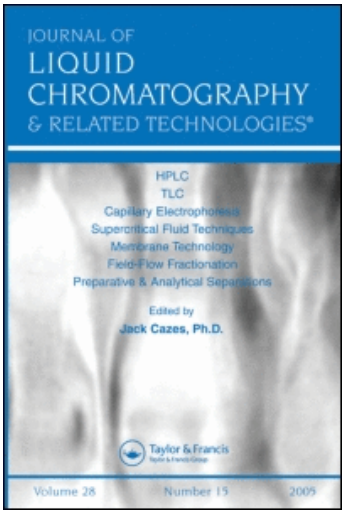

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Synthesis and Characterization of Amino-Based Columns for HPLC Made by Silanization/Hydrosilation

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Synthesis and Characterization of Amino-Based Columns for HPLC Made by Silanization/Hydrosilation

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Abstract: Synthesis and characterization of amino-based stationary phases for high performance liquid chromatography is described. The amino-based stationary phases for HPLC have been synthesized following a silanization/hydrosilation method. Three organic compounds, N-(3-butynyl) phthalimide, 4-diethylamino-2-butyn-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-butynyl) phthalimide phase, amino phase via cleavage of N-(3-butynyl) phthalimide phase (4-amino-1-butyne phase), and 3-amino-3-methyl-1-butyne bonded phases was done by sugar analysis.

Keywords: Amino-based columns, HPLC, Silanization/hydrosilation, Synthesis, Characterization

INTRODUCTION

Modified stationary phases with an alkyl chain and a polar group such as amino, amide, or diol groups are compatible with aqueous normal phase conditions, where a polar stationary phase is used with an aqueous organic mobile phase. This mode is similar to the normal phase, as a highly polar component is retained longer than a non-polar component. This method

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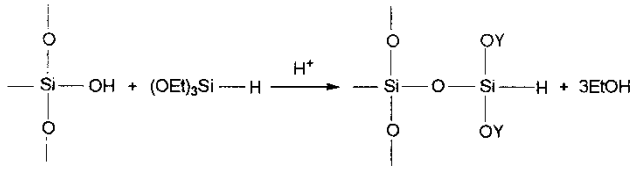
has been particularly useful for the analysis of polar compounds such as sugars.[1–3]

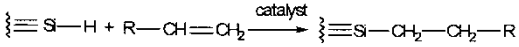
An approach developed by Pesek et al.[4] has been used to synthesize amino bonded phases. The synthetic procedure involves two steps. The first step is silanization (1a) where a silanol group (Si-OH) is converted to silica hydride (Si-H) in the presence of triethoxysilane (TES). About 95% of the OH groups are replaced by hydrides under controlled reaction conditions of temperature, concentration of TES, amount of water, acid, and reaction time. The hydride intermediate formed in this step is stable both in air and water.



(1a)

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

The second step is a hydrosilation reaction (1b) 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.



(1b)

Catalyst = Hexachloroplatinic acid or free radical initiator

The 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, referred as Speier's catalyst, is the most commonly used platinum catalyst. The main advantage of the hydrosilation method over organosilanization is the minimum number of silanol groups remaining on the surface due to conversion of most of the silanol groups to hydrides during the silanization step.[4] The acidic silanols may cause tailing or irreversible adsorption of basic compounds if they are not removed or blocked.[5]

The major goal of the present research is to prepare amino-based stationary phases via a hydrosilation of alkynes on a silica hydride surface as developed by Pesek et al.[4] 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 1. Structures III and IV are the most stable due to the formation of 5 member ring. Based on the carbon chemical shift values of the resulting bonded phase, the structure of the product could be determined.[6]

Hydrosilation product of alkynes:

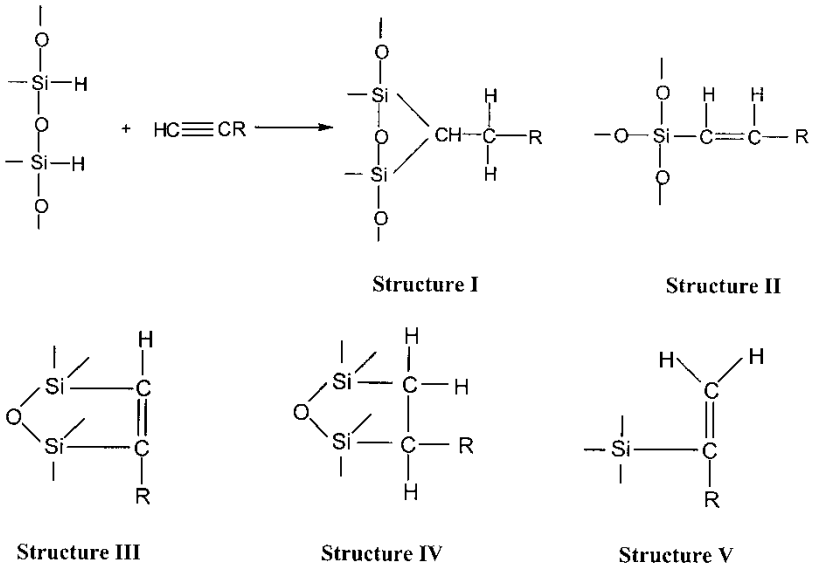
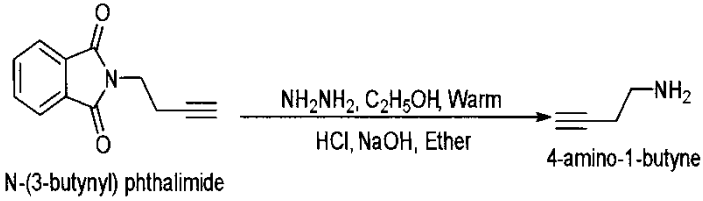


Figure 1. Possible structures of bonding of alkynes to silica hydride surface.

Three organic compounds, N-(3-butynyl) phthalimide, 3-amino-3-methyl-1-butyne, and 4-diethylamino-2-butyn-1-ol containing an alkyne group and an amino group have been chosen for the bonded moieties. Synthesis and characterization of the resulting bonded phases of these organic moieties are the major goals of this investigation. Another goal is to prepare an amino phase from the N-(3-butynyl) phthalimide bonded phase by cleavage of the phthalimide ring. The reason for the above synthetic scheme is the strong affinity of the free amino groups to the platinum catalyst. In the proposed approach, the amino groups are protected by the phtalimide ring during the hydrosilation reaction. The cleavage of the phthalamide ring can be done according to the reaction scheme depicted below:



Two sets of experimental conditions were tested and the products were evaluated by liquid state NMR. All synthesized phases were characterized

by FTIR and NMR. The final evaluation of bare silica, silica hydride, endcapped hydride, and the amino-based columns was done by HPLC analysis of sugars.

EXPERIMENTAL

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-butyn-1-ol was obtained from Aldrich Chemical Co. (Milwaukee, WI). Kromasil silica (Lot # AT0112), 5 μm average particle diameter and a specific surface area of 340 m^2/g was donated by Eka Nobel AB (Bohus, Sweden). The two catalysts, tertiary butyl peroxide and hexachloroplatinic acid, and the silanizing agent, triethoxysilane, were supplied by Aldrich Chemical Co. (Milwaukee, WI). All the solvents, dioxane, toluene, and methylene chloride used in syntheses of the bonded phases were obtained from Fischer Chemical Company (Fair Lawn, NJ). Hydrazine hydrate, sodium chloride, and diethyl ether used in the cleavage reaction were obtained from Aldrich Chemical Co. (Milwaukee, WI). De-ionized water was obtained from a Milli-Q™ purification system (Millipore Corp., Bedford, MA). Sugar samples for HPLC analysis, glucose, fructose, sucrose, maltose, lactose, xylose, raffinose, and melezitose, were obtained from Lancaster (Windham, NH). 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 columns used in the sugar analysis were obtained from Microsolv (Eatontown, NJ).

Instrumentation

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 Berendsen and DeGalan equation.[7] 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 the Berendsen and DeGalan equation shown below:

$$\alpha\ (\mu\text{mol/m}^2) = \frac{10^6 P_c}{(10^2 M_c n_c - P_c M_R) S_{BET} 2} \quad (1)$$

where p_c is the carbon percentage of the bonded material (by weight after correction for 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]

Diffuse Reflectance Infrared Fourier Transform Spectroscopy

Diffuse reflectance infrared Fourier transformation (DRIFT) spectra were obtained on a ATI Mattson Infinity Series FTIR™ spectrometer equipped with a deuterated triglycine sulfate (DTGS) detector, using a diffuse reflectance accessory (sample cup with a 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 5 mg 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 CO_2 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.

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 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-butyn-1-ol used in the hydrosilation reaction were obtained by dissolving compounds in d-chloroform.

Chromatography

Analytical liquid chromatography was performed with the use of equipment composed of a SP8800 ternary HPLC pump (SP ThermoElectron products, San Jose, CA), a Chromjet integrator, a laser light scattering detector, and a Rheodyne injector (Cotati, CA). The bonded phases were slurry packed into

150 cm × 4.6 mm stainless steel columns (Alltech Co., Deerfield, IL) at 6000 psi pressure with methanol as the driving solvent, using a Haskel pneumatic pump (Burbank, CA, US).

Procedures

Silanization

First, 10 g of dried Kromasil silica was weighed into 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 the silica and the reaction mixture was heated to 70°C. Then, 60 mL of dioxane was added to the addition funnel and was flushed with argon; afterwards 13.9 mL of triethoxysilane (TES) was added into the addition funnel. The prepared TES solution in the addition funnel was added drop wise to the reaction mixture at 70°C 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 product was filtered and washed three times each with dioxane, toluene, and diethyl ether. Then, the solid was left at room temperature overnight to evaporate ether and finally dried in a vacuum oven at 110°C for 24 hours.[8]

Bonded Phases Synthesis

N-(3-butynyl) Phthalimide Bonded Phase: Free Radical Hydrosilation

In a 250 mL three necked round bottomed flask, 4.2 g of N-(3-butynyl) phthalimide, 59 mL of toluene, and 168 μL of t-butyl peroxide were added. The mixture was heated at a constant temperature of 70°C for about an hour. While stirring, 4.2 g of Kromasil hydride was added slowly through the open neck of the flask. The flask was flushed with nitrogen and all the joints were sealed. The temperature of the reaction mixture was then raised to 100 ± 2°C and the reaction was continued for about 100 h. The final product was cooled, filtered, and washed with toluene followed by methylene chloride and diethyl ether. The solvent in the final product was allowed to evaporate at room temperature overnight and the product was dried in vacuum oven at 110°C for 24 h.[9]

Platinum Hydrosilation

A mixture of 2.2 g of N-(3-butynyl) phthalimide, 31 mL of toluene and 0.66 mL of 10 mM hexachloroplatinic acid in 2-propanol was heated at a temperature of 70 ± 2°C for 1 h with magnetic stirring. After, 1 h, 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 ± 2°C and the reaction was continued for another 100 h. After cooling the final product, it was filtered 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.

Cleavage of Phthalimide

Cleavage of N-(3-butynyl) Phthalimide to 4-Amino-1-butyne (Procedure I)

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 mM 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]

Cleavage of N-(3-butynyl) Phthalimide to 4-Amino-1-butyne (Procedure 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 mM sodium hydroxide. The solution obtained was extracted with diethyl ether. The resulting ether layer was dried and the residue was analyzed by solution state NMR.[11]

Amino Phases

Amino Phase via N-(3-butynyl) Phthalimide Bonded Phase Prepared by Free Radical Hydrosilation (from Cleavage Procedure II)

The 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 μL) 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 phthalyl hydrazide was filtered by washing with 0.1 mM sodium hydroxide, followed by water and ether. Then the silica residue was dried and analyzed by solid state NMR.

Amino Phase via N-(3-butynyl) phthalimide Bonded Phase Prepared by Platinum Hydrosilation (from Cleavage Procedure II)

The N-(3-butynyl) phthalimide bonded phase (2.1 g) prepared by platinum hydrosilation was hydrolyzed using the same procedure as described above for the N-(3-butynyl) phthalimide bonded phase prepared by free radical hydrosilation.

3-Amino-3-methyl-1-butyne Bonded Phase

The bonded phase containing 3-amino-3-methyl-1-butyne was prepared according to the same procedures used in the synthesis of the N-(3-butynyl) phthalimide bonded phase. In the free radical bonded phase synthesis, 2.16 mL of 3-amino-3-methyl-1-butyne, 67.6 mL of toluene, 136 μL 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.

4-Diethylamino-2-butyn-1-ol Bonded Phase

The 4-diethylamino-2-butyn-1-ol bonded phase was prepared according to the same procedures used in the synthesis of the N-(3-butynyl) phthalimide bonded phase. In the free radical bonded phase synthesis, 3.00 mL of 4-diethylamino-2-butyn-1-ol, 40 mL of toluene, 160 μL of t-butyl peroxide, and 2.00 g of Kromasil hydride were used. In the platinum catalyzed bonded phase, 3.00 mL of 4-diethylamino-2-butyn-1-ol, 40 mL of toluene, 0.8 mL of 10 mM hexachloroplatinic acid in 2-propanol, and 2.00 g of Kromasil hydride were used.

Chromatographic Studies

Separation of sugars was performed in the aqueous normal phase mode of 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 provide sensitive detection for samples containing weak chromophores without derivatization.

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 CO_2 gas was set at 1.25 kg/cm^2. 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 5 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 the sugars used in this study are shown in Figure 2.

RESULTS AND DISCUSSION

Elemental Analysis

The concentration of surface bonded groups, or surface coverage, α_R was calculated from the carbon content of the bonded material and the BET

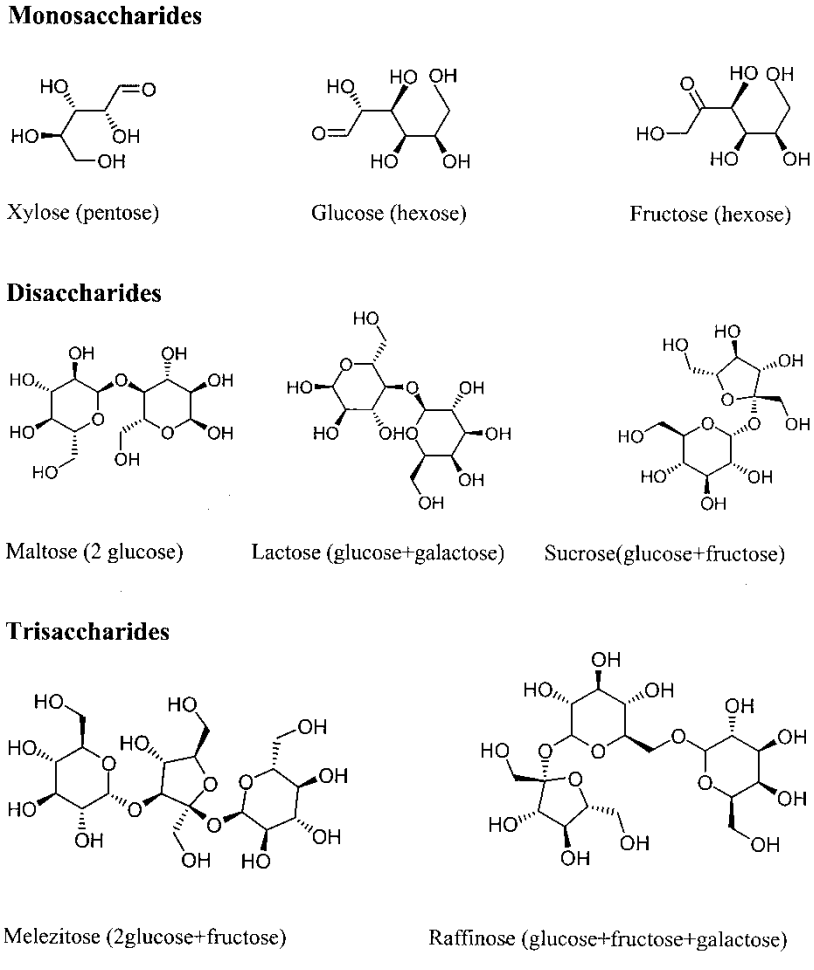


Figure 2. Structures of sugars.

specific surface area of the native silica before bonding, according to Berendsen and DeGalan as described previously.

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

Spectroscopic Characterization

Kromasil Hydride

Kromasil hydride was prepared according to the silanization procedure developed by Pesek et al.[4] In the presence of triethoxysilane and dioxane, Kromasil silica is converted to Kromasil hydride.[8] The DRIFT spectrum of Kromasil hydride, has a strong peak around 2257 cm^{-1} corresponding to the Si-H stretching vibration confirming the formation of the hydride. Also observed in a spectrum, is a broad peak between 3800 and 3000 cm^{-1} due to adsorbed water and hydrogen bonded silanols. A small peak at 3750 cm^{-1} is due to silanols that did not react with triethoxysilane in the silanization step. The remaining peaks in the spectrum are due to various fundamental vibrations of the silica matrix.[4,5]

N-(3-Butynyl) Phthalimide Bonded Phase

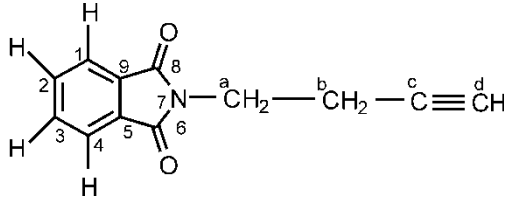
N-(3-butynyl) phthalimide was analyzed by solution state NMR so that the proton and carbon NMR chemical shifts can be compared to the compound present on the silica surface and confirm the bonding of phthalimide moiety to the silica surface. The peak assignments and chemical shifts of N-(3-butynyl) phthalimide are summarized in Table 2.[12,13]

The N-(3-butynyl) phthalimide bonded phase was prepared by the hydrosilation reaction using both a free radical catalyst (tertiary butyl peroxide)

Table 1. Surface bonding properties of bonded phases

Compound name	Type of catalyst	Carbon (%)	Surface coverage ($\mu mol/m^2$)
N-(3-butynyl) phthalimide	Free radical	3.72	0.69
N-(3-butynyl) Phthalimide	Platinum	9.65	2.14
Amino phase—after cleavage of N-(3-butynyl phthalimide)	Free radical	0.47	0.29
Amino phase—after cleavage of N-(3-butynyl phthalimide)	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

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



Proton NMR		Carbon 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

and hexachloroplatinic acid.[9] The DRIFT spectra of the N-(3-butynyl) phthalimide bonded phase prepared using a free radical catalyst and a platinum catalyst, are shown in Figures 3A and 3B, respectively. The hydrosilation product, N-(3-butynyl) phthalimide bonded phase prepared using both free radical catalyst (Figure 3A) and platinum catalyst (Figure 3B), show 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. The DRIFT spectra in Figure 3 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.

^{13}C CP-MAS NMR spectra of the N-(3-butynyl) phthalimide bonded phase prepared using a free radical catalyst (plot A) and a platinum catalyst (plot B), are shown in Figure 4. These spectra indicated that the manner in which the phthalimide reacted with silica hydride by the free radical catalyst and the platinum catalyst is quite different. In the spectrum (Figure 4A) of the N-(3-butynyl) phthalimide moiety bonded to silica using

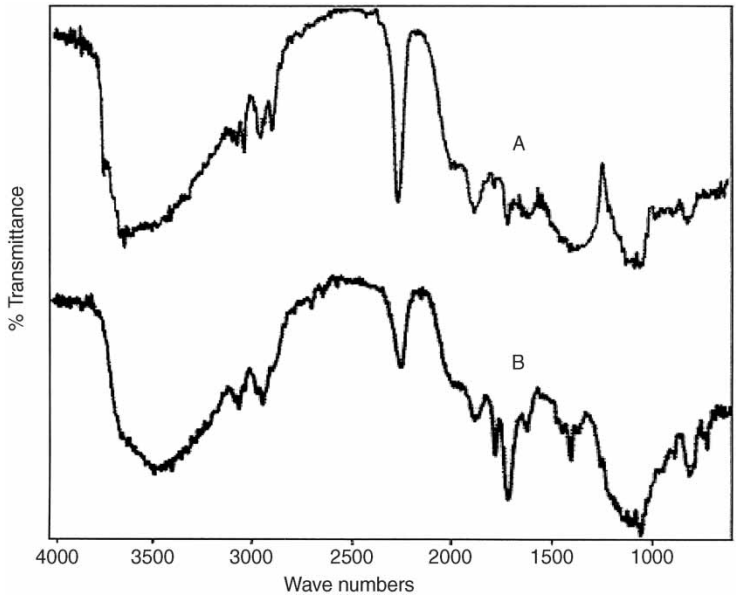


Figure 3. DRIFT spectra of N-(3-butynyl) phthalimide on Kromasil hydride prepared using (A) free radical catalyst (B) platinum catalyst.

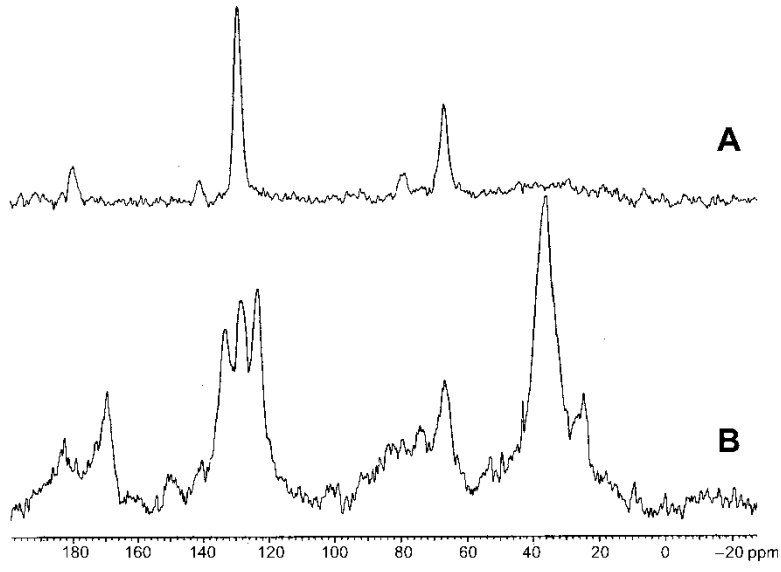


Figure 4. ^{13}C CP-MAS NMR spectra of N-(3-butynyl) phthalimide on Kromasil hydride prepared using (A) free radical catalyst (B) platinum catalyst.

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 4B), 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 1. These solid state NMR spectra further confirmed the bonding of the phthalimide moiety to Kromasil hydride with both free radical catalyst and platinum catalyst.

Amino Phases

Before the cleavage of the N-(3-butynyl) phthalimide to the amino phase on silica, the procedure was performed using the starting compound and two sets of conditions as described in the "Procedures" section. The products (dissolved in d-methanol) obtained after extraction with ether were analyzed by solution state NMR.

In the spectrum of the product obtained via Procedure II, the peaks at 3.3 and 4.8 ppm are likely due to protons attached to the aliphatic carbons of 4-amino butyne. The above mentioned peaks were missing in the spectrum of the product after Procedure I. After analyzing the NMR spectra, phthalimide cleavage procedure (II) was applied to the modified silica, the N-(3-butynyl) phthalimide bonded phase.

The DRIFT spectra of N-(3-butynyl) phthalimide bonded phases used in the synthesis of amino phases, confirmed that the organic group was bonded to Kromasil hydride with both a free radical catalyst and a platinum catalyst. Then cleavage procedure II was performed on both products. Figure 5 shows the DRIFT spectra of the obtained amino phases prepared from N-(3-butynyl) phthalimide bonded phases by the free radical catalyst and the platinum catalyst. In both amino phases (Figures 5A and 5B), 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 (Figure 5A), and peaks at 800, 1629, and 1881 cm^{-1} in the case of the platinum catalyzed amino phase (Figure 5B), 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

Figure 5. DRIFT spectra of amino phases via N-(3-butynyl) phthalimide bonded phase prepared using (A) free radical catalyst (B) platinum catalyst.

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.

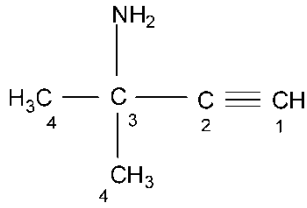
3-Amino-3-methyl-1-butyne Bonded Phase

Before preparing bonded phases from 3-amino-3-methyl-1-butyne, the parent compound was analyzed by solution state NMR. The peak assignments and chemical shifts are summarized in Table 3.[12,13]

The DRIFT spectra (not shown) confirmed that 3-amino-3-methyl-1-butyne was bonded to Kromasil hydride with a free radical catalyst, but not with a platinum catalyst.

Figure 6 shows the ^{13}C CP-MAS NMR spectra of 3-amino-3-methyl-1-butyne on Kromasil hydride prepared using a free radical catalyst (Figure 6A) and a platinum catalyst (Figure 6B). 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

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



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

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 (Figure 6B), 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, 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 result is due to the coordination of the platinum catalyst with the amino group of the 3-amino-3-methyl-1-butyne, which prevented the hydrosilation of the alkyne group. DRIFT and solid state NMR spectra of the 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 $\mu mol/m^2$) of the free radical bonded phase when compared to that of platinum catalyzed bonded phase (equivalent to 0.72 $\mu mol/m^2$).

4-Diethylamino-2-Butyn-1-ol Bonded Phase

Before the synthesis of the bonded phase containing 4-diethylamino-2-butyn-1-ol, the parent compound was analyzed by NMR by dissolving 10 μL of 4-diethylamino-2-butyn-1-ol in 0.5 mL of deuterated chloroform. The peak

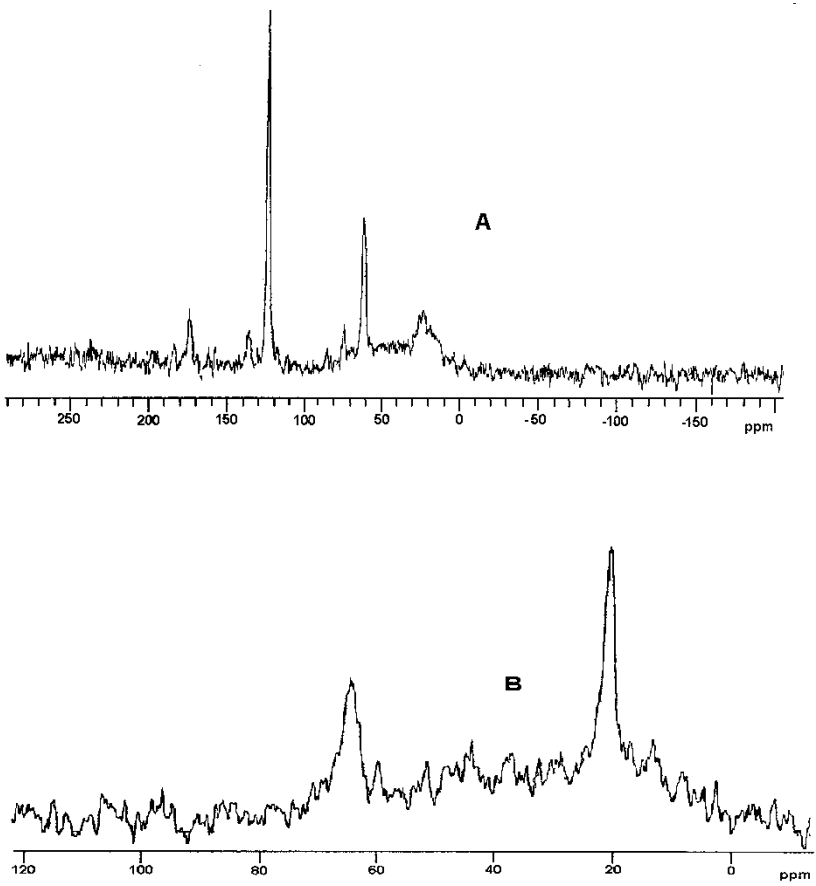


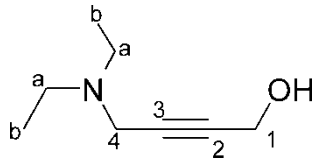
Figure 6. ^{13}C CP-MAS NMR spectra of 3-amino-3-methyl-1-butyne on Kromasil hydride prepared using (A) free radical catalyst (B) platinum catalyst.

assignments and chemical shifts of 4-diethylamino-2-butyn-1-ol are shown in Table 4.[12,13]

Bonded phases containing 4-diethylamino-2-butyn-1-ol prepared by free radical and platinum catalyst were characterized by DRIFT and NMR. The DRIFT spectra of Kromasil hydride and the 4-diethylamino-2-butyn-1-ol bonded phase prepared using platinum and free radical catalysts indicated the presence of an organic moiety.

Figure 7 shows the ^{13}C CP-MAS-NMR spectra of 4-diethylamino-2-butyn-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, where as the peak at 46 ppm corresponds to the four methylene

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



Proton NMR		Carbon 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

carbons of the bonded moiety. Since no peaks are obtained in the aromatic region, it was indicated that the organic moiety is not bonded with an olefin bond. These spectra confirmed the bonding of 4-diethylamino-2-butyn-1-ol to Kromasil hydride with both platinum and free radical catalyst. This result

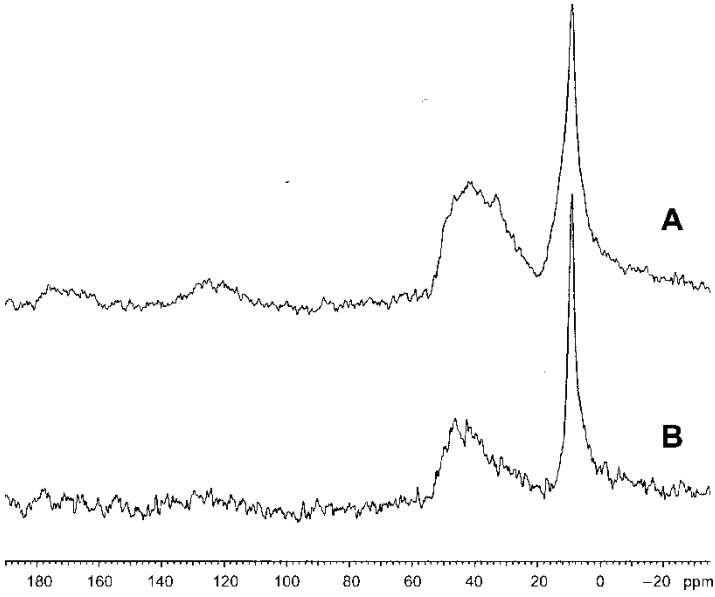


Figure 7. ^{13}C CP-MAS NMR spectra of 4-diethylamino-2-butyn-1-ol on Kromasil hydride prepared using (A) free radical catalyst (B) platinum catalyst.

is also evident in the elemental analysis (Table 1), showing significant surface coverage of both the platinum catalyzed bonded phase (1.87 $\mu mol/m^2$) and the free radical bonded phase (2.97 $\mu mol/m^2$).

Chromatographic Characterization

Chromatographic characterization of all the columns was done by performing HPLC retention studies of sugars in the aqueous normal phase mode 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 essential compounds in food sources such as sugars, flour, vegetables, and fiber. Sugar analysis is important in the generation of nutritional information, as both the sugar and total carbohydrate content must be included. Sugar samples at 5 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 composed 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.

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 8A. 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. When increasing the water concentration to 15%, only three peaks were observed and the retention times decreased (Figure 8B). This result is due to the diminished interactions between the sugars and the silanol groups. The peak identification shown in Figure 8 is the same for all the columns.

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. Chromatograms of sugars on the hydride column are shown in Figure 9. With a 10:90 water:acetonitrile mobile phase (Figure 9A), six peaks are observed. The least polar

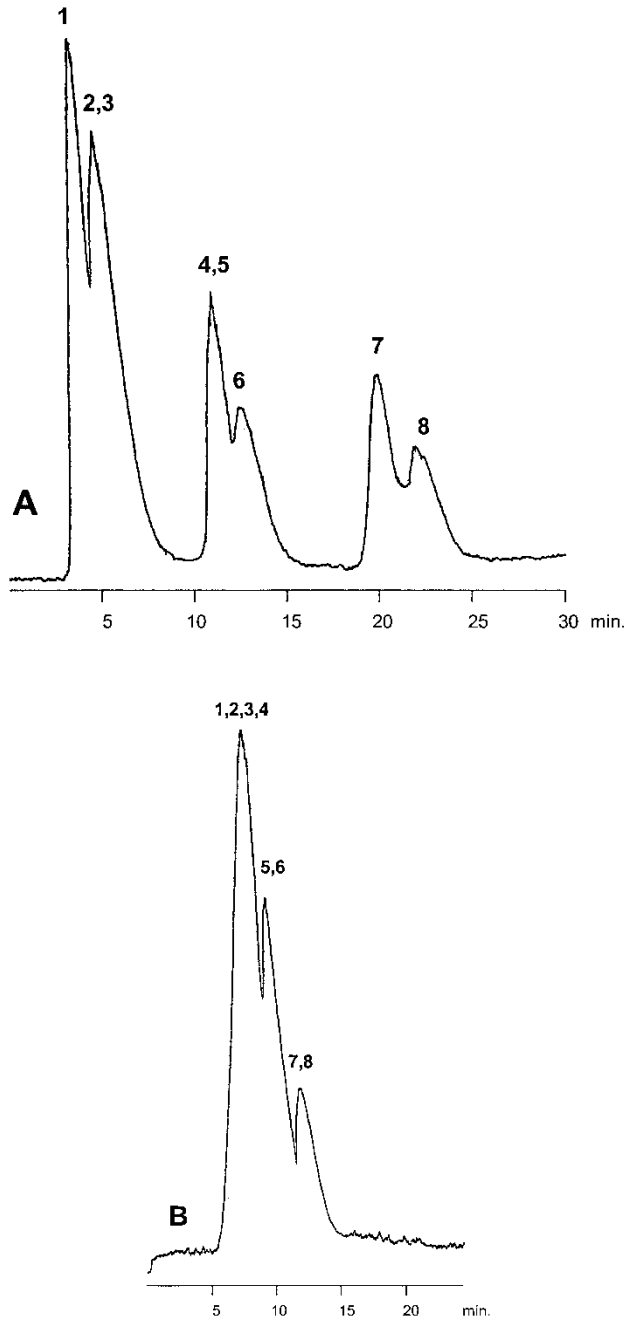


Figure 8. Chromatogram of sugars on astrosil silica with (A) 10:90 water:acetonitrile and (B) 15:85 water:acetonitrile. Samples: 1. xylose, 2. fructose, 3. glucose, 4. sucrose, 5. maltose, 6. lactose, 7. melezitose, 8. raffinose.

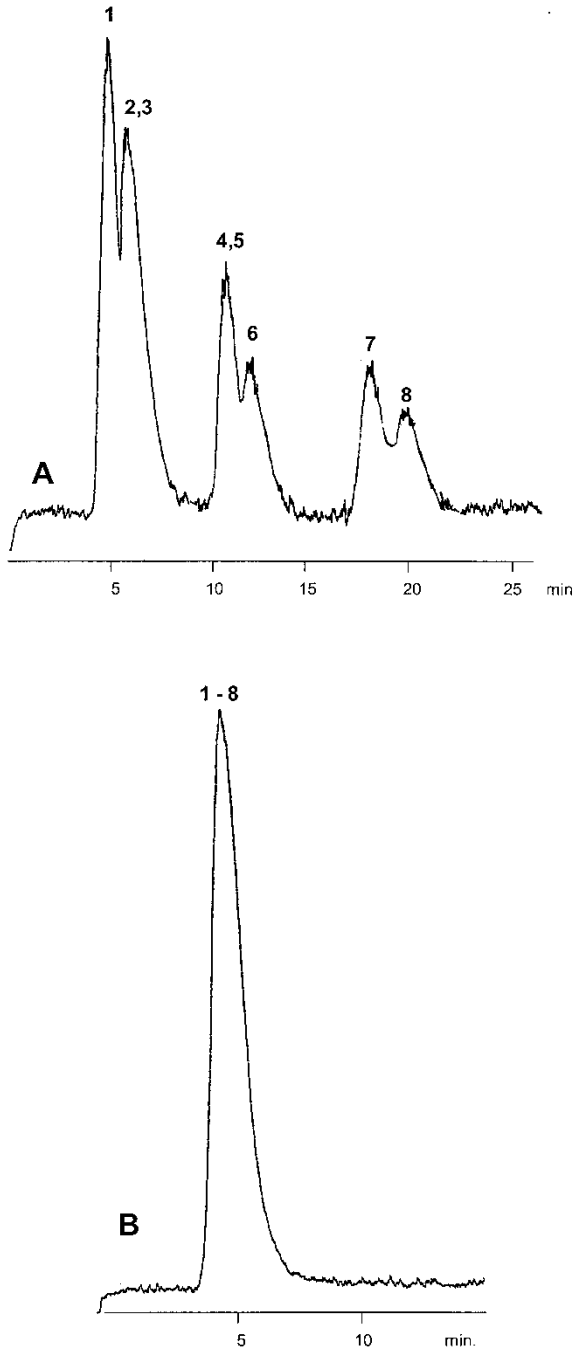


Figure 9. Chromatogram of sugars on astrosil hydride column with (A) 10:90 water: acetonitrile and (B) 15:85 water:acetonitrile. Samples as in Figure 8.

component xylose is eluted first, followed by successively more polar components fructose, glucose, sucrose, maltose, lactose, melezitose, and 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 (Figure 9B) 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 less than the bare silica, since retention on the hydride column is lower at both the 10:90 and 15:85 water:acetonitrile mobile phases.

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 not shown. 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 or near the surface.

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 chromatograms of sugars on the free radical bonded phase are shown in Figure 10. Six peaks for the eight sugar compounds were seen at 10% water (Figure 10A). Under these conditions, the fructose and glucose, and sucrose and maltose pairs were not separated. Using 15% water, retention times decreased and separation was worse (Figure 10B). The order of elution of the sugars is the same as observed on bare silica and the 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] Within the monosaccharides, the pentose (xylose) came first followed by hexoses (fructose, glucose), as xylose is

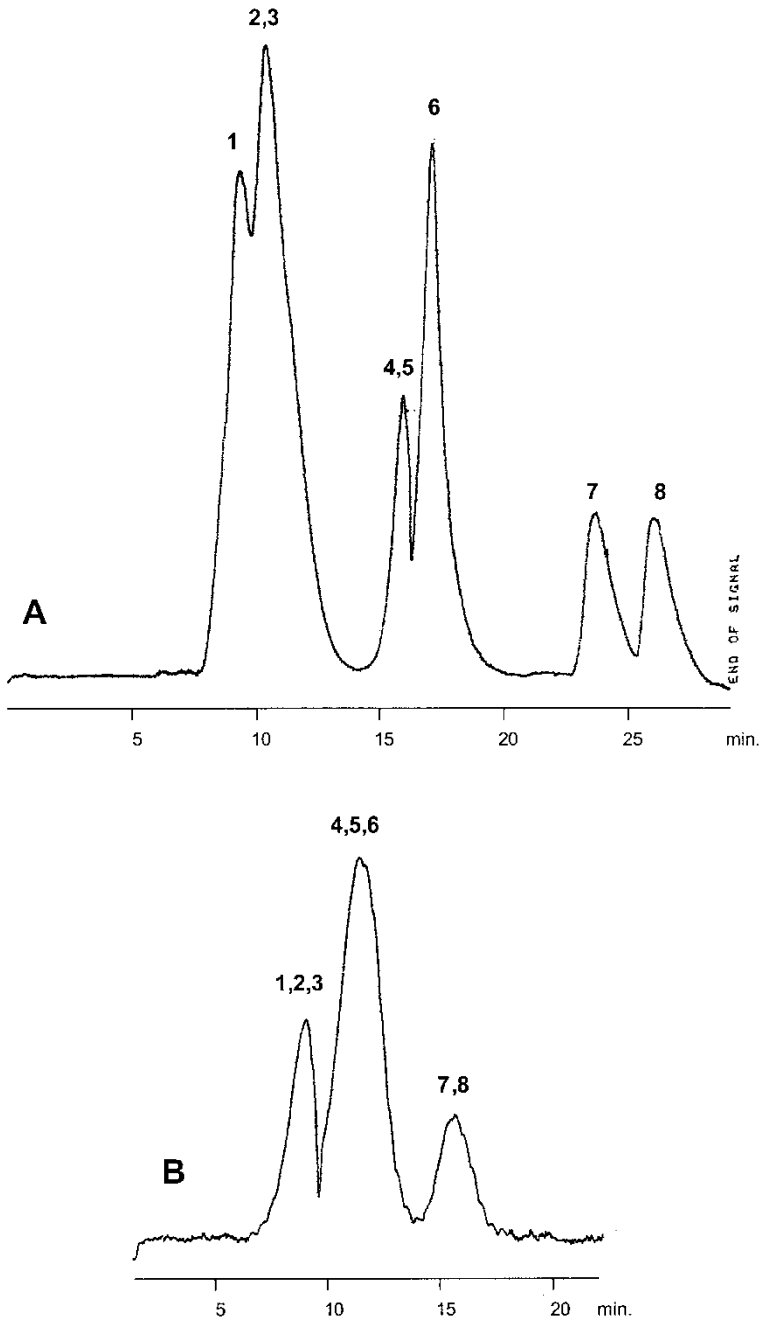


Figure 10. Chromatogram of sugars on N-(3-butynyl) phthalimide bonded phase prepared using free radical catalyst with (A) 10:90 water:acetonitrile and (B) 15:85 water:acetonitrile. Samples as in Figure 8.

the least polar with 4 hydroxyl groups when compared to glucose and fructose with five hydroxyl groups.[15] In the case of disaccharides, sucrose (glucose + fructose), maltose (2 glucoses), and lactose (glucose + galactose) have the same number of hydroxyls with glucose as a constituent in each one. So, the retention time depends on the other monosaccharide present in the molecule in the order fructose < glucose < galactose.[16] Similarly, the trisaccharides, melezitose (2 glucoses + fructose), and raffinose (glucose + fructose + galactose) with the same number of hydroxyls, have glucose and fructose in common but differ in the third monosaccharide. So, the retention time changed depending on the other monosaccharide in the order glucose < galactose.[17]

The phthalimide column made with platinum catalyst did not show any separation with both the mobile phase compositions (chromatograms not shown). This is because the phthalimide bonded phase contained a mixture of products, which are formed due to different attachment geometries, as evident from solid state NMR spectrum. Due to the mixture of products in this column, the sugars apparently did not interact well with the bonded phase and, therefore, no separation was observed.

Amino Phases

Two amino columns, one prepared via the cleavage of the N-(3-butynyl) phthalimide bonded phase made using a free radical catalyst and the second via cleavage of the N-(3-butynyl) phthalimide bonded phase made using a platinum catalyst, were characterized by sugar retention at two mobile phase compositions. 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 (Figure 11A). 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, seven peaks were obtained as shown in Figure 11B. 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.

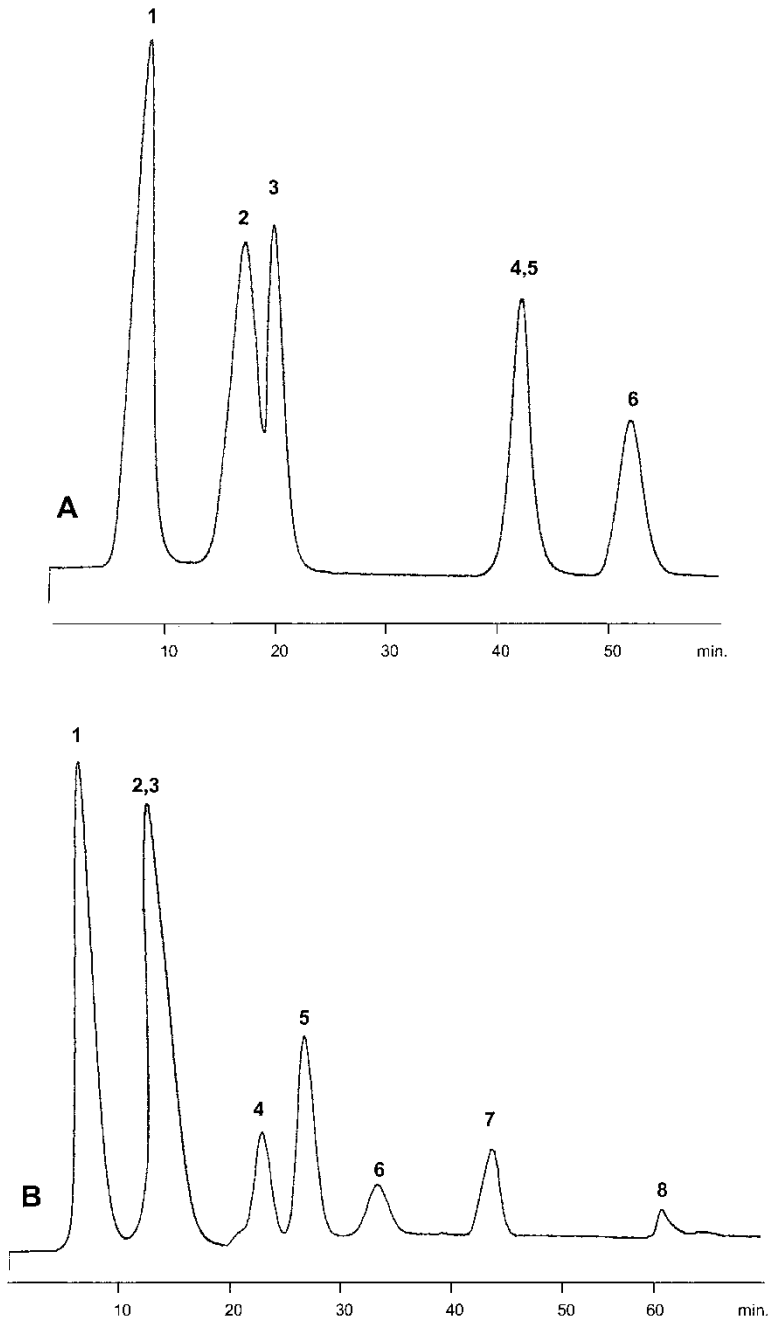


Figure 11. Chromatogram of sugars on amino phase prepared via cleavage of N-(3-butynyl) phthalimide bonded phase made with free radical catalyst at (A) 10:90 water:MeCN and (B) 15:85 water:acetonitrile. Samples as in Figure 8.

In the case of the amino phase prepared via cleavage of the N-(3-butynyl) phthalimide bonded phase made using a platinum catalyst (Figure 12A), with 10% water, good baseline 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

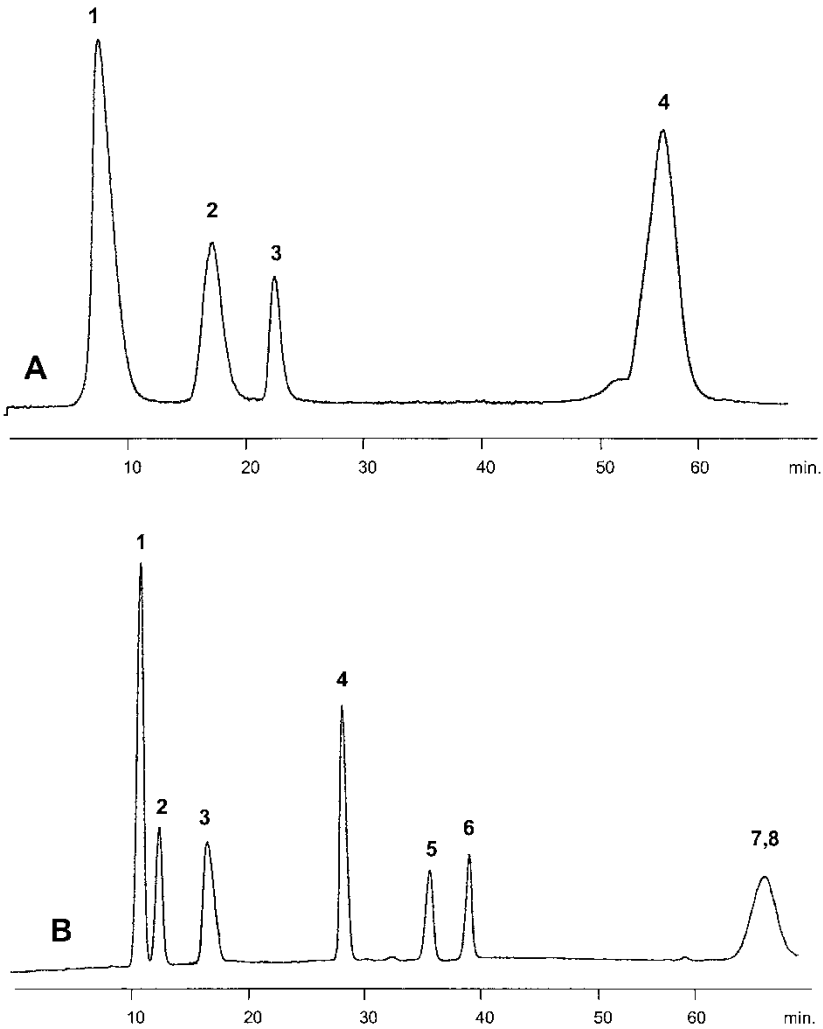


Figure 12. Chromatogram of sugars on amino phase prepared via cleavage of N-(3-butynyl) phthalimide bonded phase (made with platinum catalyst) at (A) 10:90 water:MeCN and (B) 15:85 water:acetonitrile. Samples as in Figure 8.

time. Only the melezitose-raffinose pair was not separated as shown in Figure 12B. 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-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. The fructose-glucose pair and sucrose-maltose-lactose were not separated. At 15% water in the mobile phase, a reduced retention time was observed, but five peaks were present (chromatograms not shown). 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, 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 (3 peaks vs. 5 peaks on the Pt catalyzed 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. The retention time data of the sugars on both the free radical and platinum catalyzed bonded phases is shown in Table 5.

A 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 data are shown in Table 6. Usually in normal phase HPLC, increasing the polarity of the mobile phase decreases the retention time. From the data in Table 6, it is shown 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 5. Retention data of sugars on 3-amino-3-methyl-1-butyne bonded phase

	Average retention time of sugars			
	3-Amino-3-methyl-1-butyne bonded phase (FR cat) (Water:MeCN)		3-Amino-3-methyl-1-butyne bonded phase (Pt cat) (Water:MeCN)	
Sugar sample	10:90	15:85	10:90	15:85
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

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) phthali-

Table 6. 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)	40.23	22.83
Amino phase via N-(3-butynyl) phthalimide bonded phase (Pt)	57.30	28.05
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

mide 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-butyn-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 and silica hydride, which lack an organic moiety and endcapped hydride.

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