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SYNTHESIS AND CHARACTERIZATION OF AMINO-BASED COLUMNS FOR
HPLC

A Thesis

Submitted to

The Faculty of Chemistry Department

San Jose State University

In Partial Fulfillment

of the Requirements for the Degree

Master of Science

By

Radha Suryadevara

December 2004

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ABSTRACT

SYNTHESIS AND CHARACTERIZATION OF AMINO-BASED COLUMNS FOR HPLC

by Radha Suryadevara

Synthesis and characterization of amino-based stationary phases for high performance liquid chromatography are described. The amino-based stationary phases for HPLC have been synthesized following a silanization/hydrosilation method. Three organic compounds, N-(3-butenyl) phthalimide, 4-diethylamino-2-buten-1-ol, and 3-amino-3-methyl-1-butyne were chosen as bonding moieties for the hydrosilation reaction. The synthesized bonded phases were characterized by cross polarization magic angle spinning nuclear magnetic resonance (CP MAS-NMR), diffuse reflectance infrared fourier transform (DRIFT) spectroscopy, and elemental analysis. Chromatographic evaluation of the bare silica, silica hydride, endcapped silica hydride, N-(3-butenyl) phthalimide bonded phase, amino phase, and 3-amino-3-methyl-1-butyne bonded phases was done by analysis of sugars.

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1. Introduction

Chromatography is a technique in which the components of a sample mixture are separated based on their distribution between a mobile phase and a stationary phase.

Chromatographic methods have been classified based on the nature of the mobile phase and stationary phase, e.g., gas chromatography and liquid chromatography. In the gas chromatography, the mobile phase is a gas, in liquid chromatography, the mobile phase is a liquid. In the 1970's, gas chromatography developed as a popular technique. Liquid chromatography in the paper and thin layer formats were most commonly used at that time. However, these liquid chromatographic methods had limited success because of the low mobile phase flow rates used leading to poor efficiencies and long analysis time. This led to the development of high pressure or high performance liquid chromatography, where a liquid mobile phase is forced through a solid stationary phase under pressure. HPLC is becoming the most popular chromatographic technique because of its sensitivity, reproducibility, adaptability to accurate quantitative determinations, its ability to separate nonvolatile species, and above all its applicability to wide range of substances such as pharmaceuticals, food, and biochemicals.

A typical HPLC system is shown in Figure 1. It consists of a solvent reservoir, pump, injector, analytical column, detector, recorder and waste reservoir. An HPLC system begins with the solvent reservoir, which contains the solvent used to carry the sample through the system. The solvent is filtered with an inlet solvent filter to remove any particles that could damage the system. The solvent is propelled through the system

by the pump. The next component is the sample injector, where the sample is introduced through an injection valve and often a precolumn filter is used to prevent valve and column damage due to particulate matter in the sample. Following the injector, there is a guard column prior to the analytical column to remove the particulate matter and contaminants from the solvents and the analytes that bind irreversibly to the stationary phase. Once the solvent enters the analytical column, sample components are separated based on the distribution between the mobile phase and stationary phase. The separated components pass through a detector flow cell and the electrical response from the detector is sent to a recorder, which helps to analyze and interpret the data. Finally, a backpressure regulator is often installed immediately after the detector, preventing solvent bubble formation in the flow cell, which may interfere with the detection of sample component.

HPLC is classified into two types based on the relative polarities of mobile phase and stationary phases: normal phase and reversed phase. In normal phase, highly polar stationary phases such as triethylene glycol, cyano, diol, and amino groups chemically bonded to silica and relatively non polar solvents such as hexane or isopropyl ether are used.

Reversed phase is the most popular mode of HPLC, which is usually performed on a variety of alkyl bonded silica based stationary phases with polar mobile phases like water, acetonitrile, and methanol. Alkyl groups such as butyl(C₄), octyl(C₈), and octadecyl(C₁₈) phases are commonly used for separations because of their excellent selectivity towards non polar solutes such as polycyclic aromatic hydrocarbons (PAH's)

and many polar compounds like proteins, peptides, etc. (1,2). However, mobile phases with little or no organic modifier are needed to achieve separation of many polar compounds. These aqueous mobile phases can result in collapse of octyl and octadecyl columns used for RP-HPLC, which in turn can lead to poor selectivity, retention and reproducibility of polar analytes. So modified stationary phases with an alkyl chain and a polar group such as amino or amide group have been used to overcome this problem. The increased polarity of the stationary phase close to the silica surface attributes more polar nature to the stationary phase, which prevents the collapse of alkyl chains under aqueous conditions. This is an alternative mode of chromatography, called aqueous normal phase, where a polar stationary phase with an aqueous organic mobile phases are used. This mode is similar to normal phase as a highly polar component is retained longer than non-polar component. This method has been particularly useful for polar compounds such as sugars (3).

Silica is the most widely used packing material in HPLC columns. Porous silica used in chromatography is an amorphous, noncrystalline material. Silica is the most widely used material in the synthesis of stationary phases because of its high surface area, high efficiency, good mechanical strength, modest cost and ability to withstand high pressures. In addition, silica is available in wide variety of pore sizes and shapes, and functional groups also can be easily bonded to silica surface. The high internal surface area of silica allows good analyte stationary phase interactions.

The silica matrix consists of a core of silicon atoms joined together with oxygen atoms by siloxane (Si-O-Si) bonds and residual silanol groups as shown in Figure 2.

Figure 1. Schematic of HPLC setup.

Figure 2. Structure of silica.

These silanol (Si-OH) bonds not only assign silica its hydrophilic properties, but react with silane reagents to form bonded phases. Silanol groups of silica exist on the surface in single (isolated), geminal or vicinal forms (bonded). Silanols are hydrophilic and siloxanes are hydrophobic.

Silica support is superior to other supports in terms of efficiency, rigidity, and performance. However, there are several limitations with silica based materials: limited pH stability and peak tailing of basic compounds. Silica can be used in the limited pH range of 2-8. Below pH 2, the bonded phase can undergo hydrolysis. At pH > 9.0 the silica will dissolve forming silicates. Ionic impurities such as metal ions affect the solubility and stability of the bonded phases. Further, the acidic silanols of silica may cause severe peak tailing or irreversible adsorption of basic compounds if they are not removed or blocked (4,1).

Currently, the most commonly used reaction for the modification of silica surfaces is organosilanization, where usually a dimethylalkyl silane ($X-SiR^1_2R$) is used as an organosilanization reagent as shown in reaction (1), where X is a halide, alkoxy etc., R^1 is a methyl group, and R is a second organic group which provides hydrophobic or hydrophilic properties to the modified surface. The attachment between the surface and the bonded organic moiety R is a silicon-oxygen-silicon carbon (Si-O-Si-C) linkage, which is both hydrodynamically and hydrolytically stable. This type of modified surface is referred to as a monomeric phase due to the single point of attachment between the organic group and the surface.

In organosilanization reactions, trifunctional silanes X_3SiR are used to prepare polymeric bonded phases as shown in reaction 2. The reagent will not only react with the surface but also with adjacent silane groups, thus producing the polymeric phase. But if all the adjacent bonded groups cannot cross link, some of the oxygens will have hydrogen instead of silicon atom. Thus, varying amounts of silanol groups remain on the surface depending on the reaction conditions and type of silica. Many pharmaceutical compounds are basic in nature, a fact that leads to low efficiency and asymmetric peaks in RP-HPLC separations. This problem is due to presence of residual silanol groups on the surface of silica based RP materials which often interact with the basic solutes.

(Y = Si or H)

The other approaches for the synthesis of stationary phases are shown in 3a and 3b. These two reactions produce a monomeric bonded phase. The first step of both the reactions is the conversion of silanols to halides with a thionyl chloride reagent. In the second step, this intermediate is reacted with a Grignard reagent or organolithium

reagent so that the desired ligand is bonded to the surface as shown in reaction 3a and 3b respectively. The linkage between the bonded moiety and the surface is a silicon-carbon (Si-C) bond. Though the Si-C bond is more hydrolytically stable than the Si-O-Si-C linkage, there are several drawbacks that limit the commercial use of this method. The first drawback is that the chlorinated intermediate formed is hydrolytically unstable and therefore this method has to be performed in a dry environment. The second drawback is that metal salt by-products formed in the organometallic reaction may easily contaminate the surface.

A new approach has been developed by Pesek et al. for the preparation of bonded phases, which involves two steps. The first step is silanization where a silanol group (Si-OH) is converted to silica hydride (Si-H) in the presence of triethoxysilane and all the OH groups are replaced by hydrides under controlled reaction conditions of temperature, concentration, amount of water, acid and reaction time (4a). The hydride intermediate formed in this step is stable both in air and water in contrast to the chlorinated intermediate formed in method 3.

Y = Si or H depending on the extent of cross linking

The second step is a hydrosilation reaction (4b) where an organic moiety is attached to the hydride surface. A terminal olefin is the most commonly bonded group to the silica hydride. However, other types of unsaturated compounds such as alkynes, nitriles and cyanos have also been used.

Hydrosilation

Catalyst = Hexachloroplatinic acid or free radical initiator

The most common catalysts for the hydrosilation reaction are organic and inorganic complexes of transition metals such as platinum, rhodium, palladium, ruthenium, and nickel. A solution of hexachloroplatinic acid in 2-propanol discovered in 1957 by John L. Speier referred to as Speier's catalyst is the most commonly used platinum catalyst. The main advantage of the hydrosilation method over

organosilanization is that the lesser number of silanol groups remain on the surface due to conversion of most of the silanol groups to hydrides during the silanization step (5).

The major goal of the present research is to prepare amino-based stationary phases via a hydrosilation of alkynes on silica hydride surface as developed by Pesek et al. It was found that the bonding of alkynes on silica hydride in a hydrosilation reaction can result in a product with five possible structures. The hydrosilation of alkynes and the five possible structures are shown in Figure 3. Based on the carbon chemical shift values of the resulting bonded phase, the structure of the product can be determined (6). In the current research, three organic compounds, N-(3-butyne) phthalimide, 3-amino-3-methyl-1-butyne and 4-diethylamino-2-butyne-1-ol each containing an alkyne group and an amino group, have been chosen as bonded moieties. The structures of the organic moieties chosen for the hydrosilation reaction are shown in Figure 4. Synthesis and characterization of the resulting bonded phases of these organic moieties are the major goals of this work. Another goal is to prepare amino phases from N-(3-butyne) phthalimide bonded phase and 4-diethylamino-2-butyne-1-ol bonded phase by cleavage of the phthalimide ring of N-(3-butyne) phthalimide and N-dealkylation of 4-diethylamino-2-butyne-1-ol respectively as shown in Figure 5. Characterization by FTIR and NMR is an important aspect of this work. The final goal is the chromatographic evaluation of bare silica, silica hydride, endcapped hydride and the amino-based stationary phases especially in the analysis of sugars.

Hydrosilation

Figure 3. Possible structures of bonding of alkynes to silica hydride surface.

N-(3-butynyl) phthalimide

3-amino-3-methyl-1-butyne

4-diethylamino-2-butyne-1-ol

Figure 4. Structures of organic moieties used in the hydrosilation reaction.

Cleavage of phthalimide

N-(3-butynyl) phthalimide bonded phase

Amino phase

N-dealkylation

4-diethylamino-2-butyn-1-ol bonded phase

Amino phase

Figure 5. Synthetic route of amino phases from N-(3-butynyl) phthalimide bonded phase and 4-diethylamino-2-butyn-1-ol bonded phase.

2. Experimental

2.1. *Materials*

The organic compounds that are used in the hydrosilation reaction N-(3-butynyl) phthalimide, 3-amino-3-methyl-1-butyne were purchased from GFS chemicals (Columbus, OH) and 4-diethylamino-2-butyne-1-ol was from Aldrich Chemical Co.(Milwaukee, WI). Kromasil silica (Lot # AT0112), which had a 5 μm average particle diameter and specific surface area of 340 m^2/g was purchased from Eka Nobel AB (Bohus, Sweden). Two catalysts, tertiary butyl peroxide and hexachloroplatinic acid and silanizing agent, triethoxy silane were supplied by Aldrich Chemical Co. (Milwaukee, WI). All the solvents dioxane, toluene, and methylene chloride used in syntheses of bonded phases were obtained from Fischer Chemical Company (Fair lawn, NJ). Hydrazine hydrate, sodium chloride and diethyl ether used in the Gabriel synthesis was obtained from Aldrich Chemical Co.(Milwaukee, WI). All of the deuterated solvents used in the NMR analysis of samples d-chloroform, d-methanol and d-benzene were purchased from Cambridge isotopic laboratories (Andover, MA). De-ionized water was obtained from a Milli-QTM purification system (Millipore Corp., Bedford, MA). Sugar samples, glucose, fructose, xylose and melezitose, were supplied by Sigma Chemical Company (St.Louis, MO) and sucrose, maltose, lactose and raffinose were obtained from Fisher Chemical Company (Fairlawn, NJ). The HPLC grade solvents methanol and acetonitrile used in the analysis of sugars were obtained from Fisher Chemical Company (Fair lawn, NJ). Astrosil silica, Astrosil hydride, and endcapped Astrosil hydride used in sugar analysis were obtained from Microsolv Company (Long Branch, NJ).

2.2. *Instrumentation*

2.2.1. Elemental analysis

Elemental analysis was carried out by Desert Analytics (Tucson, AZ). The carbon percentage of the synthesized bonded phases was performed using the combustion method. The carbon percentage was used to calculate surface coverage of the bonded phases, which provides quantitative information about the amount of organic moiety bonded on the silica surface. The relationship between carbon percentage determined by elemental analysis and surface coverage is given by the Brendsen and DeGalan equation (7).

2.2.2. Diffuse reflectance infra red Fourier transform spectroscopy

Diffuse reflectance infrared Fourier transformation (DRIFT) spectra were obtained on a ATI Mattson Infinity Series FTIRTM spectrometer equipped with a deuterated triglycine sulfate (DTGS) detector, using a diffuse reflectance accessory (Sample cup of 3 mm diameter and 2 mm depth). Both the sample and KBr were dried for several hours in a vacuum oven at 110°C and the DRIFT sample was prepared by adding 5mg of KBr to 100 mg of sample. This sample mixture was finely ground with a mortar and pestle and transferred into the sample cup. Finally the sample was pressed with a slide in order to obtain a smooth surface and the sample compartment was purged with nitrogen for 15 min to remove CO₂ and water. Spectra were recorded in the range of 4000-450 cm⁻¹ with a resolution of 4 cm⁻¹ and averaged over 100 scans.

2.2.3. Nuclear magnetic resonance spectroscopy

Solid state NMR was performed with a combination of cross polarization (CP) and magic angle spinning (MAS). All ^{13}C CP-MAS NMR spectra were recorded on a Varian NMR Inova 400 spectrometer at a spinning rate of 5000 Hz. Solid samples of 100 mg to 150 mg were packed into double bearing ZrO_2 rotors. The spectra were obtained with a cross polarization contact time of 5 ms, a pulse interval of 5 sec and a pulse width of 6.5 μs . Chemical shifts were referenced to tetramethylsilane using external glycine as the standard for ^{13}C spectra acquisition. Solution state NMR spectra of three organic moieties, N-(3-butynyl) phthalimide, 3-amino-3-methyl-1-butyne and 4-diethylamino-2-butyne-1-ol used in hydrosilation reaction were obtained by dissolving compounds in d -chloroform.

2.2.4. Chromatography

Analytical liquid chromatography was performed with the use of equipment composed of a SP8800 ternary HPLC pump (SP ThermoSeparation products, San Jose, CA), Chromjet integrator, laser light scattering detector, and Rheodyne injector (Cotati, CA). The N-(3-butynyl) phthalimide bonded phase made with free radical catalyst was packed into 10 cm $\times$ 4.6 mm stainless steel column (Alltech Co., Deerfield, IL) and other bonded phases, N-(3-butynyl) phthalimide bonded phase made with platinum catalyst, amino phase and 3-amino-3-methyl-1-butyne bonded phase were slurry packed into columns of dimensions 150 mm $\times$ 4.6 mm (Alltech Co., Deerfield, IL) at 6000 psi

pressure using methanol as the driving solvent using a Haskel pneumatic pump (Burbank, CA, US).

2.3. Procedures

2.3.1. Silanization

First, Kromasil silica was dried in vacuum oven at 120°C. The dried Kromasil silica (10g) was placed in a 500 mL three necked round bottomed flask equipped with a condensor, thermometer, addition funnel, heating mantle and a magnetic stirrer. Then 200 mL of dioxane and 4.86 mL of 2.3 M HCl was added to silica and the reaction mixture was heated to 70°C. Then dioxane (60.1 mL) was added to the addition funnel and was flushed with argon and then 13.9 mL of triethoxysilane was added into the addition funnel in such a way that the TES solution doesn't go directly into the round bottomed flask. At 70°C, the prepared TES solution in the addition funnel was added drop wise to the reaction mixture over a period of 1 hr. Then the temperature was raised to 90°C and reflux was continued for another 90 min. After a cooling period, the reaction mixture was centrifuged (at 1500 rpm for 10 min), washed three times with each of dioxane, toluene and diethyl ether. The final product was dried at room temperature overnight to evaporate ether and finally dried in a vacuum oven at 110°C for 24 hours (8).

2.3.2. Bonded phases synthesis

2.3.2.1. N-(3-butynyl) phthalimide bonded phase

2.3.2.1.1. *Free radical hydrosilation*

In a 250 mL three necked round bottomed flask, 4.2 g of N-(3-butynyl) phthalimide, 59.14 mL of toluene and 168 μ L of t-butyl peroxide were added. The mixture was heated at a constant temperature of 60 – 70°C for about an hour. While stirring, 4.2 g of Kromasil hydride was added slowly through the open neck of the flask. Then the flask was flushed with nitrogen and all the joints were properly sealed. The temperature of the reaction mixture was then raised to $100 \pm 2^\circ\text{C}$ and the reaction was continued for about 100 h. The final product was cooled and centrifuged. The supernatant was decanted. Finally, washing with toluene followed by methylene chloride and diethyl ether was done as described in the Kromasil hydride synthesis. The solvent in the modified silica was allowed to evaporate at room temperature overnight and dried in vacuum oven at 110°C for 24 h (9).

2.3.2.1.2. *Platinum hydrosilation*

A mixture of 2.2 g of N-(3-butynyl) phthalimide, 30.8 mL of toluene and 0.66 mL of 10 mM hexachloroplatinic acid in 2-propanol was heated at a temperature of $70 \pm 2^\circ\text{C}$ for 1 h with magnetic stirring. After 1 h heating, a clear solution was obtained. Then 1.65 g of Kromasil hydride was added slowly to the solution while stirring. The temperature of the reaction mixture was then raised to $100 \pm 2^\circ\text{C}$ and the reaction was continued for another 96 h. After cooling, the final product was centrifuged and washed with four 30 mL portions each of toluene, followed by methylene chloride and diethyl

ether. Then the silica product was dried overnight and then in a vacuum oven at 110 °C for 24 h.

2.3.2.2. Cleavage of phthalimide

2.3.2.2.1. *Cleavage of N-(3-butynyl) phthalimide to 4-amino-1-butyne*

A solution of 100 mg of N-(3-butynyl) phthalimide (0.5 mmol) in 1 mL of ethanol was transferred into a beaker. Hydrazine hydrate (24 µL) was added to the solution and warmed. Then the precipitate obtained was decomposed by warming with an excess of 0.1 M hydrochloric acid until litmus paper changed color. The insoluble phthalyl hydrazide was filtered using a syringe filter and washed with water. The filtrate was transferred into a beaker and concentrated to remove ethanol by heating. After cooling, the concentrated filtrate was made alkaline with 0.1 M sodium hydroxide and extracted with ether in a separatory funnel. The resulting solution was dried and the residue was analyzed by solution state NMR (10).

2.3.2.2.2. *Cleavage of N-(3-butynyl) phthalimide to 4-amino-1-butyne (II)*

N-(3-butynyl) phthalimide (200 mg) was placed in a 50 mL round bottomed flask. Hydrazine hydrate (48 µL) in 20 mL of ethanol was added to the phthalimide and the solution was allowed to reflux at room temperature for 48 h. After refluxing, 0.1 M

hydrochloric acid was added and heated until a white precipitate of phthalyl hydrazide was seen. Then the insoluble phthalyl hydrazide was filtered using a syringe filter and washed with water. The filtrate was concentrated to remove ethanol and the cooled solution was made alkaline with 0.1 M sodium hydroxide. Half of the solution obtained was extracted with ether and half with sodium chloride. Extraction was tried with ether and sodium chloride solution separately to check the isolation of product in both the extraction methods. So, half of the solution obtained in the cleavage reaction is extracted with ether and half with sodium chloride solution (sodium chloride in ether). The resulting ether layers from both the extractions were dried and the residues were analyzed by solution state NMR (11).

2.3.2.3. Amino Phases

2.3.2.3.1. Amino phase via N-(3-butynyl) phthalimide bonded phase prepared by free radical hydrosilation

N-(3-butynyl) phthalimide bonded phase (2 g) prepared by free radical hydrosilation was placed in a 50 mL round bottomed flask. Hydrazine hydrate (48 μ L) in 20 mL of ethanol was added to the bonded phase and the slurry was allowed to reflux at room temperature for 48 h. After refluxing, 0.1 M hydrochloric acid was added and heated until a white precipitate of phthalyl hydrazide was seen. Then the solution containing modified silica and phthalyl hydrazide was transferred to a filter funnel and the insoluble phthalyl hydrazide was filtered by washing with 0.1 M sodium hydroxide,

followed by water and ether. Then the silica residue was dried and analyzed by solid state NMR.

2.3.2.3.2. *Amino phase via N-(3-butynyl) phthalimide bonded phase prepared by platinum hydrosilation*

N-(3-butynyl) phthalimide bonded phase (2.1 g) prepared by platinum hydrosilation was placed in a 50 mL round bottomed flask. Hydrazine hydrate (48 μ L) in 20 mL of ethanol was added to the bonded phase and the slurry was allowed to reflux at room temperature for 48 h. After refluxing, 0.1 M hydrochloric acid was added and heated until a white precipitate of phthalyl hydrazide was seen. Then the solution containing modified silica and phthalyl hydrazide was transferred to a filter funnel and the insoluble phthalyl hydrazide was filtered by washing with 0.1 M sodium hydroxide, followed by water and ether. Then the silica residue was dried and analyzed by solid state NMR.

2.3.2.4. 3-amino-3-methyl-1-butyne bonded phase

Bonded phases containing 3-amino-3-methyl-1-butyne were prepared according to the same procedures used in the synthesis of the N-(3-butynyl) phthalimide bonded phase. In free radical bonded phase synthesis, 2.16 mL of 3-amino-3-methyl-1-butyne, 67.6 mL of toluene, 136 μ L of t-butyl peroxide and 2.4 g of Kromasil hydride were used. In the synthesis of the platinum catalyzed bonded phase, 1.44 mL of 3-amino-3-methyl-1-

butyne, 32 mL of toluene, 0.638 mL of 10 mM hexachloroplatinic acid in 2-propanol and 2.4 g of Kromasil hydride were used.

2.3.2.5. 4-diethylamino-2-butyn-1-ol bonded phase

Bonded phases containing 4-diethylamino-2-butyn-1-ol were prepared according to the same procedures used in the synthesis of N-(3-butynyl) phthalimide bonded phase. In the free radical bonded phase synthesis, 0.75 mL of 4-diethylamino-2-butyn-1-ol, 10 mL of toluene, 40 μ L of t-butyl peroxide, and 0.5 g of Kromasil hydride were used. In the case of the platinum catalyzed bonded phase, 0.75 mL of 4-diethylamino-2-butyn-1-ol, 10 mL of toluene, 0.2 mL of 10 mM hexachloroplatinic acid in 2-propanol and 0.75 g of Kromasil hydride were used.

2.3.3. Separation procedure

Separation of sugars was performed in the aqueous normal phase HPLC with varying mobile phase compositions of water and acetonitrile. The chromophores of sugars are too weak to be detected by UV. Sugars must be derivatized with a chromophore to be detected by UV. The laser light scattering detector can detect samples like sugars containing weak chromophores without derivatization. A schematic diagram of the laser light scattering detector used in this study is shown in Figure 6. The detection mechanism consists of three stages: nebulization, evaporation and detection. First, the nebulizer transforms the liquid mobile phase coming from HPLC column into tiny

Figure 6. Schematic of laser light scattering detector.

droplets by CO₂ gas. Then these tiny droplets are carried by the CO₂ carrier gas into the drift tube which is pre heated to a temperature of 63°C. In this heated zone, the mobile phase evaporates leaving the dried analyte particles, which is called the evaporation stage. Finally, the dried analyte particles coming from the drift tube pass through an incident laser light beam and the light scattered from the solid particles is measured by a photomultiplier. The signal from the photomultiplier is sent to the recorder.

The laser light scattering detector drift tube temperature was set at 63°C and the detection cell temperature at 60°C. The pressure of the driving CO₂ gas was set at 1.25 kg/cm². The HPLC pump was set at a maximum pressure of 2000 psi. The flow used during the experiments was 0.35 mL/min. The integrator attenuation and chart speed were set at 64 and 0.25 respectively. Samples were prepared by dissolving solid sugars in 10:90 methanol:water. Sugars at concentrations of 15 mg/ml were analyzed on all amino based columns with water:acetonitrile (10:90 and 15:85) mobile phases. All the sugar samples were filtered using a 20 µm nylon membrane before injection. Sugar samples were injected with a Rheodyne injector equipped with a 20 µL loop. The structures of all sugars used in this study are shown in Figure 7.

Monosaccharides

Xylose (pentose)

Glucose (hexose)

Fructose (hexose)

Disaccharides

Maltose (2 glucose)

Lactose (glucose+galactose)

Sucrose (glucose+fructose)

Trisaccharides

Melezitose (2glucose+fructose)

Raffinose (glucose+fructose+galactose)

Figure 7. Structures of sugars.

3. Results and Discussion

3.1. *Elemental analysis of bonded phases*

The concentration of surface bonded groups, or surface coverage, α_R was calculated from the carbon content of the bonded material and the BET specific surface area of the native silica before bonding according to Brendsen and Degalan equation shown below

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

Where p_c is the carbon percentage of the bonded material (by weight after correction from any carbon present before bonding), M_c is the atomic weight of carbon, n_c is the number of carbon atoms in the bonded organic group, M_R is the molecular weight of the attached group, and S_{BET} is the specific surface area of the native substrates (7).

The percentage carbon and the surface coverage values for the different bonded silica based packings in this study are shown in Table 1.

3.2. *Spectroscopic characterization*

3.2.1. Kromasil hydride

Kromasil hydride was prepared according to the silanization procedure developed by Pesek et al, in presence of triethoxy silane and dioxane in refluxing toluene, where Kromasil silica is converted to Kromasil hydride (8). Figure 8 shows the DRIFT spectrum of Kromasil hydride, which has a strong peak around 2257 cm^{-1} corresponding

to the Si-H stretching vibration confirming the formation of Kromasil hydride. The broad peak between 3800 and 3000 cm^{-1} is due to adsorbed water and hydrogen bonded silanols. The peak at 3750 cm^{-1} is due to silanols that did not react with triethoxysilane in the silanization step. The remaining peaks are due to various fundamental vibrations of the silica matrix (4,5).

3.2.2. N-(3-butynyl) phthalimide bonded phase

First, N-(3-butynyl) phthalimide was analyzed by solution state NMR so that the proton and carbon NMR chemical shifts of N-(3-butynyl) phthalimide can be compared to the compound present on the modified silica surface to confirm the bonding of phthalimide moiety. The ^1H NMR spectrum of N-(3-butynyl) phthalimide is shown in Figure 9. The peak assignments and chemical shifts of N-(3-butynyl) phthalimide are summarized in Table 2. The peak at 1.9 ppm is due to proton attached to carbon 'd'. The peaks at 2.62 and 3.88 ppm are due to protons attached to carbons 'b' and 'a' respectively. The peak at 7.86 ppm is due to two equivalent protons attached to carbons 1 and 4. The peak at 7.72 ppm can be assigned to two equivalent protons attached to carbons 2 and 3.

The ^{13}C NMR spectrum of N-(3-butynyl) phthalimide is shown in Figure 10. The peak assignments and chemical shifts of N-(3-butynyl) phthalimide are shown in Table 2. The peak at 70.4 ppm is due to acetylene carbon 'd'. The peaks at 38.8, 18.5 and 81 ppm can be assigned to carbons 'a', 'b' and 'c' respectively. The peak at 168.5 ppm is due to two carbonyl carbons 6 and 8. The peaks at 123.5 and 132 ppm are due to aromatic

Table 1. Surface bonding properties of bonded phases.

Compound name	Type of catalyst	% Carbon	Surface coverage ($\mu\text{mole/m}^2$)
N-(3-butynyl) phthalimide	Free radical	3.72	0.69
N-(3-butynyl) phthalimide	Platinum	9.65	2.14
Amino phase	Free radical	0.47	0.29
Amino phase	Platinum	2.44	1.22
3-amino-3-methyl- 1-butyne	Free radical	8.99	1.81
3-amino-3-methyl- 1-butyne	Platinum	6.12	0.72
4-diethylamino-2- butyn-1-ol	Free radical	4.01	2.97
4-diethylamino-2- butyn-1-ol	Platinum	1.94	1.87

Figure 8. DRIFT spectrum of Kromasil hydride.

Figure 9. Proton NMR spectrum of N-(3-butynyl) phthalimide.

Figure 10. Carbon NMR spectrum of N-(3-butenyl) phthalimide.

Table 2. Proton and carbon NMR chemical shifts of N-(3-butynyl) phthalimide.

N-(3-butynyl) phthalimide

¹ H NMR		¹³ C NMR	
Proton	ppm	Carbon	ppm
a (2H)	3.88	a (1C)	38.8
b (2H)	2.62	b (1C)	18.5
d (1H)	1.90	c (1C)	81.0
1,4 (2H)	7.86	d (1C)	70.4
2,3 (2H)	7.72	1,4 (2C)	123.5
		2,3 (2C)	134.2
		5,9 (2C)	132.0
		6,8 (2C)	168.5

carbons 1,4 and 5,9 respectively. The peak at 134.2 ppm can be assigned to aromatic carbons 2 and 3 (12,13).

The N-(3-butynyl) phthalimide bonded phase was prepared by the hydrosilation reaction using both free radical catalyst i.e. tertiary butyl peroxide and hexachloroplatinic acid (9). DRIFT spectra of the N-(3-butynyl) phthalimide bonded phase prepared using free radical catalyst and platinum catalyst are shown in Figures 11A and 11B respectively. The hydrosilation product, N-(3-butynyl) phthalimide bonded phase prepared using free radical catalyst (11A) showed a strong Si-H stretching band at 2260 cm^{-1} , but reduced in intensity when compared to the Si-H peak in Kromasil hydride. This decrease in intensity indicated bonding of the phthalimide moiety to the silica hydride. In addition, there are peaks at 2952 cm^{-1} and 3036 cm^{-1} which are due to aliphatic and aromatic C-H stretching vibrations of the bonded moiety. The peaks at 1710 , 1350 and 808 cm^{-1} are due to carbonyl stretching, aliphatic C-H bending and aromatic C-H out of plane bending vibrations of the bonded moiety respectively. These results indicate that N-(3-butynyl) phthalimide is bonded to Kromasil hydride.

The N-(3-butynyl) phthalimide bonded phase prepared using platinum catalyst (Fig 11B) showed a Si-H peak at 2248 cm^{-1} , which is reduced in intensity when compared to that of Kromasil hydride indicating bonding of the phthalimide moiety to the Kromasil hydride. In addition, the peaks at 2931 and 3065 cm^{-1} are due to aliphatic and aromatic C-H stretching vibrations of N-(3-butynyl) phthalimide. The peaks at 1714 , 1398 , and 795 cm^{-1} correspond to carbonyl stretching, aliphatic C-H bending and aromatic C-H out of plane bending vibrations of the phthalimide moiety respectively.

The DRIFT spectra in Figure 11 indicate that N-(3-butynyl) phthalimide has been successfully bonded to Kromasil hydride with both a free radical catalyst and a platinum catalyst, since the respective spectra clearly showed IR bands for the aliphatic and aromatic groups of the bonded moiety.

¹³C CP-MAS NMR spectra of the N-(3-butynyl) phthalimide bonded phase prepared using free radical catalyst (plot A) and platinum catalyst (plot B) are shown in Figure 12. These spectra indicated that the manner in which the phthalimide reacted with the free radical catalyst and the platinum catalyst is quite different. In the spectrum (Figure 12A) of the N-(3-butynyl) phthalimide moiety bonded to silica using a free radical catalyst, the peaks between 60 and 90 ppm correspond to the aliphatic carbons of N-(3-butynyl) phthalimide, and the peaks between 120 and 190 ppm correspond to the aromatic ring of the bonding moiety. Fewer peaks are observed in the aliphatic region, which may be due to polymerization in the free radical hydrosilation reaction.

In the spectrum of the N-(3-butynyl) phthalimide bonded phase prepared using a platinum catalyst (Figure 12B), the peaks between 20 and 80 ppm can be assigned to the aliphatic chain, and the peaks between 120 and 160 ppm correspond to the aromatic carbons of the bonding moiety. In addition, two peaks at 169 and 182 ppm correspond to carbonyl carbons of the phthalimide ring indicating that more than one product is formed in this reaction. This mixture of products formed in this reaction might be due to different attachment geometries as shown in Figure 3. These solid state NMR spectra further confirmed the bonding of the phthalimide moiety to Kromasil hydride with both free radical catalyst and platinum catalyst (12,13).

Figure 11. DRIFT spectra of N-(3-butynyl) phthalimide on Kromasil hydride prepared using (A) Free radical catalyst (B) Platinum catalyst.

Figure 12. ^{13}C CP-MAS NMR spectra of N-(3-butynyl) phthalimide on Kromasil hydride prepared using (A) Free radical catalyst (B) Platinum catalyst.

3.2.3. Amino phase

Before the synthesis of the amino phase, the synthesis of amine from N-(3-butynyl) phthalimide was done following a cleavage procedure so that if this reaction is successful, it can be used for the conversion of the N-(3-butynyl) phthalimide bonded phase to the amino phase. The reaction scheme for cleavage of phthalimide is shown in Figure 13. The product obtained after extraction with ether was analyzed by solution state NMR.

Figure 14 shows the ^1H NMR spectrum of the amine product obtained by dissolving 1mg of amine in 0.5 mL of d-benzene. The expected product in this reaction is 4-amino-1-butyne. Three strong peaks at 1.66, 1.8 and 6.7 ppm are observed. The peaks at 1.66 and 1.8 ppm can be assigned to protons of the butynyl moiety of N-(3-butynyl) phthalimide. The peak at 6.7 ppm corresponds to the proton of the aromatic ring. This peak at 6.7 ppm indicated that there is still an aromatic group in the product and phthalimide is not cleaved completely. Figure 15 shows the ^{13}C NMR spectrum of the amine product, which has only one peak at 127 ppm corresponding to solvent deuterated benzene. No other peaks were seen. This carbon NMR spectrum did not give any information, which is probably because the concentration of the sample was too low. But, proton NMR spectrum showed that the product obtained in the cleavage of phthalimide reaction is not the expected amine as shown in Figure 14.

Then synthesis of amine was done following another procedure for the cleavage of phthalimide (II). The reaction scheme is shown in Figure 16. In this reaction, a byproduct, phthalyl hydrazide, is formed. It was analyzed by solution state NMR.

Figure 13. Reaction scheme for cleavage of N-(3-butynyl) phthalimide.

Figure 14. Proton NMR spectrum of aminobutyne obtained in cleavage of phthalimide.

Figure 15. Carbon NMR spectrum of amino butyne obtained in cleavage of phthalimide.

Figure 16. Reaction scheme for cleavage of phthalimide (II).

Figure 17 shows the ^1H NMR spectrum of phthalyl hydrazide (dissolved in d-benzene). The peaks at 3.5, 2.25 and 1.57 ppm correspond to the protons attached to carbons 'a', 'b' and 'd' of N-(3-butynyl) phthalimide. The peaks at 6.78 and 7.37 ppm can be assigned to the aromatic protons attached to carbons 2,3 and 1,4 of N-(3-butynyl) phthalimide respectively. But in the spectrum of phthalyl hydrazide, the expected peaks are only those of the phthalimide ring. Additional peaks are observed which correspond to the butynyl group. This spectrum indicated that phthalyl hydrazide formed in the cleavage of phthalimide (II) is not the expected hydrazide.

The ^{13}C NMR spectrum of phthalyl hydrazide is shown in Figure 18. The peaks at 36.3, 18.1 and 70.4 ppm correspond to carbons 'a', 'b' and 'd' of N-(3-butynyl) phthalimide. The peak at 127 ppm corresponds to the solvent d-benzene. In addition, the peaks at 122 and 132 ppm can be assigned to aromatic carbons 1,4 and 2,3 respectively. In this spectrum, aliphatic carbon peaks are not expected peaks, which indicates that phthalyl hydrazide is not formed as predicted.

The final product obtained in the cleavage of phthalimide (II) was analyzed by solution state NMR. Figure 19 shows the ^1H NMR spectrum of final product, 4-aminobutyne (dissolved in d-methanol). The peaks at 3.3 and 4.8 ppm are likely due to protons attached to the aliphatic carbons of 4-amino butyne. Though these two peaks do not confirm the amine product, the phthalimide cleavage procedure (II) was applied to the modified silica, the N-(3-butynyl) phthalimide bonded phase, to prepare amino phases in order to determine if this procedure can be applied to the bonded phase.

Figure 17. Proton NMR spectrum of phthalyl hydrazide obtained in cleavage of phthalimide (II).

Figure 18. Carbon NMR spectrum of phthalyl hydrazide obtained in cleavage of phthalimide (II).

Figure 19. Proton NMR of amino butyne obtained in cleavage of phthalimide (II).

Amino phases were prepared using N-(3-butynyl) phthalimide bonded phases made with free radical catalyst and platinum catalyst following the phthalimide cleavage procedure (II) as shown in Figure 20. Figure 21 shows the DRIFT spectra of N-(3-butynyl) phthalimide bonded phases used in the synthesis of amino phases. The spectrum of the N-(3-butynyl) phthalimide bonded phase prepared using a free radical catalyst (Fig. 21A) is the same as that shown in Figure 11A because the same bonded phase is used for the amino phase synthesis. But the N-(3-butynyl) phthalimide bonded phase prepared using a platinum catalyst is different (Fig. 21B), with a peak at 2247 cm^{-1} , which corresponds to an Si-H bond, but reduced in intensity when compared to that of Kromasil hydride indicating the bonding of the phthalimide moiety to Kromasil hydride. In addition, peaks at 2945 and 3064 cm^{-1} correspond to aliphatic and aromatic C-H stretching vibrations of the bonded phthalimide moiety. The peaks at 1714 and 835 cm^{-1} correspond to carbonyl stretching and aromatic C-H out of plane bending vibrations of the phthalimide moiety. These results indicate that N-(3-butynyl) phthalimide is bonded to Kromasil hydride with both a free radical catalyst and a platinum catalyst.

Figure 22 shows DRIFT spectra of amino phases prepared from N-(3-butynyl) phthalimide bonded phases made by free radical catalyst and platinum catalyst. In both amino phases (Figures 22A and 22B), no Si-H peak is observed, which is supposed to be seen and no other structural peaks of the amino moiety were observed. The peaks at 793 , 1630 , and 1867 cm^{-1} in the case of the free radical amino phase (Fig. 22A) and peaks at 800 , 1629 and 1881 cm^{-1} in the case of the platinum catalyzed amino phase (Fig. 22B) are the same peaks seen in the DRIFT spectrum of Kromasil hydride, which are due to the

fundamental vibrations of the silica matrix. These DRIFT spectra of the amino phases did not indicate the presence of an amino organic moiety on the silica surface and based on these results, it is difficult to predict the success of the phthalimide cleavage. But, from the carbon percentage values shown in Table 1, less carbon is observed in the free radical and platinum catalyzed amino phases when compared to the corresponding phthalimide bonded phases indicating the loss of some bonded material after the cleavage of phthalimide.

3.2.4. 3-amino-3-methyl-1-butyne bonded phase

Before preparing bonded phases from 3-amino-3-methyl-1-butyne, the parent compound was analyzed by solution state NMR. Figure 23 shows the ^1H NMR spectrum obtained by dissolving 10 μL of 3-amino-3-methyl-1-butyne in 0.5 mL of deuterated chloroform. The peak assignments, proton and carbon NMR chemical shifts of 3-amino-3-methyl-1-butyne are summarized in Table 3. The peak at 1.67 ppm can be assigned to the proton attached to carbon 1. The protons attached to carbon 4 are responsible for the peak at 1.39 ppm.

The ^{13}C NMR spectrum of 3-amino-3-methyl-1-butyne is shown in Figure 24 and peak assignments are shown in Table 3. The peak at 67.9 ppm can be assigned to carbon 1. Carbons 2 and 3 are responsible for the peaks at 92 and 45.2 ppm respectively. The peak at 31.8 ppm can be assigned to carbon 4.

Bonded phases containing 3-amino-3-methyl-1-butyne were analyzed by DRIFT and ^{13}C CP-MAS-NMR. DRIFT spectra of 3-amino-3-methyl-1-butyne bonded phases

Figure 20. Reaction scheme for synthesis of amino phase via N-(3-butynyl) phthalimide bonded phase.

Figure 21. DRIFT spectra of N-(3-butynyl) phthalimide bonded phases used in amino phase synthesis prepared using (A) Free radical catalyst (B) Platinum catalyst.

Figure 22. DRIFT spectra of amino phases via N-(3-butynyl) phthalimide bonded phase prepared using (A) Free radical catalyst (B) Platinum catalyst.

Figure 23. Proton NMR spectrum of 3-amino-3-methyl-1-butyne.

Figure 24. Carbon NMR spectrum of 3-amino-3-methyl-1-butyne.

Table 3. Proton and carbon NMR chemical shifts of 3-amino-3-methyl-1-butyne.

3-amino-3-methyl-1-butyne

¹ H NMR		¹³ C NMR	
Proton	ppm	Carbon	ppm
1	1.67	1	67.9
4	1.39	2	92.0
		3	45.2
		4	31.8

prepared using a free radical catalyst (curve A) and a platinum catalyst (curve B) is shown in Figure 25. Curve A shows a Si-H peak at 2252 cm^{-1} , but reduced in intensity when compared to that of Kromasil hydride. This less intense Si-H peak indicates the bonding of 3-amino-3-methyl-1-butyne to Kromasil hydride. In addition peaks at 2889 and 805 cm^{-1} correspond to aliphatic C-H stretching and N-H bending vibrations of the bonded moiety respectively. The 3-amino-3-methyl-1-butyne bonded phase prepared using a platinum catalyst (curve B) shows a strong peak at 2256 cm^{-1} corresponding to the Si-H bond. The peak at 2980 cm^{-1} corresponds to an aliphatic C-H stretching vibration indicating the presence of an organic moiety. The peak at 3739 cm^{-1} is due to unreacted silanols present on the surface of Kromasil hydride. These DRIFT spectra showed that 3-amino-3-methyl-1-butyne was bonded to Kromasil hydride with both a free radical catalyst and a platinum catalyst.

Figure 26 shows the ^{13}C CP-MAS NMR spectra of 3-amino-3-methyl-1-butyne on Kromasil hydride prepared using a free radical catalyst (Fig. 26A) and a platinum catalyst (Fig. 26B). In the free radical bonded phase, peaks at 124 and 174 ppm indicate that the organic moiety is bonded to silica hydride with an olefin bond. The peaks at 23 and 62 ppm correspond to the methyl and methylene resonance of an ethoxy moiety present on the surface of Kromasil silica hydride. This spectrum confirmed the bonding of 3-amino-3-methyl-1-butyne to Kromasil hydride. In the case of the platinum catalyzed bonded phase of 3-amino-3-methyl-1-butyne (Fig. 26B), only two peaks were obtained. The peaks at 20 and 64 ppm correspond to methyl and methylene resonance of an ethoxy moiety present on the surface of Kromasil silica hydride. In the DRIFT spectrum of this

platinum catalyzed bonded phase as shown in Figure 25B, the aliphatic C-H peak at 2980 cm^{-1} indicated the presence of an organic moiety. But the solid state NMR confirmed that the aliphatic C-H peak seen in DRIFT spectrum is due to a residual ethoxy group and not due to the expected bonded phase organic moiety. This confirmed that 3-amino-3-methyl-1-butyne is not bonded to Kromasil hydride with a platinum catalyst. This is because the coordination of the platinum catalyst with the amino group of the 3-amino-3-methyl-1-butyne prevented the hydrosilation of the alkyne group. DRIFT and solid state NMR spectra of 3-amino-3-methyl-1-butyne bonded phase indicated that the organic moiety is bonded to Kromasil hydride only with a free radical catalyst. This result is also evident by the elemental analysis from the higher surface coverage ($1.81\text{ }\mu\text{mol/m}^2$) of the free radical bonded phase when compared to that of platinum catalyzed bonded phase ($0.72\text{ }\mu\text{mol/m}^2$).

3.2.5. 4-diethylamino-2-butyne-1-ol bonded phase

Before the synthesis of the bonded phase containing 4-diethylamino-2-butyne-1-ol, the parent compound was analyzed by NMR by dissolving $10\text{ }\mu\text{L}$ of 4-diethylamino-2-butyne-1-ol in 0.5 mL of deuterated chloroform. The ^1H NMR spectrum of 4-diethylamino-2-butyne-1-ol is shown in Figure 27. The peak assignments and chemical shifts of 4-diethylamino-2-butyne-1-ol are shown in Table 4. The peak at 4.25 ppm corresponds to the proton attached to carbon 1. The peak at 3.4 ppm corresponds to two protons attached to carbon 4. The peak at 2.5 ppm corresponds to four protons of the two

Figure 25. DRIFT spectra of 3-amino-3-methyl-1-butyne on Kromasil hydride prepared using (A) Free radical catalyst (B) Platinum catalyst.

Figure 26. ^{13}C CP-MAS NMR spectra of 3-amino-3-methyl-1-butyne on Kromasil hydride prepared using (A) Free radical catalyst (B) Platinum catalyst.

Figure 27. Proton NMR spectrum of 4-diethylamino-2-butyne-1-ol

Table 4. Proton and carbon NMR chemical shifts of 4-diethylamino-2-butyne-1-ol.

4-diethylamino-2-butyne-1-ol

¹ H NMR		¹³ C NMR	
Proton	ppm	Carbon	ppm
1	4.25	1	51.1
4	3.4	2	80.8
a	2.5	3	84
b	1.03	4	41.1
		a	47.3
		b	12.5

equivalent carbons 'a'. Finally, the peak at 1.03 ppm corresponds to six protons attached to two equivalent carbons 'b'.

Figure 28 shows the ^{13}C NMR spectrum of 4-diethylamino-2-butyne-1-ol. The peak assignments of 4-diethylamino-2-butyne-1-ol are shown in Table 4. The peak at 51.1 ppm can be assigned to carbon 1. Carbons 2 and 3 are responsible for the peaks at 80.8 ppm and 84 ppm respectively. The peak at 41.1 ppm corresponds to carbon 4. The peaks at 47.3 and 12.5 ppm can be assigned to carbons 'a' and 'b' respectively.

Bonded phases containing 4-diethylamino-2-butyne-1-ol prepared by free radical and platinum catalyst were characterized by DRIFT and NMR. Figure 29 shows the DRIFT spectra of Kromasil hydride and the 4-diethylamino-2-butyne-1-ol bonded phase prepared using platinum catalyst. In the platinum catalyzed bonded phase (Curve B), no Si-H peak is observed when compared to that of Kromasil hydride (Curve A). This may be due to reduction of hydride by the platinum catalyst. However, the peaks at 2979 and 1452 cm^{-1} were observed which correspond to aliphatic C-H stretching and bending vibrations of the bonded moiety respectively indicating the presence of an organic moiety.

Figure 30 shows the ^{13}C CP-MAS-NMR spectra of 4-diethylamino-2-butyne-1-ol on Kromasil hydride prepared using a free radical catalyst (Curve A) and a platinum catalyst (Curve B). Both spectra showed only two peaks, the peak at 10 ppm corresponds to a methyl resonance of the bonded moiety, whereas the peak at 46 ppm corresponds to the four methylene carbons of the bonded moiety. Since no peaks are obtained in the aromatic region, it indicated that the organic moiety is not bonded with an olefin bond.

This spectra confirmed the bonding of 4-diethylamino-2-butyne-1-ol to Kromasil hydride with both platinum and free radical catalyst (12,13). This result is also evident by the elemental analysis, showing the high surface coverages of both the platinum catalyzed bonded phase ($1.87\mu\text{mol}/\text{m}^2$) and the free radical bonded phase ($2.97\mu\text{mol}/\text{m}^2$) as shown in Table 1.

3.3. *Chromatographic characterization*

Chromatographic characterization of all the bonded phases was done by performing retention studies of sugars in the aqueous normal phase HPLC with laser light scattering detection. Sugars (carbohydrates) are widely distributed in nature. These compounds are the key intermediates of metabolism, structural components of plants (cellulose), and key components of food sources such as sugars, flour, vegetables, and fiber. Sugar analysis is important in the generation of nutrition panels as both the sugar and total carbohydrate content must be included. Sugar samples at 15 mg/ml in 10:90 methanol:water were injected onto the columns. A mixture of eight sugars, maltose, sucrose, glucose, fructose, lactose, xylose, raffinose and melezitose, was injected for each analysis. Mobile phases of 10:90 water:acetonitrile and 15:85 water:acetonitrile were used. A comparative study of the separation performance of all the columns was done through the sugar analysis.

Figure 28. Carbon NMR spectrum of 4-diethylamino-2-butyne-1-ol.

Figure 29. DRIFT spectra of (A) Kromasil hydride (B) 4-diethylamino-2-butyne-1-ol on Kromasil hydride prepared using platinum catalyst.

Figure 30. ^{13}C CP-MAS NMR spectra of 4-diethylamino-2-butyn-1-ol on Kromasil hydride prepared using (A) Free radical catalyst (B) Platinum catalyst.

3.3.1. Astrosil silica

Astrosil silica without any surface modification was analyzed by sugar retention. With a 10:90 water:acetonitrile mobile phase, six peaks in the eight sugar mixture are observed. The order of elution for the sugars is based on the polarity of the sugars. Generally, the least polar component is eluted first in normal phase HPLC. The least polar component xylose is eluted first on this column as shown in Figure 31. The separation of sugars on the Astrosil silica is due to polar-polar interactions between the hydrophilic silanol groups of silica and the polar hydroxyl groups of the sugars. By increasing the water concentration to 15%, only three peaks were observed and the retention time decreased. This result is due to the diminished interactions between the sugars and the silanol groups. The peak identification shown in Figure 31 is same for all the columns.

3.3.2. Astrosil hydride

Astrosil hydride, which is similar to Kromasil hydride was studied by sugar retention in order to compare the separation performance of the hydride and organic modified silica based bonded phases. The retention time data of the sugars on the Astrosil hydride column are shown in Table 5. Chromatograms of sugars on the hydride column are shown in Figure 32. With a 10:90 water:acetonitrile mobile phase, six peaks are observed. The least polar component xylose is eluted first followed by successively more polar components fructose, glucose, sucrose, maltose, lactose, melezitose, and

Figure 31. Chromatogram of sugars on Astrosil silica with (A) 10:90 Water:Acetonitrile and (B) 15:85 Water:Acetonitrile.

1.Xylose 2. Fructose 3. Glucose 4.Sucrose 5. Maltose 6. Lactose 7. Melezitose 8. Raffinose.

Table 5. Retention time data of sugars on Astrosil hydride column.

Sample no	Sugar samples	Average retention time(min) of sugars on Hydride column	
		10:90 Water:MeCN	15:85 Water:MeCN
1	Xylose	5.43	4.07
2	Fructose	6.87	4.43
3	Glucose	7.07	4.83
4	Maltose	9.73	4.90
5	Sucrose	10.17	4.90
6	Lactose	12.93	5.13
7	Melezitose	20.10	5.50
8	Raffinose	23.95	5.67

Figure 32. Chromatogram of sugars on Astrosil hydride column with (A) 10:90 Water : Acetonitrile and (B) 15:85 Water: Acetonitrile.

1. Xylose 2. Fructose 3. Glucose 4. Sucrose 5. Maltose 6. Lactose 7. Melezitose 8. Raffinose.

raffinose. Glucose and fructose appeared as a single peak. Sucrose and maltose also are eluted as single peak because of their close retention times. Since the astrosil hydride is slightly hydrophobic and the sugars are hydrophilic, the separation of sugars on this column is probably due to interaction between the remaining hydrophilic silanol groups present on the surface of Astrosil silica hydride and the polar hydroxyl groups of sugars. Using 15% water, no separation was observed. This result is consistent with normal phase behavior because by increasing the concentration of water to 15%, the interaction of sugars with the silanols on the hydride column is reduced. Overall the polarity of the hydride phase is approximately the same as the bare silica since the retention on the two columns is similar in the 10:90 water:acetonitrile mobile phase. However, the retention is lower on the hydride column with the 15:85 water:acetonitrile mobile phase.

3.3.3. Endcapped Astrosil hydride

Endcapped Astrosil hydride is prepared from the starting Astrosil hydride. The residual silanol (unreacted) groups of Astrosil hydride were endcapped with a trimethylchloro silane reagent to prevent interaction between the silanol groups and the hydroxyl groups of sugars. The chromatograms of sugars on the endcapped Astrosil hydride are shown in Figure 33. No separation was observed at both mobile phase conditions as the silanol groups are blocked with silane reagent. This result confirmed that the separation of sugars on the Astrosil silica and the Astrosil hydride is due to the acidic silanols present on the surface.

3.3.4. N-(3-butynyl) phthalimide bonded phase

The N-(3-butynyl) phthalimide bonded phases prepared using a free radical catalyst and a platinum catalyst were characterized chromatographically by the retention of sugars. The retention times (t_R) of the sugars on this column are shown in Table 6. The chromatograms of sugars on the phthalimide bonded phase are shown in Figure 34. Six peaks for the eight sugar samples were seen at 10 % water. Under these conditions, fructose and glucose, and sucrose and maltose pairs were not separated. Using 15% water, retention times decreased and separation was worse. The order of elution of the sugars is the same as observed on bare silica and hydride column. The order is xylose<fructose<glucose<sucrose<maltose<lactose <melezitose<raffinose. This result can be attributed to the fact that the monosaccharides (xylose, fructose, glucose) are eluted first followed by the disaccharides (sucrose, maltose, lactose) and then the trisaccharides (melezitose, raffinose) depending on the number of hydroxyl groups(14). With in the monosaccharides, the pentose (xylose) came first followed by hexoses (fructose, glucose) as xylose is least polar with 4 hydroxyl groups when compared to glucose and fructose with five hydroxyl groups (15). In case of disaccharides as shown in Figure 7, sucrose(glucose+fructose), maltose(2 glucose) and lactose (glucose+galactose) have same number of hydroxyls and glucose is one of the monosaccharides. So retention time changed with other monosaccharide in the order fructose<glucose<galactose which is already determined with monosaccharides (16). Similarly trisaccharides, melezitose (2 glucose+fructose) and raffinose (glucose+fructose+galactose) with same number of hydroxyls have glucose, fructose in

Figure 33. Chromatogram of sugars on endcapped Astrosil hydride with (A) 10:90 Water:Acetonitrile and (B) 15:85 Water:Acetonitrile.

Table 6. Retention time data of sugars on N-(3-butynyl) phthalimide bonded phase.

Sugar sample	Average retention time of sugars (t_R)			
	N-(3-butynyl) phthalimide bonded phase (Free radical catalyst)		N-(3-butynyl) phthalimide bonded phase (Platinum catalyst)	
	10:90Wat:MeCN	15:85Wat:MeCN	15:85Wat:MeCN	15:85Wat:MeCN
Xylose	9.43	7.97	4.95	4.47
Fructose	10.93	8.87	5	4.60
Glucose	11.23	8.97	5	4.57
Sucrose	15.17	10.47	5	4.57
Maltose	16.07	10.53	5	4.40
Lactose	18.03	11.63	5	4.53
Melezitose	24.10	13.73	4.95	4.87
Raffinose	27.10	14.63	4.90	4.80

Figure 34. Chromatogram of sugars on N-(3-butynyl) phthalimide bonded phase prepared using free radical catalyst with (A) 10:90 Water:Acetonitrile and (B) 15:85 Water:Acetonitrile.

common and differ in one monosaccharide. So retention time changed with the other monosaccharide in the order glucose < galactose (17). Whereas phthalimide column made with platinum catalyst did not show any separation with both the mobile phase compositions (Figure 35). This is because the phthalimide bonded phase contained mixture of products which are formed due to different attachment geometries as evident from solid state NMR spectrum shown in Figure 12B. Due to the mixture of products in this column, sugars are not interacting well with the bonded phase and therefore no separation was observed.

3.3.5. Amino phase

Two amino columns, one prepared via N-(3-butynyl) phthalimide bonded phase using a free radical catalyst and the second via N-(3-butynyl) phthalimide bonded phase using a platinum catalyst were characterized by sugar retention at two mobile phase compositions. The retention times of sugars on both amino phases are shown in Table 7. Amino columns have been popular for sugar analysis. It was believed that separation of sugars is due to intermolecular hydrogen bonding between the amino group and the hydroxyl group of carbohydrates, and the total number of hydroxyls and their distribution play an important role in retention (14). In the case of the free radical amino phase, five peaks were observed in the eight sugar mixture at 10% water. The sucrose-maltose pair was not separated. In addition, raffinose and melezitose were not eluted since these sugars are strongly polar and retained longer on the stationary phase. However at 15% water, all eight sugars were separated although only partial resolution of fructose and

Figure 35. Chromatogram of sugars on N-(3-butynyl) phthalimide bonded phase prepared using platinum catalyst with (A) 10:90 Water:MeCN and (B) 15:85 Water: Acetonitrile.

Table 7. Retention time data of sugars on amino phases.

Sugar sample	Average retention time of sugars			
	Amino phase via N-(3-butynyl) phthalimide bonded phase (FR cat)		Amino phase via N-(3-butynyl) phthalimide bonded phase (Pt cat)	
	10:90Wat:MeCN	15:85Wat:MeCN	15:85Wat:MeCN	15:85Wat:MeCN
Xylose	10.17	9.13	12.97	11.00
Fructose	14.27	12.47	19.13	14.53
Glucose	16.00	13.57	23.63	17.17
Sucrose	30.00	22.83	48.30	30.05
Maltose	35.70	26.90	No peak	37.60
Lactose	48.25	33.10	No peak	47.00
Melezitose	No peak	43.83	No peak	48.00
Raffinose	No peak	63.00	No peak	56.50

glucose is achieved and the baseline is also good as shown in Figure 36. Although the DRIFT spectrum of this phase did not indicate the presence of an amino moiety, when the chromatograms of sugars on the N-(3-butynyl) phthalimide bonded phase (free radical catalyst) and the amino phase prepared from the phthalimide bonded phase are compared, the best separation was observed in the amino phase. This indicated the presence of a polar amino group on this bonded material, and therefore strong interaction between the amino group and the hydroxyl groups of the sugars through hydrogen bonding resulted in the best separation. This optimum separation of sugars on the amino phase indicated that the cleavage of the phthalimide was successful.

In the case of the amino phase prepared via the N-(3-butynyl) phthalimide bonded phase made using a platinum catalyst (Figure 37), with 10% water, good base line separation of four sugars xylose, fructose, glucose and sucrose was seen. But maltose, lactose, melezitose and raffinose were not eluted. This is due to the fact that the most polar components are retained longer on the polar amino phase. However at 15% water, seven peaks were observed and good baseline separation was achieved in a shorter analysis time. Only the melezitose- raffinose pair was not separated as shown in Figure 37 (15,16,17). Although the DRIFT spectrum of this amino phase also did not indicate the presence of an amino moiety, the best separation of sugars was achieved on this phase and indicated that the cleavage of phthalimide is successful. Separation on both the amino phases was better than the bare silica indicating that some entity other than the silanols is controlling the retention.

3.3.6. 3-amino-3-methyl-1-butyne bonded phase

Two columns, 3-amino-3-methyl-1-butyne on Kromasil hydride prepared with a free radical catalyst and a platinum catalyst were characterized by sugar retention using two mobile phase compositions. In the case of the free radical bonded phase, four peaks for the eight sugar mixture were observed with 10% water in the mobile phase. Fructose-glucose pair and sucrose-maltose-lactose were not separated. At 15% water in the mobile phase, reduced retention time was obtained as shown in Figure 38. This behavior is consistent with the normal phase mode, where by increasing the polarity of the mobile phase, the retention time of the solutes decreases.

In the case of the platinum catalyzed bonded phase (Figure 39), with 10% water in the mobile phase, fructose-glucose, sucrose-maltose-lactose and melezitose-raffinose components were not well separated. At 15% water in the mobile phase, retention times were decreased, but separation is even poorer when compared to that of the free radical bonded phase. This is because in the platinum catalyzed bonded phase, the platinum catalyst coordinated with the amino group and prevented significant hydrosilation of the alkyne group. So this phase has very little organic moiety, but has the hydride surface. So the separation observed with this column is due to the remaining silanols on the surface of Kromasil hydride and the effects of the hydride layer. Therefore, the chromatograms of sugars on this column shown in Figure 39 are quite similar to that of Astrosil hydride column shown in Figure 32. The retention time data of the sugars on both the free radical and platinum catalyzed bonded phases is shown in Table 8.

Figure 36. Chromatogram of sugars on amino phase prepared using N-(3-butynyl) phthalimide bonded phase made with free radical catalyst at (A) 10:90 Water:MeCN and (B) 15:85 Water: Acetonitrile.

Figure 37. Chromatogram of sugars on amino phase prepared using N-(3-butynyl) phthalimide bonded phase (made with platinum catalyst) at (A) 10:90 Water:MeCN and (B) 15:85 Water: Acetonitrile.

Figure 38. Chromatogram of sugars on 3-amino-3-methyl-1-butyne bonded phase prepared using free radical catalyst at (A) 10:90 Water:MeCN and (B) 15:85 Water: Acetonitrile.

Figure 39. Chromatogram of sugars on 3-amino-3-methyl-1-butyne bonded phase prepared using platinum catalyst at (A) 10:90 Water:MeCN and (B) 15:85 Water: Acetonitrile

Table 8. Retention time data of sugars on 3-amino-3-methyl-1-butyne bonded phase.

Sugar sample	Average retention time of sugars			
	3-amino-3-methyl-1-butyne bonded phase (FR cat)		3-amino-3-methyl-1-butyne bonded phase (Pt cat)	
	10:90Wat:MeCN	15:85Wat:MeCN	10:90Wat:MeCN	15:85Wat:MeCN
Xylose	6.97	6.83	6.47	5.47
Fructose	7.70	7.77	7.53	5.90
Glucose	7.93	7.90	7.90	5.93
Sucrose	11.73	10.40	10.77	7.03
Maltose	12.83	10.43	11.23	6.97
Lactose	16.00	12.13	13.13	7.93
Melezitose	22.77	14.97	18.97	9.83
Raffinose	29.40	17.33	21.97	10.90

3.3.7. 4-diethylamino-2-butyne-1-ol bonded phase

The 4-diethylamino-2-butyne-1-ol moiety was successfully bonded to Kromasil hydride with both a free radical catalyst and a platinum catalyst as evident from the DRIFT spectra, solid state NMR spectra and elemental analysis. These phases have not been packed into columns because initial characterization of this bonded phase indicated that the hydrosilylation of this organic moiety was unsuccessful. But from high carbon percentage and solid state NMR spectra, it is confirmed that hydrosilylation is successful. So, these phases have to be converted into amino phases by dealkylation of 4-diethylamino-2-butyne-1-ol bonded phases, packed into columns and characterized by sugar retention, which will be the future work of this research project.

Comparison of the retention of sugars on all the columns was made. The average retention time of sucrose on all of the columns was selected at the two mobile phases 10:90 water:acetonitrile and 15:85 water:acetonitrile. The average retention time data for sucrose on all the columns is shown in Table 9 and a comparison plot is shown in Figure 40. Usually in normal phase HPLC, increasing the polarity of the mobile phase decreases the retention time. The comparison plot (Figure 40) shows that the behavior of sucrose on all the columns is consistent with normal phase behavior. The behavior of 3-amino-3-methyl-1-butyne bonded phase prepared with platinum catalyst is quite similar to that of Astrosil hydride as the platinum catalyzed bonded phase has little organic moiety, which is evident from the solid state NMR spectra and the retention observed on this phase is only due to hydride surface.

Table 9. Average retention time of sucrose on different columns analyzed in this study at mobile phases 10:90 and 15:85 Water: Acetonitrile.

Columns	Avg t_R of sucrose at 10% water	Avg t_R of sucrose at 15% water
Astrosil silica	11.4	7.9
Astrosil hydride	10.17	4.90
Endcapped Astrosil hydride	3.60	3.30
N-(3-butynyl) phthalimide bonded phase (FR)	15.17	10.47
N-(3-butynyl) phthalimide bonded phase (Pt)	5.00	4.57
Amino phase via N-(3-butynyl) phthalimide bonded phase(FR)	30.23	22.83
Amino phase via N-(3-butynyl) phthalimide bonded phase(Pt)	48.30	30.05
3-amino-3-methyl-1-butyne bonded phase (FR)	11.73	10.4
3-amino-3-methyl-1-butyne bonded phase (Pt)	10.77	7.03

Ast silica – Astrosil silica

EndcapAshyd – Endcapped Astrosil Hydride

N3but – N-(3-butynyl) phthalimide bonded phase

3a3m1b – 3-amino-3-methyl-1-butyne bonded phase

Pt – Platinum catalyst

Ast hyd – Astrosil hydride

Amph – Amino phase

FR- Free radical catalyst

Figure 40. Comparison plot of average retention time of sucrose on different columns at 10% water and 15% water.

4. Conclusions

N-(3-butynyl) phthalimide is bonded to Kromasil hydride both with free radical and platinum catalyst as evident from DRIFT and NMR spectra. The DRIFT spectra of the amino phase prepared from N-(3-butynyl) phthalimide bonded phase did not indicate the cleavage of phthalimide, but the best separation was achieved on the amino phase which confirmed the cleavage of phthalimide. From solid state NMR spectra, it is evident that 3-amino-3-methyl-1-butyne is bonded to Kromasil hydride only with a free radical catalyst and not with a platinum catalyst as the platinum catalyst coordinated with the amino group and prevented the hydrosilation of the alkynes. 4-diethylamino-2-butyne-1-ol is bonded to Kromasil hydride both with free radical and platinum catalyst as evident from NMR and DRIFT spectra, and needs further characterization by HPLC.

Chromatographic characterization of all the columns by sugar retention showed normal phase behavior. From the chromatographic data, it is also confirmed that the best separation of sugars was achieved on the bonded phases containing organic moieties when compared to bare silica, silica hydride which lack an organic moiety and endcapped hydride.

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