixture and allowed to react for 1 hour. The 2.4 g of Vydac hydride was added to the flask and reaction mixture heated to 68 °C. The reaction was allowed to continue at this temperature for 90 hours.

After the 90 hour reaction time, the mixture was allowed to cool, centrifuged for ten minutes at about 1500 rpm and then the top level drained. The product was washed three times with 40 mL aliquots of ethanol and allowed to dry overnight in a hood. The

product was then further dried in a vacuum oven at 110 °C for 24 hours. To evaluate the success of the hydrosilation reaction in creating the bonded organic stationary phase, elemental analysis, DRIFT and CP-MAS NMR spectroscopy were utilized.

6. Column Packing

The two synthesized stationary phases, C8 endcapped with AMPS and the AMPS bonded to Vydac silica were used to pack two stainless steel columns (15 cm x 4.6 mm internal diameter, Alltech Associate Inc., Deerfield, IL). The two columns were packed using a Haskell Pump (Haskell, Burbank, CA) column packer. HPLC-grade methanol and carbon tetrachloride (Fisher Scientific, Fair Lawn, NJ) were used as the solvents in the packing procedure. For packing each column, about 2 g of the bonded silica was stirred in a 90:10 carbon tetrachloride:methanol solution and then poured into the pump reservoir. Each column was packed under high pressure using nitrogen gas. After packing, the columns were allowed to relax for 30 minutes before removal from the apparatus. After removal from the column packer, each column was capped with plastic fittings and was ready for evaluation studies.

C. Characterization of Bonded Stationary Phases

1. Diffuse Reflectance Infrared Fourier Transform Spectroscopy (DRIFT)

Using the ATI Mattson Infinity Series FT-IR spectrophotometer interfaced with a Hewlett Packard Venturis FX-2 computer, infrared spectra of the Vydac hydride intermediate, C8 on Vydac, C8 endcapped with AMPS and the AMPS on Vydac stationary phases were collected. The plotter was a Hewlett Packard Desk Jet 722C. The IR spectra of the solid bonded phases were collected using a diffuse reflectance

accessory (Spectra Tech., Stamford, CT) to enhance sensitivity. Each sample for analysis was prepared using a 95:5 ratio of solid sample to ground IR-grade KBr. The sample was well mixed and placed in a sample cup of 2 mm diameter x 2 mm depth and the top leveled using a spatula. The sample was then placed in the DRIFT accessory in the FT-IR and scanned. Before sample analysis, a reference background was obtained using pure KBr. Each prepared sample was scanned 200 times at a resolution of 2 cm^{-1} over a range of $4000 - 450\text{ cm}^{-1}$ and normalized to 100% transmittance.

2. Carbon ^{13}C CP-MAS NMR

The CP MAS ^{13}C NMR spectra were collected using a Varian (Palo Alto, CA) INOVA 400 MHz spectrometer with the cross-polarization and magic angle spinning techniques. Each solid sample of about 200-300mg was placed into the ZrO_2 double bearing rotor. The rotor spun each sample at a rate of 4700 – 5200 Hz at room temperature. For ^{13}C , glycine was used as the external reference and for ^{29}Si , polyhydrosiloxane. The spectra were collected using a 5 second contact time and a 5 second repetition rate.

3. High Performance Liquid Chromatography Instrumentation

The HPLC system used for this project was a Perkin Elmer (Perkin Elmer, Norwalk, CT) system. It consisted of an injector, a 200 series LC pump, and an UV-VIS multi-wavelength absorbance detector. The system was interfaced with a Hewlett Packard Model 3395 integrator to generate the chromatograms. The synthesized stationary phases packed into stainless-steel columns were used to conduct the HPLC studies. To degas the system, Compressed helium flowing at 50 mL/min was used to

degas the system and to continuously spurge the eluents.

For gradient elution studies, a Hewlett-Packard (Avondale, PA) model 1050 liquid chromatographic system was used. It was equipped with a quaternary gradient pump, automatic injector, variable wavelength detector and a computer data station (ChemStation Software). HPLC grade methanol (EM Science, Gibbstown, NJ), and water were used as the mobile phase components for the chromatographic studies. For the HPLC analysis, the Perkin Elmer Universal Test Mix was purchased from Perkin Elmer and the Supelco Reverse Phase Test Mix from Supelco. Two groups of basic organic compounds including a tetracycline mix, (oxytetracycline, minotetracycline, democycline, chlorotetracycline and doxycycline) and some anilines (aniline, diphenylaniline and n,n- dimethylaniline) were purchased from Sigma (St. Louis, MO).

4. Elemental Analysis

For elemental analysis, the two synthesized organic samples, C8 endcapped with AMPS and AMPS on vydac were sent to Desert Analytics in Tucson, AZ to determine the carbon percentages. Using a micro-combustion analysis, the carbon content of both samples was successfully determined. The carbon percentage (%C) results provided from the elemental analysis were used to calculate the surface coverage of the bonded silica.

D. Surface Coverage

Surface coverage for samples can be easily calculated if the carbon percentages of the bonded phases is known by using the Berendsen and de Galan equation shown below [18]:

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

Where, P_c is the carbon percentage by weight of the bonded stationary phase, M_c is the atomic number of carbon, and n_c , the number of carbons in the bonded organic moiety or moieties. S_{BET} (Brunauer, Emmet and Teller) is the specific surface area (m^2/g) of the silica substrate, and M_R the molecular weight of the stationary phase [5,18].

CHAPTER III. RESULTS AND DISCUSSION

A. Elemental Analysis Results

Using the carbon percentage values provided by Desert Analytics in Tucson, AZ the surface coverage for the two bonded phases were calculated. The reported results were 7.91%C for the C8 endcapped with AMPS sample and 4.64%C for the AMPS on Vydac sample. The Maximum surface coverage that can be obtained for silica is 8 $\mu\text{mol}/\text{m}^2$ [6,19]. The calculated surface coverage was 6.38 $\mu\text{mol}/\text{m}^2$ for the C8 endcapped with AMPS stationary phase and 7.79 $\mu\text{mol}/\text{m}^2$ for the AMPS on Vydac stationary phase, very high values. The high surface coverage values can be attributed to the successful attachment of AMPS to silica partly because of the less bulky size of this compound. **Table 3** provides the calculated surface coverage values for the bonded stationary phases.

Table 3. Surface Coverage and Carbon Percentage Data Table

Synthesized Stationary Phase	% Carbon	Surface Coverage ($\mu\text{mol}/\text{m}^2$)
C8 endcapped w/ AMPS	7.91	6.38
AMPS on Vydac	4.64	7.79

B. Characterization Studies of Silica Hydride

1. DRIFT Spectra

a. TES Silanization

To determine the success of the TES Silanization reaction in creating the silica hydride, DRIFT spectroscopy was used to characterize the silica hydride. **Figure 4**

shows the DRIFT spectrum of the silica hydride sample. The spectrum shows a band at around 2250 cm^{-1} indicating the presence of a silica-hydride bond. A band in this region of the spectrum is indicative of a Si-H bond stretching [7].

2. CP-MAS ^{13}C Spectra

As an example of the carbon 13 CP-MAS NMR studies of Vydac-hydride, **Figure 5** shows a spectrum from a previous study [20]. In this spectrum, there are two distinct peaks at 16 ppm and 60 ppm. These peaks are indicative of methyl and methylene moieties of the ethoxy groups respectively. These two groups are usually found on almost all synthesized Vydac hydride surfaces by TES silanization [10].

3. CP-MAS ^{29}Si Spectra

Figure 6 from a previous study shows the silicon 29 CP-MAS NMR spectrum for Vydac hydride [20]. The presence of a peak at -110 ppm is indicative of a silicon atom with four siloxane linkages. The next peak at -100 ppm indicates that a silicon atom with three siloxane linkages and a hydroxyl group is present [7]. The peak at -85 ppm is indicative of a silicon atom with three-siloxane linkages and a hydride attached. The last peak at -75 ppm indicates the presence of a silicon atom with two siloxane linkages, one hydroxyl group and one hydride [10]. The information provided by **Figure 6** confirmed that Vydac hydride was created by TES silanization.

C. Bonding of 1-Octene, Endcapping With AMPS and Bonding AMPS to Silica

1. DRIFT Spectra

a. C8 endcapped with AMPS, AMPS on Vydac and Vydac Hydride

To determine the success of the hydrosilation reaction for the attachment of 1-

Figure 4. DRIFT Spectrum of Vydac Silica Hydride

Figure 5. ^{13}C CP-MAS NMR of Vydac Hydride

Figure 6. ^{29}Si CP-MAS NMR of Vydac Silica Hydride

octene to silica through the Vydac hydride intermediate, AMPS to Vydac through the Vydac hydride intermediate and C8 and AMPS to Vydac, DRIFT spectroscopy was utilized. **Figure 7** shows the DRIFT spectra of the AMPS and C8 Vydac bonded phase, (A), the AMPS Vydac bonded phase, (B) and the Vydac hydride intermediate, (C). In **Figure 7**, the DRIFT spectrum (C) of the Vydac hydride shows a distinct stretching band at 2250 cm^{-1} representing the Si-H bond. In the DRIFT spectrum of AMPS on Vydac, (B) and AMPS and C8 on Vydac, (A), the Si-H stretching is still present at 2250 cm^{-1} , however, there is a significant decreased in the Si-H peak compared to that of the spectrum of the Vydac hydride intermediate (C). These results indicate that Si-H sites are being removed in the attachment of 1-octene and AMPS to Vydac. In the DRIFT spectrum (A) of AMPS and C8 on Vydac, and (B) of AMPS on Vydac, there are stretching bands at 2571 cm^{-1} and 2683 cm^{-1} representing the OH and CH_2 bonds present in AMPS. This result indicates the successful attachment of AMPS to Vydac. In spectrum A of AMPS and C8 on Vydac there is a band at 2952 cm^{-1} representing a C-H bond stretching. The appearance of the C-H bond stretching at 2952 cm^{-1} confirms that the organic moiety 1-octene, has been successfully attached to Vydac through the Vydac hydride intermediate [7].

b. C8 Endcapped with AMPS, AMPS on Vydac and C8 on Vydac

Figure 8 shows the DRIFT spectra of A) C8 endcapped with AMPS (C8 and AMPS on Vydac), B) AMPS on Vydac and C) C8 on Vydac. In **Figure 8**, spectrum A of C8 endcapped with AMPS shows a smaller peak of the residual Si-H bond stretching at 2250 cm^{-1} , the OH and CH_2 band stretching at 2571 cm^{-1} and 2683 cm^{-1} from AMPS

and the C-H bond stretching from C8 near 3000 cm^{-1} . Spectrum B of AMPS on Vydac also shows the residual Si-H groups at 2250 cm^{-1} and the OH and CH_2 band stretching from AMPS at 2571 cm^{-1} and 2683 cm^{-1} . Spectrum C of the C8 on Vydac stationary phase shows the residual Si-H groups at 2250 cm^{-1} and the C-H bond stretching from C8 attachment to Vydac near 3000 cm^{-1} . From review of the spectra in **Figure 8**, it is clearly indicated that the hydrosilation reactions for the attachment of AMPS to Vydac, C8 to Vydac and AMPS to C8 Vydac to create the bonded stationary phases were highly successful [10].

c. Vydac Silica vs. Kromasil Silica

By examining **Figures 7 and 8**, the C-H bond stretch peak for the C8 attachment though present, is however not very prominent. **Figure 9** shows two DRIFT spectra, one spectrum of C8 attachment to Kromasil silica via a Kromasil hydride intermediate and the other of a C8 attachment to Vydac silica via the Vydac hydride intermediate. By comparison, the DRIFT of the C8 attachment to Kromasil shows a much stronger C-H stretching peak near 3000 cm^{-1} than the C8 attachment to Vydac. This can be attributed to the fact that Kromasil silica has 3 times as much surface area when compare to Vydac silica. The surface area of Kromasil silica is about $300\text{ m}^2/\text{g}$, where as the surface area of Vydac silica is about $100\text{ m}^2/\text{g}$ thus providing a greater amount of C8 per gram of sample for Kromasil and a stronger C-H bond stretching signal.

2. CP-MAS ^{13}C Spectra

a. 1-Octene Bonded Phase

Additional spectroscopic studies were conducted to confirm the attachment of 1-

Figure 7. DRIFT Spectra of
A: C8 Endcapped With AMPS Silica Hydride
B: AMPS Vydac Silica Hydride
C: Vydac Silica Hydride

AMPS on C8 Sophie James. Fri Nov 30 10:11:38 2001

Figure 8. DRIFT Spectra of
A: C8 Endcapped with AMPS Vydac Silica Hydride
B: AMPS Vydac Silica Hydride
C: C8 Vydac Silica Hydride

Figure 9. DRIFT Spectra of Vydac Silica vs. Kromasil Silica

octene and AMPS to Vydac through the Vydac hydride intermediate CP-MAS ^{13}C NMR was selected to provide structural information of the synthesized organic phases. **Figure 10** shows the carbon-13 NMR spectrum of the 1-octyl bonded stationary phase. The spectrum shows a peak at about 12 ppm representing the Si-C linkage from C8 to silica and a peak at around 22 ppm representing the CH_3 group present in C8. There are also three distinct peaks at 28 ppm, 32 ppm, and 36 ppm representing the CH_2 groups present in the 1-octyl attachment to silica. Results of the ^{13}C CP-MAS NMR further confirm the success of the hydrosilation reaction in the attachment of 1-octene to Vydac silica through the hydride intermediate.

b. AMPS Bonded Phase

To confirm the success of the hydrosilation reaction in the direct attachment of AMPS to Vydac through the Vydac hydride intermediate the bonded stationary phase was also characterized by CP-MAS NMR. **Figure 11** shows the ^{13}C CP-MAS NMR spectrum of the AMPS on Vydac bonded stationary phase. In **Figure 11**, the peak at around 16 ppm represents the Si-C linkage resulting from the attachment of AMPS to Vydac silica. The peaks at 24 ppm and 27 ppm are due to the two CH_3 groups present in AMPS. **Figure 11** also shows a peak at about 54 ppm, representing the $\text{CH}_2\text{-C=O}$ group present in AMPS and another peak at 58 ppm resulting from the $\text{CH}_2\text{-S}$ group present in AMPS. The chemical structure of 2-acrylamido-2-methyl-1-propanesulfonic acid (AMPS) is provided in **Figure 11**. All relevant carbon peaks are identified and assigned in the ^{13}C CP-MAS NMR of the AMPS bonded stationary phase. The results

of this characterization study thus confirm the successful attachment of AMPS to Vydac silica via the Vydac hydride intermediate.

c. AMPS & 1-Octene Bonded Phase

Figure 12 shows the ^{13}C CP-MAS NMR spectrum of the C8 bonded stationary phase endcapped with AMPS. In **Figure 12**, the peak at 12 ppm represents the Si-C linkage resulting from the attachment of C8 to Vydac and the peak at 16 ppm represents the Si-C linkage from AMPS attachment to Vydac. The chemical shift at 22 ppm represents the CH_3 group in C8. **Figure 12** also shows chemical shifts at 24 ppm and 27 ppm resulting from the two CH_3 groups in AMPS. The chemical shift at 32 ppm represents the CH_2 groups present in the 1-octyl attachment to Vydac silica. **Figure 12** shows a peak at 54 ppm representing the $\text{CH}_2\text{-C=O}$ group in AMPS and another peak at 58 ppm representing the $\text{CH}_2\text{-S}$ group also present in AMPS. From the structural information provided by the ^{13}C CP-MAS NMR, it is clearly indicated that 1-octene was successfully attached to Vydac silica through the Vydac hydride intermediate by the hydrosilation reaction. This study showed that the C8 bonded stationary phase was successfully endcapped by the attachment of AMPS to Vydac through the Vydac-hydride intermediate.

D. Chromatographic Studies

HPLC Reverse Phase Chromatographic studies were conducted to evaluate the efficiencies of the previously synthesized C8 column, the C8 endcapped with AMPS column and the AMPS on Vydac column. HPLC studies were also conducted to generate comparative data of the three columns. As stated earlier, one of the goals of

C8:

C8 on silica:

Figure 10. Carbon 13 CP-MAS NMR of C8 Vydac

Figure 11. Carbon 13 CP-MAS NMR of AMPS on Vydac – AIBN free radical

AMPS:

AMPS on silica:

C8:

C8 on silica:

Figure 12. Carbon 13 CP-MAS NMR of C8 endcapped with AMPS

this project was to enhance column efficiency by reducing the amount of residual silanol groups present on the C8 stationary phase by endcapping using AMPS. The presence of AMPS on the C8 Vydac stationary phase should enhance the hydrophilicity of the column and provide better separation of basic organic compounds.

Isocratic separations were conducted using the Perkin Elmer Universal Test Mix (PE-UTM) consisting of five compounds and a Supelco Reverse Phase Test Mix consisting of four different compounds. In addition to this, several basic tetracycline and aniline compounds were tested to generate comparative data and evaluate the log of k' , and peak asymmetry factors.

1. Isocratic Separation of Perkin Elmer Universal Test Mix

a. Separation of C8 vs. C8 Endcapped with AMPS

Figure 13 shows the chromatographic separation of the Perkin Elmer Universal Test Mix (PE-UTM) consisting of toluene, ethylbenzene, isopropylbenzene, t-butylbenzene and anthracene on the C8 column and the C8 endcapped with AMPS column. The PE-UT mix was analyzed on the two columns using a flow rate of 0.5 mL/min and UV detection at 254 nm. For the C8 column, the mobile phase consisted of a 50:50, (v/v) methanol/water composition. For the C8 endcapped with AMPS column, the mobile phase consisted of a 40:60, (v/v) methanol/water composition. **Figure 13** shows that both the C8 column and the C8 endcapped with AMPS columns successfully separated all five compounds of the PE-UT mix with almost equal performance.

Separation was unsuccessful for the AMPS on Vydac column.

2. Isocratic Separation of Supelco Reverse Phase Test Mix

C8 vs. C8 Endcapped with AMPS

Figure 13. Isocratic Separation of Perkin Elmer Test Mix
Mobile Phase: 50/50 (v/v) MeOH/H₂O for C8 column and 40/60 (v/v) MeOH/H₂O for C8 endcapped with AMPS column.
Flow rate: 0.5 mL/min. **UV detection:** UV 254 nm.
Samples: 1 = Toluene, 2 = Ethylbenzene, 3 = Isopropylbenzene
4 = t- Butylbenzene, 5 = Anthracene

a. Separation of C8 vs. C8 Endcapped with AMPS

In order to generate additional comparative data, the Supelco Reverse Phase Test Mix was tested on the C8 column, C8 endcapped with AMPS column and the AMPS on Vydac column. The Supelco Reverse Phase Test Mix consists of acetophenone, benzene, toluene and uracil. This mix is widely used to test the performance of reverse phase columns. **Figure 14** shows the separation of the Supelco Reverse Phase Test Mix on the C8 column and the C8 endcapped with AMPS column. All three columns were tested using a mobile phase of 60/40 (v/v) methanol/water (MeOH/H₂O), a flow rate of 0.5 mL/min and UV detection at 254 nm. In **Figure 14**, the chromatograph of the HPLC separation on the C8 column shows the separation of only three of the four compounds of the Supelco Reverse Phase Test Mix. One of the compounds of the test mix was not successfully separated. On the other hand, the chromatograph of the C8 endcapped with AMPS column shows the successful separation of all four compounds of the Supelco Reverse Phase test Mix within less than 6 minutes.

From the results of this study, it can be interpreted that endcapping the C8 stationary phase with AMPS successfully enhanced the ability of the column when compared to the C8 column. For the separation of the Supelco Reverse Phase Test Mix, the C8 endcapped with AMPS column shows a greater efficiency than the C8 column. Separation by the C8 column and the C8 endcapped with AMPS column occur by hydrophobic interactions between the solutes and the mobile phase. Both columns exhibiting reverse phase column characteristics. Separation of the Supelco Reverse

Column: C8

Column:
C8 endcapped with AMPS

Figure 14. Isocratic Separation of Supelco Reverse Phase Test Mix
Mobile Phase: 60/40 (v/v) methanol/water.
Flow rate: 0.5 mL/min. UV detection: 254 nm
Samples: 1 = uracil, 2 = Acetophenone, 3 = Benzene, 4 = Toluene

Phase Test Mix on the AMPS on Vydac column was unsuccessful at these conditions. This can be attributed to the fact that the AMPS on Vydac column is very hydrophilic due to the presence of the sulfonic acid group in AMPS. The polar nature of this group allows the column to exhibit normal phase column characteristics.

3. Evaluation of Basic Organic Compounds

a. Separation of Tetracyclines on C8 vs. C8 Endcapped with AMPS

Tetracyclines are a very important group of drugs that have a common basic structure. Tetracyclines are widely used as an antibiotic for the treatment of several diseases. The separation of the different tetracyclines compounds are very important clinically because they differ to some extent in their degree of chemical activity and toxicity in the treatment of diseases. For chromatographic studies, a tetracycline test mix containing oxytetracycline, minotertacyline, democycline, chlorotetracycline and doxycycline was analyzed on the C8 column and the C8 endcapped with AMPS column. As stated earlier, it is widely known that after the modification of silica there are usually some residual silanol groups present on the surface. The presence of these residual silanol groups sometimes interferes with the separation of basic compounds through an ion exchange process. This interaction, in most cases, results in peak tailing, band broadening and poor separation of basic compounds [21].

One goal of this project was to reduce the amount of residual silanol groups on silica and to prevent the interaction of basic compounds with these residual groups by endcapping the C8 stationary phase with AMPS. **Figure 15** shows the chromatograph of the tetracycline mix separation on the C8 column and the C8 endcapped with AMPS

column. The tetracycline mix was run using a mobile phase of 90/10 (v/v) acetonitrile/sodium perchlorate buffer at pH=2, a flow rate of 0.5 mL/min and UV detection of 280 nm. The chromatograph of the tetracycline mix separation on the C8 column shows the successful separation of four of the five tetracycline compounds. Chlorotetracycline was not successfully separated on the C8 column. However, for the C8 endcapped with AMPS column, the chromatograph shows the successful separation of the five basic tetracycline compounds. From this data it is shown that the endcapping procedure greatly enhanced the efficiency of the column in the separation of the basic tetracycline compounds by reducing the amount of residual groups and protecting the basic compounds from these groups, therefore providing better separation of basic compounds.

4. Separation of Aniline Compounds

a. Separation on C8, C8 Endcapped with AMPS and AMPS on Vydac Columns

To gather additional comparative data, chromatographic studies were conducted on the C8 column, the C8 endcapped with AMPS column and the AMPS on Vydac column using a group of aniline compounds. Anilines are basic compounds that can be used to test the efficiencies of hydrophobic stationary phases. Aniline, diphenylaniline and n,n-dimethylaniline were tested on the three columns using various mobile phase compositions. **Figure 16** shows the separation of the three aniline compounds on the C8 column using mobile phase compositions of 20/80 (v/v), 40/60 (v/v), 60/40 (v/v) and 80/20 (v/v) methanol/water, a flow rate of 0.5 mL/min and UV detection of 254 nm. **Figure 16** shows the separation of the aniline, diphenylaniline and n,n-dimethylaniline

C8 vs. C8 Endcapped with AMPS

Figure 15. Evaluation of Basic Organic compounds

Mobile Phase: 90/10 (v/v) acetonitrile/buffer pH=2 (sodium perchlorate), **Flow rate:** 0.5 mL/min. **UV detection:** 280 nm. **Samples:** 1 = oxytetracycline, 2 = minotetracycline, 3 = democycline, 4 = chlorotetracycline, 5 = doxycycline

first occurring at the 60/40 (v/v) MeOH/H₂O mobile phase composition for the C8 column. For the C8 column, the mobile phase composition of 40/60 (v/v) MeOH/H₂O shows the best separation of aniline, diphenylaniline and n,n-dimethylaniline.

Figure 17 shows the chromatographic separation of the three aniline compounds on the C8 endcapped with AMPS column. For this column, **Figure 17** shows the best separation of the three aniline at the mobile phase composition of 40/60 (v/v) methanol/water composition. **Figure 18** shows the chromatographic separation of aniline, diphenylaniline and n,n-dimethylaniline on the AMPS on Vydac column. In **Figure 18** the separation of the aniline compounds occurs at 60/40 (v/v) methanol/water mobile phase composition but with little improvement in separation as the water composition of the mobile phase increases. For the various mobile phase compositions, the separation of aniline, diphenylaniline and n,n-dimethyl aniline is about the same for the AMPS on Vydac column exhibiting typical ion-exchange column characteristics. For the C8 column and the C8 endcapped with AMPS column, **Figures 16 and 17** show that the separation of the aniline compounds improves as the water composition of the mobile phase increases. Both columns exhibit typical reverse phase column characteristics. The results from this study showed that aniline, diphenylaniline and n,n-dimethylaniline were successfully separated on the C8 column, the C8 endcapped with AMPS column and the AMPS on Vydac column.

5. Evaluation of the Log k'

In HPLC, there are several factors that can have an effect on the efficiency of the technique. One of those factors is the sample capacity factor (k') which as stated

Figure 16. Separation of Basic Aniline compounds.
Column: C8 on Vydac. **Flow rate:** 0.5 mL/min. **UV detection:** 254 nm.
Samples: 1 = aniline, 2 = diphenylaniline, 3 = N,N-dimethylaniline

Figure 17. Separation of Basic aniline compounds. Column: C8 endcapped with AMPS. Flow rate: 0.5 mL/min. UV detection: 254 nm. Samples: 1 = aniline, 2 = diphenylaniline, 3 = N,N-dimethylaniline

Figure 18. Separation of Basic Aniline Compounds. Column: AMPS on Vydac. Flow rate: 0.5 mL/min. UV detection: 254 nm. Samples: 1 = aniline, 2 = diphenylaniline, 3 = N,N-Dimethylaniline

earlier, can be obtained from the equation: $k' = (t_r - t_0)/t_0$, where t_r represents the retention time of a retained peak and t_0 represents the retention time of a reference peak. The capacity factor is widely used to describe the ability of a stationary phase to retain components. It is a measure of the time a solute spends in the stationary phase relative to the time it spends in the mobile phase. The capacity factor is a relative measurement and is normally linear with respect to the mobile phase composition. A $k' > 0$ can lead to good separation whereas a $k' = 0$ is for peaks that are not retained by the column [1,2,3].

Using a group of basic organic compounds and KNO_3 as the reference compound, the column capacity factors for the C8 column, the C8 endcapped with AMPS column and the AMPS on Vydac were determined. The compounds selected for the determination of the capacity factors were aniline, diphenylaniline, n,n-dimethylaniline, p-toluidine and pyridine. **Figure 19** shows the graph of the log of k' for these compounds at different percentages of a methanol/water mobile phase on the C8 column. For each compound in **Figure 19**, the graph shows a nonlinear relationship for the log k' vs. % methanol in water with the value of the k' increasing with an increase in the polarity of the % methanol in water mobile phase. **Figure 19** also shows a different orientation of the log of k' graph for each of the organic compounds. **Figure 20** shows the graph of the log of k' vs. % methanol in water for these compounds on C8 endcapped with AMPS column. The graph of the C8 endcapped with AMPS column also shows a nonlinear relationship of the log of k' vs. % methanol in water for each compound. The graph of the C8 endcapped with AMPS column shows a similar

orientation for the log of k' to that of the C8 column. The elution order of the organic compounds for both columns confirm that the interactions between the solutes and the mobile phase are mostly hydrophobic, a typical characteristic of reverse phase columns.

Figure 21 shows the graph of the log of k' vs. % methanol in water of aniline, diphenylaniline, n,n-dimethylaniline, p-toluidine and pyridine on the AMPS on Vydac column. The graph of the AMPS on Vydac column shows a more complex relationship of the log of k' vs. % methanol in water indicating a different mechanism of separation on this column. **Figure 21** of the AMPS on Vydac column also shows a different graph orientation for each organic compound compared to the C8 column and C8 endcapped with AMPS column, with the AMPS on Vydac column exhibiting typical ion-exchange column or normal phase column characteristics.

6. Evaluation of Peak Asymmetry Factor

Chromatographic studies were also conducted to evaluate the peak symmetry factor of the C8 column, the C8 endcapped with AMPS column and the AMPS on Vydac column. The presence of residual groups on a silica surface after modification can sometimes result in peak tailing effects for basic organic compounds. To evaluate the success of the endcapping procedure in enhancing the efficiency of the C8 stationary phase and to generate performance data, the peak asymmetry factors of the three columns were determined using aniline, diphenylaniline, n,n-dimethylaniline, p-toluidine and pyridine. When a sample is injected for HPLC analysis, the normal dispersion of component molecules through a given column is represented by a bell-shaped Gaussian peak [1,2,3]. Ideally, a chromatographic peak should be symmetrical

Figure 19. Log k' determination on C8 column

Figure 20. Log k' determination on C8 Endcapped with AMPS

Figure 21. Log of k' determination on AMPS on Vydac

but are sometimes asymmetrical due to a peak tailing effect. Peak tailing is usually the result of unwanted interactions of compounds with residual groups present on the silica surface causing longer retention of these compounds by the stationary phase. The peak asymmetry factor can be defined by the equation:

$$A_s = b/a,$$

where A_s is the peak asymmetry factor with the values of a and b determined at 1/2 peak height. For symmetrical peaks, $a=b$ and the asymmetry factor is one or close to one. The closer the asymmetry factor is to one the better the peak symmetry. An example for the determination of peak asymmetry is shown in **Figure 22** [3].

Figure 22. Determination of Peak asymmetry factor

$$A_s = b/a$$

A_s = peak asymmetry factor

For symmetrical peaks, $a = b$ and the asymmetry is one

Figure 23 shows plots of the peak symmetry factor vs. % methanol in water of aniline on the C8 column, the C8 endcapped with AMPS column and the AMPS on Vydac column. **Figure 24** shows the peak symmetry factor vs. % methanol in water of diphenylaniline on the three columns. The two graphs in **Figures 23 and 24** show a symmetry factor close to or equal to one for the C8 endcapped with AMPS follow by the C8 column and the AMPS on Vydac column with peak symmetry factors greater than 1. **Figures 25, 26, and 27** show the graphs of the peak symmetry factor vs. %

Figure 23. Chromatographic Determination Asymmetry Factors of Aniline

Figure 24. Chromatographic Determination of Asymmetry Factors of Diphenylaniline

Figure 25. Chromatographic Determination of Asymmetry Factor of Pyridine

Figure 26. Chromatographic Determination of Asymmetry Factor of P-toluidine

Figure 27. Chromatographic Determination of Asymmetry Factor of N, N-dimethylaniline

methanol in water for pyridine, p-toluidine and n,n-dimethylaniline respectively on the C8 column, C8 endcapped with AMPS column and the AMPS on Vydac column. The three graphs in **Figures 25, 26 and 27** also show symmetry factors close to or equal to one for the C8 endcapped with AMPS column whereas the C8 and the AMPS on Vydac columns show peak symmetry factors greater than one. The results of this study confirmed that the column efficiency of the C8 column was greatly enhanced by endcapping with AMPS.

CHAPTER IV. CONCLUSIONS

For this research project, two different bonded phases for High Pressure Liquid Chromatography were successfully synthesized via TES silanization followed by a hydrosilation reaction. After synthesis, the phases were characterized by Diffuse Reflectance Infrared Fourier Transform spectroscopy, Cross-polarization Magic Angle Spinning NMR and Elemental Analysis. Spectroscopic characterization studies confirmed that the two stationary phases, C8 endcapped with AMPS and AMPS on Vydac silica were successfully created.

Chromatographic studies were conducted using the Perkin Elmer Universal Test Mix, a Supelco Reverse Phase Test Mix and several basic organic compounds to evaluate the performance of the two new bonded phases and a previously synthesized C8 bonded phase. Results of the study showed a successful separation of the Perkin Elmer Universal Test Mix on the C8 and C8 endcapped with AMPS stationary phases. Results of the study also showed a successful separation of the Supelco Reverse Phase Test Mix on the C8 and C8 endcapped with AMPS but with better efficiency on the C8

endcapped with AMPS stationary phase. The log of k' and asymmetry factors were evaluated on the C8, C8 endcapped with AMPS and the AMPS on Vydac stationary phases and results showed a better performance for the C8 endcapped with AMPS column. Based on the results of this study, it can be concluded that the endcapping of the C8 stationary phase with AMPS was very successful and that the column efficiency was enhanced by the endcapping procedure.

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