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Synthesis and characterization of ion exchange bonded phases for HPLC

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SYNTHESIS AND CHARACTERIZATION OF ION EXCHANGE BONDED PHASES FOR HPLC

A Thesis Presented to
The Faculty of the Department of Chemistry
San Jose State University

In Partial Fulfillment
of the Requirement for the Degree
Master of Science

by
Surekha Gangakhedkar
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ABSTRACT

SYNTHESIS AND CHARACTERIZATION OF ION EXCHANGE BONDED PHASES FOR HPLC

by Surekha Gangakhedkar

The aim of this research project was the synthesis of cation-exchange stationary phases for high performance liquid chromatography (HPLC). The synthesis of silica hydride was followed by the hydrosilation reaction, where the hydride surface is modified with alkyl compounds bearing cation exchange groups. The hydrosilation reaction occurs in the presence of a platinum catalyst. The compounds that were used for the synthesis of the stationary phases were 10-undecynoic acid, undecylenic acid, 4-pentenoic acid and 4-styrene sulfonic acid. The former three compounds were used to synthesize weak cation-exchangers and the latter compound was used to synthesize a strong cation-exchanger.

The techniques used to characterize the synthesized stationary phases were elemental analysis, cross polarization-magic angle spinning nuclear magnetic resonance spectroscopy (CP-MAS NMR) and diffuse reflectance infrared fourier transform spectroscopy (DRIFT). Undecylenic acid, 10-undecynoic acid and 4-pentenoic acid stationary phases were packed into columns. Chromatographic evaluation of these columns was carried out. Separation of PTH-amino acids, nucleic acids and other compounds was tested on these columns.

ACKNOWLEDGMENTS

I would like to thank my advisor, Dr. Joseph Pesek, and his project scientist, Dr. Maria Matyska-Pesek for giving me an opportunity to work on this project and for their support and guidance. I greatly appreciate their patience and help with understanding the subject and use of instrumentation used for this work.

I would also like to thank my committee members, Dr. Pamela Stacks and Dr. Sam Perone for their valuable time in reviewing my manuscript. I appreciate their encouragement and advice.

Finally, I am thankful to my husband for his support and encouragement during this project.

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I. INTRODUCTION

A. High Performance Liquid Chromatography

Chromatography can be defined as, "a technique in which the components of a mixture are separated on an adsorbent column in a flowing system" [1]. The various chromatographic methods are classified according to their mobile and stationary phases. For example, in gas-liquid chromatography the mobile and stationary phases are gaseous and liquid respectively, whereas in liquid-liquid chromatography they are immiscible liquids, one of which is bound to an inert solid support. Prior to the 1970's, the most commonly used chromatographic techniques were paper chromatography, thin-layer chromatography and open-column chromatography. However, these chromatographic techniques were inadequate for quantification of compounds and resolution between similar compounds. This paved the way for the development of high pressure liquid chromatography (HPLC) in the mid-1970's. Since then advances in the development of column packing materials, on-line detectors, computers and automation have added to the convenience of HPLC. Today, HPLC is widely used as an indispensable analytical technique in a wide variety of fields ranging from biotechnology, cosmetics, food, and environmental industries.

The basic components of an HPLC system are:

- Eluent containers for the mobile phase - Mobile phase refers to the solvent being continuously applied to the column, or stationary phase. The mobile phase acts as a carrier for the sample solution.

- Injector - The sample solution is injected into the mobile phase through the injector port.
- Column - It contains the solid support (stationary phase) over which the mobile phase continuously flows. As the sample solution flows with the mobile phase through the stationary phase, the components of that solution will migrate according to the non-covalent interactions of the compounds with the stationary phase.
- Pump - to move the mobile phase and sample through the system
- Detector - to visualize the separated components.
- Data collection device to assist in interpretation and storage of results
- Waste container for the solvent.

B. Silica

Silica is most commonly used for packings in HPLC columns. Porous silica used in chromatography is an amorphous, non-crystalline material [2]. Silica is used as the base material for the synthesis of stationary phases because of its high surface area, excellent mechanical strength, high efficiency, and modest cost. Silica can be used in both aqueous and non aqueous environments. The high internal surface area of silica allows for good analyte - stationary phase interaction.

1. Structure of Silica

The structure of silica is shown in Figure 1. The matrix of the primary silica gel consists of core of Si atoms joined together with oxygen atoms by siloxane (Si-O-Si)

bonds. In addition, the surface also contains residual, uncondensed OH groups from the original polymeric silicic acid. These hydroxyl groups form the reactive silanols (Si-OH) bonds that not only confer upon silica gel its polar properties, but react with silane reagents to form bonded phases. Silanols can exist on the surface in single (isolated), geminal or vicinal forms [2]. The various types of silanols are shown in Figure 2. Silanols are hydrophilic while siloxanes are hydrophobic. Water can also be hydrogen bonded to the OH groups. When the silica surface is fully hydroxylated, silica has a maximum silanol density of 8.0 $\mu mol/m^2$.

2. Limitations of Silica

The pore structure of silica is very sensitive to treatment with aqueous solutions. Prolonged treatment of silica with aqueous solutions decreases the specific surface area [3]. Silica can be used in the limited pH range of 2-8. Below pH 2, the bonded organic phase can undergo hydrolysis. At pH>9.0 the samples dissolve forming silicates. Ionic impurities, such as metal ions affect the solubility and stability of products. Further, the acidic silanols may cause tailing or irreversible adsorption of basic compounds if they are not removed or covered.

C. Methods of Silica Modification

A number of reactions can be used to modify the silica surface with a variety of organic ligands. The resulting stationary phases can be hydrophobic, hydrophilic or

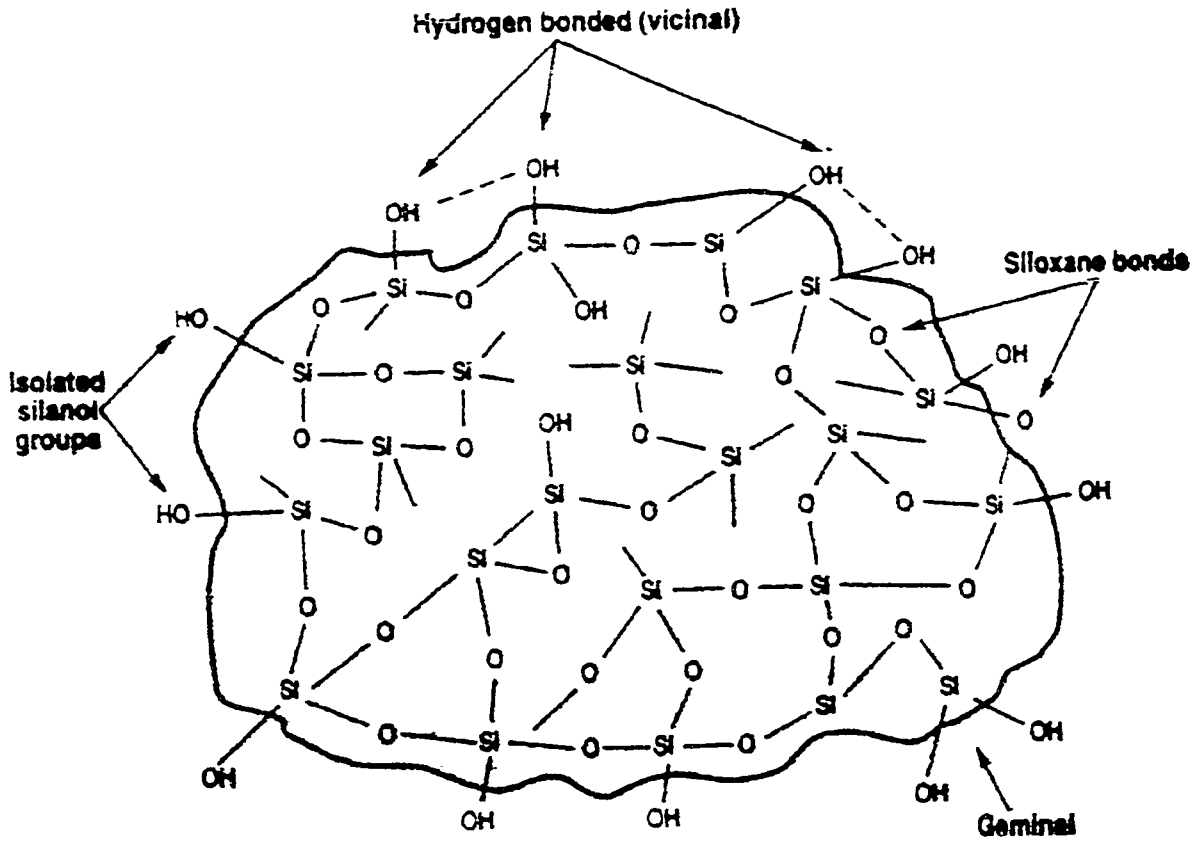


Figure 1 : Structure of silica

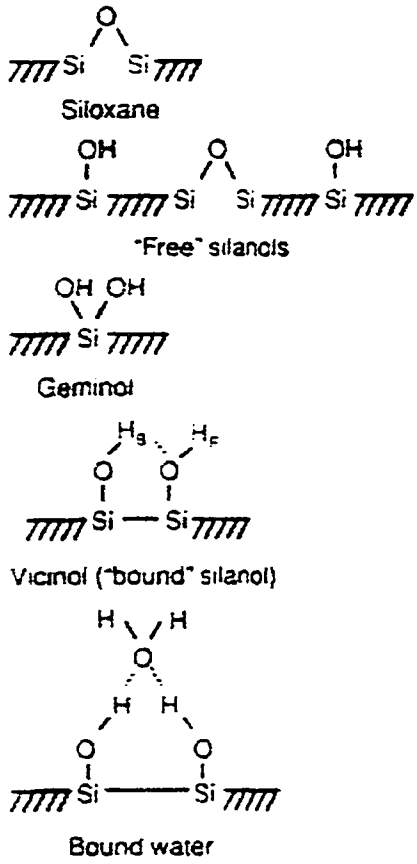


Figure 2 : Types of silanols

ionic depending upon the type of organic ligand attached. A brief description of the various modification reactions follows.

1. Esterification

This reaction involves coupling of the hydroxyl groups of an alcohol and silanol [4]. This type of reaction results in a Si-O-C linkage at the surface. This type of linkage though thermodynamically stable is unstable in the presence of water. Hence, stationary phases synthesized by this method cannot be used with aqueous mobile phases.

$$\text{Si-OH} + \text{R-OH} \rightarrow \text{Si-OR} + H_2O \qquad (1)$$

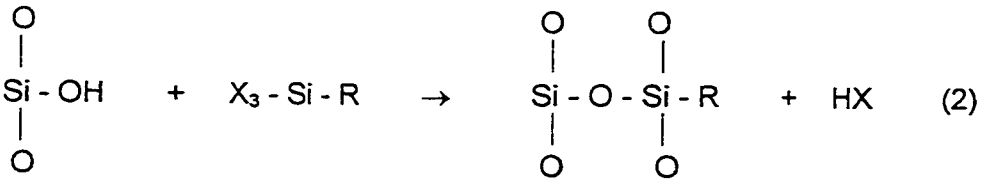
2. Organosilanization

In the early 1970's commercial phases utilizing organosilanes became available. The reaction of surface silanols with organoalkoxysilanes produces Si-O-Si-C linkages. When organomonochlorosilanes are used a monolayer of organo phase is obtained. Figure 3 represents the monomeric reaction where only one reactive group is present on the silane and only one point of attachment to the silica surface is possible [5]. Such a materials referred to as a 'monomeric phase'. The reaction scheme can be represented as follows:

$$\equiv \text{Si-OH} + \text{X - Si R'}_2\text{ R} \rightarrow \equiv \text{Si - O - Si R'}_2\text{R} + \text{HX} \qquad (1)$$

where X = halide, R = alkyl

The second approach involves the use of trichlorosilanes (an organosilane with three reactive groups). Figure 4 represents a typical derivatization reaction. A 'polymeric phase' results because bonding not only occurs at the silica surface but there is extensive crosslinking between adjacent organosilane molecules [5]. This type of reaction yields more bonded mass but less well defined surface coverage, because different silanes may anchor to the surface or to other silanes at some distance from the surface. The reaction can be represented as follows:



where, Y = H or Si

Stationary phases synthesized via this method are both thermodynamically and hydrolytically stable. The pH stability range can be extended by making the R' groups bulkier leading to the steric protection of surface in the presence of aggressive aqueous solutions [6].

3. Chlorination followed by Reaction of Grignard Reagents

Another approach that can be used is the chlorination of surface silanols followed

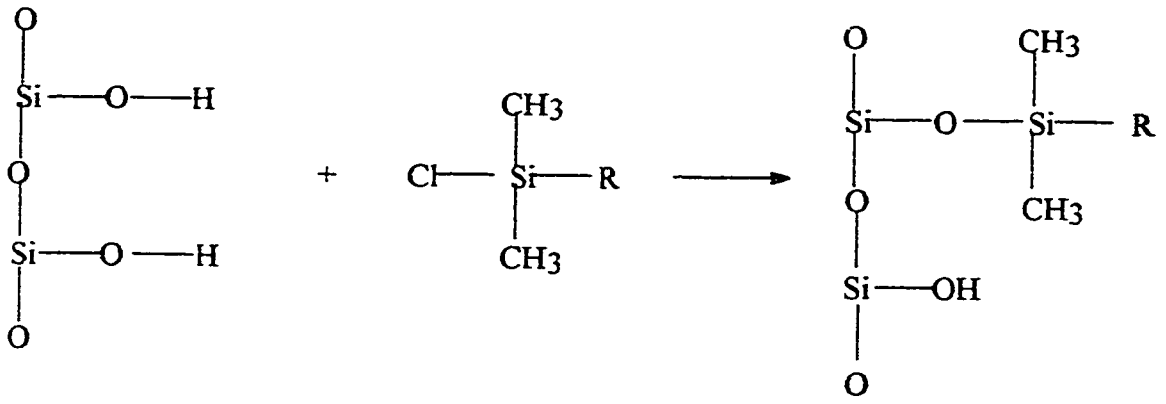


Figure 3 : Synthesis of a monomeric phase

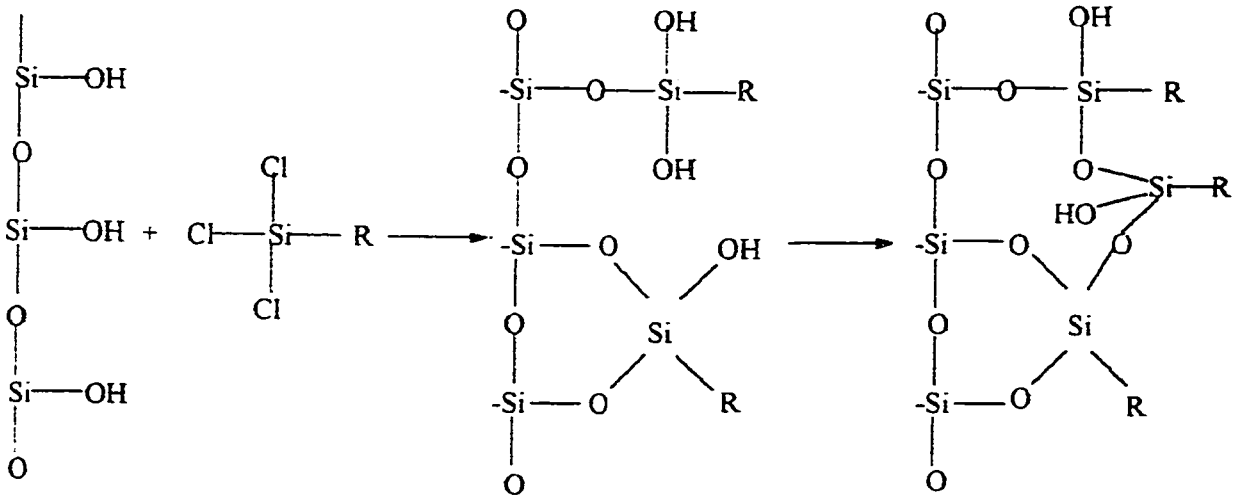


Figure 4: Synthesis of a polymeric phase

by treatment with either a grignard reagent or an organolithium compound. The later step bonds the desired organic moiety to the silica surface. This type of reaction gives a surface Si-C bond which is hydrolytically stable. The limitations of this method are that the chlorinated intermediate is unstable in the presence of water and hence requires dry working conditions. Further, the presence of residual metal salts on the silica surface is not desirable because they may result in non-uniform retention characteristics in the final product.

$$\equiv Si - OH + SOCl_2 \xrightarrow{\text{toluene}} \equiv Si - Cl + SO_2 + HCl \qquad (1)$$

$$\equiv Si - Cl + BrMgR \rightarrow \equiv Si - R + MgClBr \qquad (2a)$$

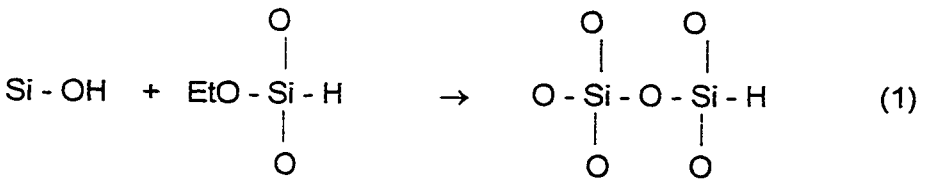
or

$$\equiv Si - Cl + Li - R \rightarrow \equiv Si - R + LiCl \qquad (2b)$$

4. TES Silanization followed by Hydrosilation

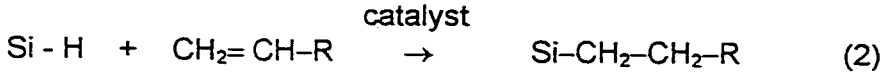
This method developed by Pesek et al.[4] produces a hydride modified support that serves as an intermediate for the synthesis of chromatographic stationary phases. This method was used to modify the silica surface in this project. This is a two step process. In the first step, the surface silanols are converted to hydrides by reaction with triethoxysilane ($HSi(OCH_2CH_3)_3$. The reaction occurs in the presence of water, an acid catalyst and an appropriate solvent, resulting in the replacement of the

surface silanols and the deposition of a hydride monolayer. Stationary phases for HPLC require high alkyl density with a minimal amount of residual silanols. This can be accomplished by using the smallest possible silane, TES.



(1)

Hydrosilation can be defined as the addition reactions of organic and inorganic silicon hydrides to multiple bonds such as -C≡C-, -C=C-, =C=O, =C=N-, -C=N, -N=N-, -N=O [8]. Hydrosilation reactions can proceed either by a free radical mechanism or catalyzed by transition metal salts, nucleophilic-electrophilic reactions. Catalysts most commonly used for this reaction are organic and inorganic complexes of transition metals such as platinum, rhodium, palladium, ruthenium, iridium, and nickel (group VIII). In 1957, John L. Speier from Dow Corning Co. discovered that hexachloroplatinic acid ($H_2PtCl_6.6H_2O$) is an effective homogenous catalyst for hydrosilation. A solution of chloroplatinic acid hexahydrate dissolved in 2-propanol and referred to as Speier's catalyst is the most widely used platinum catalyst. An important characteristic of the H_2PtCl_6-solvent system is the requirement of an induction period before a fast exothermic hydrosilation reaction.



(2)

The hydrosilation reaction results in the replacement of the hydrides on the surface with the desired organic moiety. Usually, the amount of olefin used is in excess to

the number of silicon hydride groups available. The advantages of this method are increased alkyl density and enhanced coating stability [4]. This type of reaction results in a Si-C linkage at the surface. The hydride intermediate is stable in the presence of air and water.

D. Ion Exchange Chromatography

In ion-exchange chromatography, species are separated on the basis of differences in electric charge. An ion-exchanger in aqueous solution consists of anions, cations, and water, where either the cations or the anions are chemically bound to an insoluble matrix. The chemically bound ions are referred to as fixed ions and the ions of opposite charge are referred to as the counter-ions. The ion-exchanger is classified as cation-exchange when the fixed ion carries a negative charge and exchanges cations from solution [9].

$$\underset{\text{(stationary phase)}}{R^-Y^+} + \underset{\text{(solution)}}{X^+} \Leftrightarrow \underset{\text{(stationary phase)}}{R^-X^+} + \underset{\text{(solution)}}{Y^+}$$

In an anion exchanger, the fixed ion carries a positive charge and exchanges anions from solution.

$$\underset{\text{(stationary phase)}}{R^+Y^-} + \underset{\text{(solution)}}{X^-} \Leftrightarrow \underset{\text{(stationary phase)}}{R^+X^-} + \underset{\text{(solution)}}{Y^-}$$

Ion Exchangers may further be classified as

- Strong acid or base
- Weak acid or base

Strong ion exchangers retain the charge on the fixed ion over a wide pH range, whereas weak exchangers are ionized only over a much narrower pH range. Table 1 summarizes the possible functional groups on synthetic ion-exchange materials. The most commonly used substituent groups are sulfonate or trialkylammonium groups for the strong ion-exchangers and carboxylate or amine groups for weak ion-exchangers. Other substituent groups have so far not found significant use in HPLC.

E. Characterization Techniques

1. Diffuse Reflectance Infrared Fourier Transform Spectroscopy (DRIFT)

Infrared spectroscopy is one of the most important analytical techniques used to characterize inorganic and organic molecules by absorption frequencies that are characteristic of certain functional groups in the compound. This technique is based on the vibrations of the atoms of a molecule. An infrared spectrum is obtained by passing radiation through a sample and determining what fraction of the incident radiation is absorbed at a particular energy. The energy at which any peak in an absorption spectrum appears corresponds to the frequency of a vibration

Table 1: Functional groups on some synthetic ion-exchange materials.

Type	Functional group	Classification
Cation-exchangers		
Sulfonic acid	$-SO_3^-H^+$	Strong
Carboxylic acid	$-COO^-H^+$	Weak
Phosphonic acid	$-PO_3H^-H^+$	Weak
Phosphinic acid	$-PO_2H^-H^+$	Weak
Phenolic	$-O^-H^+$	Weak
Arsonic acid	$-AsO_3H^-H^+$	Weak
Selenoic acid	$-SeO_3^-H^+$	Weak
Anion-exchangers		
Quartenary amine	$-N(CH_3)_3^+OH^-$	Strong
Tertiary amine	$-NH(CH_3)_2^+OH^-$	Weak
Secondary amine	$-NH_2(CH_3)^+OH^-$	Weak
Primary amine	$-NH_3^+OH^-$	Weak

of a part of a sample molecule. The uses of the infrared spectrum are that, every different bond has a different natural frequency of vibration, and since the same type of bond in two different compounds is in a slightly different environment, no two molecules of different structure will have exactly the same infrared spectrum [10]. Also, the absorptions of each type of bond (N-H, C-H, O-H, C-O, C-C, C=C, C≡C etc.) are found only in certain small portions of the vibrational infrared region. Hence, a small range of absorption can be defined for each type of bond.

Infrared spectroscopy can be used to study the bonding of organic compounds at the silica surface. Functional group information about molecules that are either adsorbed on or chemically bound to the surface can be obtained. The Fourier transform mode facilitates signal averaging hence, spectra of low surface area materials can be obtained [6]. An increase in sensitivity is obtained by signal averaging so that samples with a low concentration of bonded or absorbed species can be characterized. Collection of spectra via diffuse reflectance provides for a further increase in sensitivity. In the case of samples with high surface area, an ordinary transmission spectrum can be obtained if the sample is mixed with KBr.

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

NMR is a valuable spectroscopic technique for the identification and characterization of a wide range of organic and inorganic compounds. NMR

provides information about the number of each type of nuclei as well as the nature of the immediate environment of each of these types of nuclei.

NMR spectra of solids are generally characterized by broad and featureless resonances due to fixed orientation of bonds. Magic angle spinning (MAS) is a technique employed to eliminate line broadening due to chemical shift anisotropy. In MAS the samples are spun at a high speed (several KHz) at an angle of 55° (the "magic angle") with respect to the applied magnetic field [6]. This averages the chemical shift variations so that a single sharp line is obtained for each chemically different type of a particular nucleus. In surface characterization, complications can occur due to the low sensitivity of the nucleus to be observed (carbon-13 and silicon-29). In such cases, cross polarization (CP) can be used to match the frequencies between the insensitive nucleus and hydrogen such that some of the energy associated with protons can be transferred to a nuclei of low natural abundance like ^{13}C and ^{29}Si [7]. This results in an increase in signal intensity. Further, use of CP shortens the time needed to acquire the spectrum of solid samples containing carbon-13 or silicon-29. Thus, more data sets can be obtained resulting in a higher signal to noise ratio.

a) CP-MAS Carbon-13 NMR

Structural information about the molecule bonded to the oxide surface can be obtained by carbon-13 NMR. In many cases resonances of individual carbon atoms

can be identified which will confirm the correct structure of the bonded molecule as well as to confirm bonding.

b) CP-MAS Silicon-29 NMR

Silicon-29 NMR can be used to directly monitor the changes occurring at the silica surface or any other oxide which has been modified by TES silanization to produce a hydride intermediate. The use of cross polarization has greatly reduced the relaxation time of ^{13}C and ^{29}Si nuclei in the solid state. Since the efficiency of cross polarization is dependent on the Si-H distance, internal Si atoms do not contribute to the CP-MAS spectrum [6].

3. Elemental Analysis

Combustion analysis is widely used to determine the chemical composition of a wide range of compounds. For example, the method of determining the amounts of carbon and hydrogen in a substance involves combustion to carbon dioxide and water. In the case of surface modification of oxides, elemental analysis provides information about the presence of an organic moiety on the surface as well as quantitative information based on the known structure of the compound. Carbon analysis is carried out after the surface modification of silica because it does not have this element under normal conditions. The carbon percentage is an indication of the alkyl density on the silica surface.

F. Compounds used in Stationary Phase Synthesis

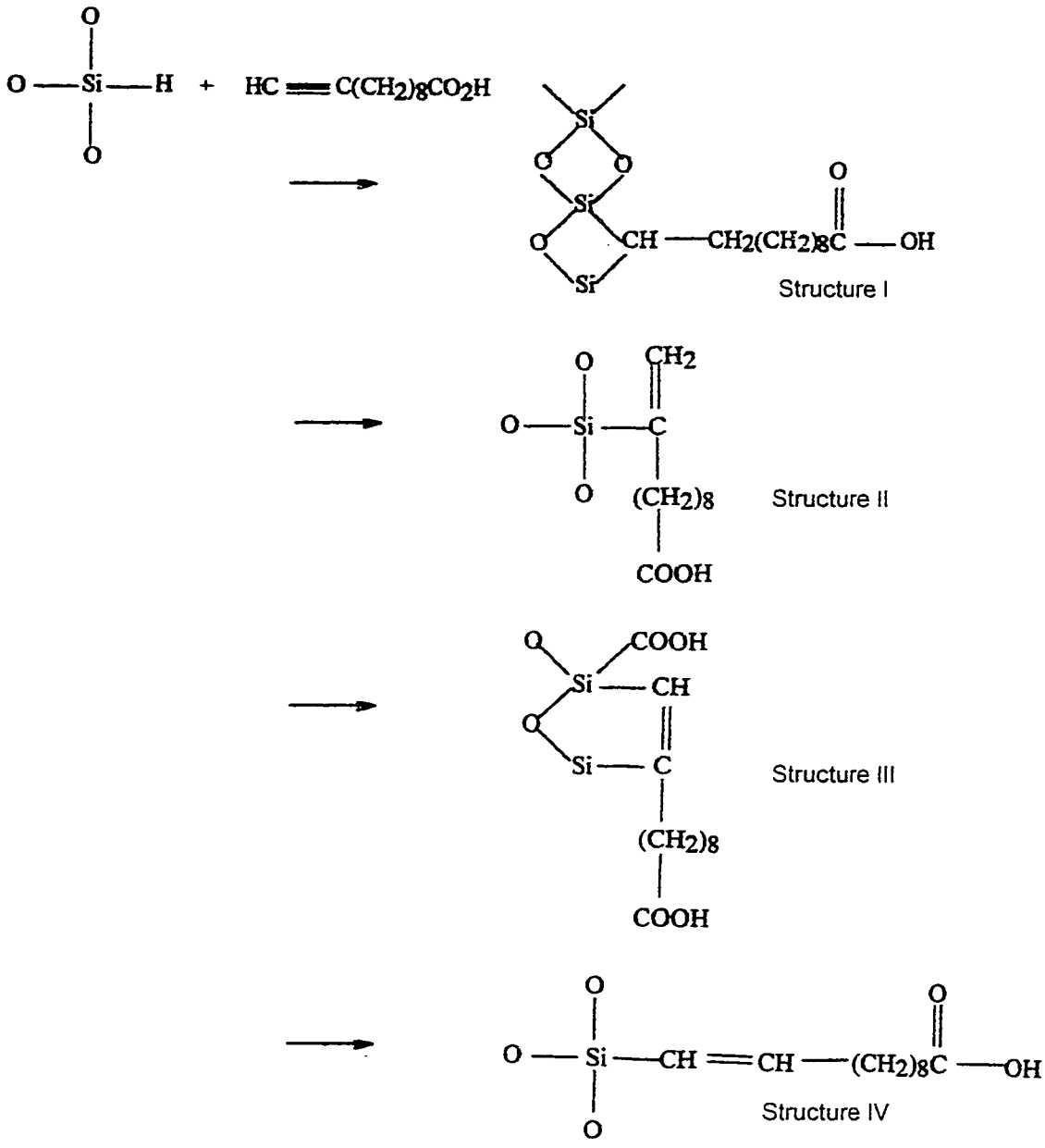
Four different compounds were selected to determine their use as HPLC stationary phases. The compounds are listed in Table 2.

Table 2 : Compounds used in Stationary Phase Synthesis

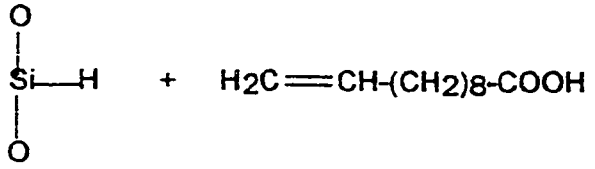
Compound	Molecular Formulae	Molecular Weight
10 - Undecynoic acid	$HC \equiv C(CH_2)_8COOH$	182.27
Undecylenic acid	$H_2C = CH(CH_2)_8COOH$	184.28
4 - Pentenoic acid	$H_2C = CHCH_2CH_2COOH$	100.12
4 - Styrene sulfonic acid	$H_2C = CHC_6H_4SO_3Na$	206.20

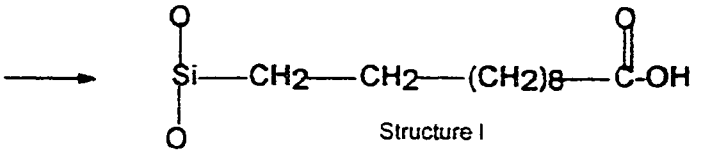
These compounds were used to synthesize silica stationary phases bearing cation exchange functional groups. Synthesis of cation exchange stationary phases has not been carried out using the hydrosilation reaction. Various bonded phases have been synthesized via the hydrosilation reaction using acetylene and olefin compounds. In hydrosilation, acetylene compounds are known to form stable linkages where the terminal carbon is attached to two silicon atoms (page 19- Structure 1). One of the objectives of this project was to evaluate the stability and chromatographic properties of stationary phases made from acetylene and olefin compounds. Further, a comparison in the properties of long and short alkyl chain columns can be made. Hydrosilation reactions of compounds used in this study and their possible products are shown in the following pages.

1. 10 - Undecynoic acid

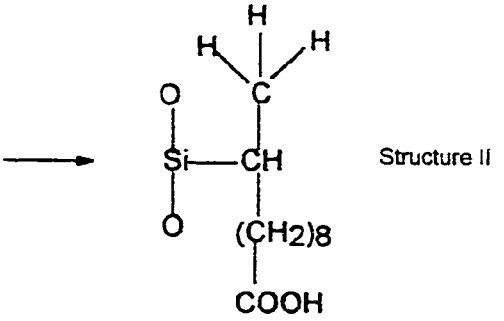


2. Undecylenic acid

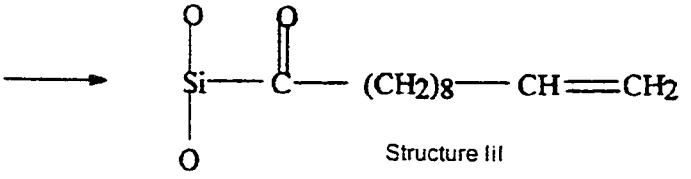




Structure I

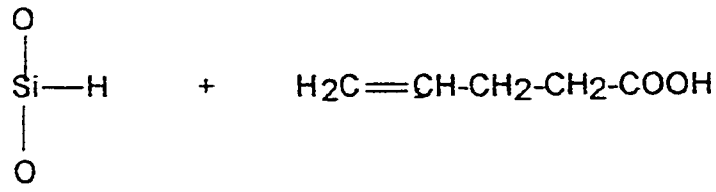


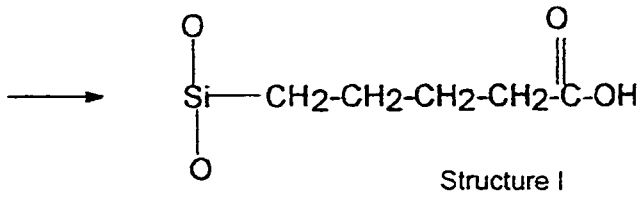
Structure II



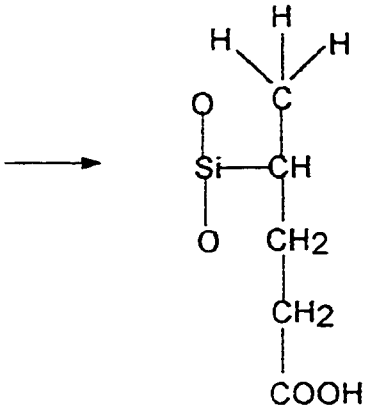
Structure III

3. 4 - Pentenoic acid

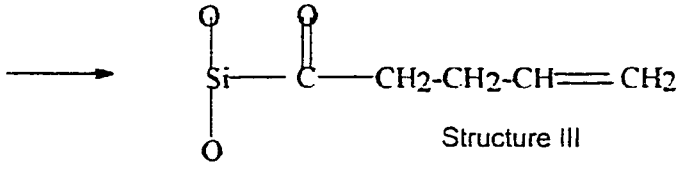




Structure I

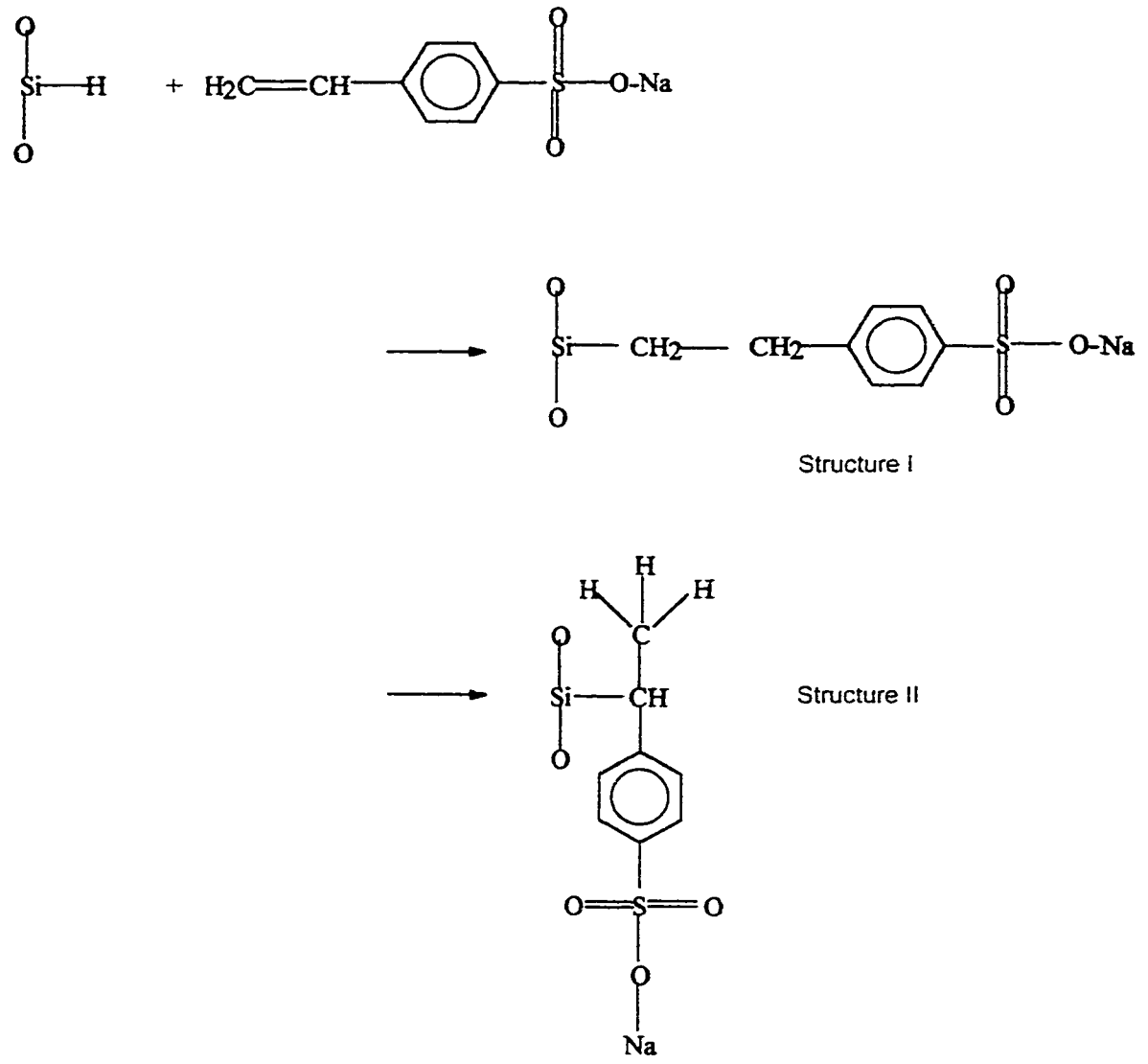


Structure II



Structure III

4. 4 - Styrene sulfonic acid



G. Goals of the Research

The primary goal in this research was to synthesize cation-exchange stationary phases for HPLC. The surface modification of silica to yield the bonded phase was carried out in two steps. First, a layer of Si-H monolayer was synthesized by TES silanization of the surface silanols. In the next step, organic moieties were bonded on the hydride intermediate by a hydrosilation reaction to give the corresponding bonded phases. The synthesized stationary phases were characterized using various analytical techniques like diffuse reflectance infrared fourier transform spectroscopy (DRIFT) , ^{13}C and ^{29}Si cross polarization magic angle spinning nuclear magnetic resonance spectroscopy (CP-MAS NMR) and elemental analysis. The final goal was the chromatographic evaluation of the bonded phases. This was carried out by the separation of PTH-amino acids, nucleic acids and other compounds.

II. EXPERIMENTAL

A. Materials

All the materials used for this research are listed in tables. All the chemicals were used as received.

Table 3 : Chemicals used in Stationary Phase Synthesis

Name	Manufacturer	CAS Number
Acetic acid	Aldrich Chemical Co. Milwaukee, WI	[64-19-7]
Acetonitrile	Aldrich Chemical Co. Milwaukee, WI	[75-05-8]
1,4-Dioxane	Fisher Scientific Fair Lawn, NJ	[123-91-1]
Ethyl Ether	Fisher Scientific Fair Lawn, NJ	[60-29-7]
Hydrochloric acid	Aldrich Chemical Co. Milwaukee, WI	[7647-01-0]
Hexachloroplatinic acid (38-40 % Pt)	Strem Chemicals. Newbury Port, MS	[16941-12-1]
Methylene Chloride	J.T Baker, Inc. Phillipsburg, NJ	[75-09-2]
Methanol	Fisher Scientific Fair Lawn, NJ	[67-56-1]
4 - Pentenoic acid	Aldrich Chemical Co. Milwaukee, WI	[591-80-0]
Potassium Bromide	Aldrich Chemical Co. Milwaukee, WI	[7758-02-3]
4 - Styrene sulfonic acid, sodium salt hydrate	Aldrich Chemical Co. Milwaukee, WI	[2695-37-6]
Triethoxysilane	Huls Petrach Systems. Huls, Bristol, PA	[998-30-1]
Tetrahydrofuran	Fisher Scientific Fair Lawn, NJ	[109-99-9]
Toluene	EM Science Gibstown, NJ	[108-88-3]
10 - Undecynoic acid	Farchan labs, Inc. Gainesville, Florida	[2777-65-3]
Undecylenic acid	Aldrich Chemical Co. Milwaukee, WI	[112-38-9]

Table 4 : Compounds used in HPLC Analysis

Chemicals	CAS Number
PTH - Aspartic acid	[5624-13-5]
PTH - Asparagine	[5624-08-8]
PTH - Cysteic acid	[108321-85-3]
PTH - Glutamine	[10567-96-9]
PTH - Hydroxyproline	[81703-65-3]
PTH - Isoleucine	[5066-94-4]
PTH - Leucine	[4399-40-0]
PTH - Methionine sulfone	[68984-76-9]
PTH - Proline	[2963-99-2]
PTH - Serine	[5789-22-0]
PTH - Tryptophan	[5789-24-2]
Aminophylline	[1603-40-3]
Caffeine	[58-08-2]
2,3 dihydroxypropyl theophylline	[479-18-5]
Theophylline	[58-55-9]
Adenine	[73-24-5]
Adenosine	[58-61-7]
Cytosine	[71-30-7]
Thymine	[65-71-4]
Uracil	[66-22-8]

The solvents were reagent grade. The silica used was Vydac TP 106 obtained from The Separations Group, Hesperia, CA.

B. Synthetic Procedures - Silica Derivatization

1. Preparation of Silica Hydride

The preparation of silica hydride was carried out according to the following procedure. Fifteen grams of Vydac silica (101 TP # 900201, with particle diameter of 6.583 μm, an average pore diameter of 300 A° and a specific surface area (S_{BET}) of 106.5 m^2/g), was placed in a vacuum oven (VWR Scientific Model 1410) at 110° for twelve hours. All required glassware was cleaned and placed in an oven (Precision Scientific Company Model 17) at 110°C overnight. In a N_2-filled glove box, 12.67 mL of 1.00 M of triethoxysilane (TES) was added to 54.8 mL of distilled 1,4-dioxane. The TES-dioxane mixture was transferred to a 125 mL pressure-equalizing addition funnel in the glove box. A 500 mL, 3-neck, round bottom flask was equipped with a drying tube, west condenser tube, thermometer, magnetic stirrer, heating mantle, and an electric heater. The silica, 15 mL of 2.3 M HCl, and 367.5 mL of dioxane were transferred to the round bottom flask. The joints were sealed with parafilm. The reaction mixture was heated to reflux at 93°C until a clear solution was observed. At this stage, the TES - dioxane mixture was added drop wise from the addition funnel (~25 - 30 minutes). After the addition of TES was completed, the system was maintained at a temperature of 93°C for an hour. The mixture was centrifuged (IEC HN-S Centrifuge, Needham Heights, MA) for ten

minutes at 1500 rpm and the solid was washed with 15-20 mL portions of toluene, methylene chloride, and diethyl ether (three times with each solvent). The product was dried overnight in the hood at room temperature and then under vacuum at 110°C for an additional 24 hours. The silica hydride product was then characterized by DRIFT.

2. Preparation of Speier's Catalyst

This synthesis was carried out in a glove box that is flushed with vacuum and nitrogen alternately three times. 0.4 g of chloroplatinic acid hexahydrate (Strem Chemicals, Newbury Port, MS) was dissolved in 100 mL of distilled isopropanol at 93°C to give 10 mM Speier's catalyst. The solution was then stored in the refrigerator.

3. Hydrosilation Reaction

Four grams of silica hydride were placed in a vacuum oven at 110° overnight and all required glassware was treated as above. About 53 mL of toluene, 8.4 mL of 1 mM speier's catalyst, and olefin compound equivalent to 1.512 mmoles were added to a round bottom flask equipped with a drying tube, west condenser tube, thermometer, magnetic stirrer, heating mantle, and an electric heater. The mixture was heated at a constant temperature of 70 - 80 °C for an hour. The silica hydride was then added slowly through a funnel to the solution in the flask and heated at 100 °C for 96 hours. The mixture was centrifuged (IEC HN-S Centrifuge, Needham Heights, MA) for ten minutes at 1500 rpm and the solid was washed with 15-20 mL portions

of toluene, methylene chloride, and diethyl ether (three times with each solvent). The product was dried overnight in the hood at room temperature and then under vacuum at 110°C for an additional 24 hours. The product was then characterized by DRIFT, CP-MAS NMR, elemental analysis, and HPLC.

C. Column Packing

Silica hydride modified with 10-undecynoic acid, undecylenic acid and 4-pentenoic acid was slurry packed under nitrogen into three 15 cm x 0.46 cm internal diameter stainless steel columns (Alltech Co., Deerfield, IL) using a Haskel (Burbank, CA) pneumatic pump at 6,000 psi. Two grams of the synthesized stationary phase were added to about 20 mL of carbon tetrachloride : methanol (90:10) mixture and the slurry was added to the pump reservoir. Filtered HPLC- grade methanol (Fisher Scientific) was used as the driving solvent. After the column was packed, it was left on the packing apparatus for 30 minutes. The columns were then capped with plastic fittings.

D. Hydrolytic Stability Tests

1. Low pH hydrolysis

For the acid hydrolysis, 5 mL of 10 mM hydrochloric acid (pH 2.02) containing 10% methanol was added to 200 mg of bonded phase in a centrifuge tube (1.24 X 4 inches). The mixture was magnetically stirred for 24 hours at room temperature. The solution was centrifuged for ten minutes at 1500 rpm. The

supernatant was discarded and the solid was washed with 10-15 mL portions of toluene, methylene chloride, and diethyl ether (three times with each solvent). The product was dried overnight in the hood at room temperature and then under vacuum at 110°C for 24 hours.

Another hydrolysis reaction was carried out on the synthesized bonded phases under similar conditions but at a temperature of 50°C.

2. High pH hydrolysis

For the base hydrolysis, 5 mL of 10 mM sodium hydroxide (pH 9) containing 10% methanol was added to the bonded phase and treated as above.

Base hydrolysis of the samples at a temperature of 50°C was carried out. The conditions were as mentioned above. The final products were characterized by DRIFT.

E. Instrumental Procedures

1. Diffuse Reflectance Infrared Fourier Transform Spectroscopy (DRIFT)

DRIFT spectra of the silica samples were taken using an ATI Mattson Infinity series FT-IR spectrometer with a diffuse-reflectance accessory. The spectrometer was equipped with a Hewlett Packard Venturis FX-2 computer containing Winfirst software. The plotter used was a Hewlett Packard Desk Jet 722C. The KBr used

for the background spectrum was ground using a mortar and pestle (~5 minutes) and stored in an oven at 110°C until use. The silica samples were made with 5% IR-grade KBr. The sample compartment was purged with nitrogen gas for 15 minutes to remove CO_2 and water. Spectra were collected at a resolution of 2 cm^{-1} in the region of 4000 - 450 cm^{-1} and averaged over 100 scans.

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

The ^{13}C and ^{29}Si CP-MAS NMR spectra were taken on a Burker (Billerica, MS) MSL 300 MHz spectrometer. About 200-300 mg of solid sample was placed in a ZrO_2, double-bearing rotor. The spinning speed was 5 KHz. The probe temperature was 20 ± 2°C. Chemical shifts were referenced to tetramethylsilane using external glycine as the standard for ^{13}C spectra acquisition. In the case of ^{29}Si spectra, chemical shifts were referenced to external poly(hydrido)siloxane. Pulse widths of 6.5 μsec for ^{13}C and 5.0 μsec for ^{29}Si were used. A 5 μs contact time and a 5 sec repetition time was used for both ^{13}C and ^{29}Si analyses.

F. Elemental Analysis (EA)

Elemental analysis was carried out by Desert Analytics (Tucson, AZ). Carbon percentage of the synthesized stationary phases was carried out using the combustion method. The carbon percentage was used to calculate surface

coverage. This provides quantitative information about the amount of organic moiety bonded on the silica surface. The relationship between the carbon percentage determined by elemental analysis and surface coverage is given by the following equation developed by Berendsen and de Galan [11]:

$$\alpha_R\ (\mu mol/m^2) = \frac{10^6 p_C}{(100\ M_C n_C - p_C M_R) S_{BET}}$$

where,

p_C = carbon percentage by weight of the bonded phase,

M_C = atomic weight of carbon,

M_R = molecular weight of the bonded organic moiety,

n_C = number of carbon atoms in the organic moiety, and

S_{BET} (m^2/g) = Brunauer, Emmet, and Teller (BET) specific surface area of silica hydride. Usually, as the length of the alkyl chain increases the surface coverage decreases.

G. High Performance Liquid Chromatography

The HPLC system used in this study consisted of a Hitachi L-6200 pump, UV detector (Hitachi L-4000), Spectra Physics Chromjet intergrator, and a manual injection valve (Rheodyne) with 20 μL loop. The solvents were degassed with high purity grade helium gas at 50mL/min for ~10 minutes and maintained at 5 mL/min during analysis. Helium gas was used to prevent the formation of air bubbles in the tubing. All solvents were filtered through a 0.20μm nylon membrane (Alltech,

Deerfield, IL). PTH-amino acid samples were prepared by dissolving the solids in water : methanol (1:1). The theophylline samples were prepared by dissolving the solids in deionized water. In the case of the nucleic acids, the solids were dissolved in water : isopropanol (1:1) mixture. All of these samples were kept in capped sample vials (Thermo Separation Products, San Jose, CA) and stored in the refrigerator. A sonicator (Bransonic 220, Branson Cleaning Equipment, Shelton, CT) was used to break up and help dissolve particles in some of the samples.

III. RESULTS AND DISCUSSION

A. Confirmation of the formation of Silica hydride

1. Diffuse Reflectance Fourier Transform Infrared Spectroscopy results

DRIFT provides important information in surface analysis. Spectra of molecules that are either chemically bonded to surfaces or adsorbed can be obtained. Figure 5 is the DRIFT spectrum of silica hydride. The characteristic feature of the spectrum is the broad band between 3000 and 4000 cm^{-1} which is due to adsorbed water and H-bonded OH groups. The band at 3750 cm^{-1} can be attributed to the non-hydrogen bonded (isolated) silanols (≡Si-OH) groups on the surface. After the native silica is modified with TES via the silanization reaction to give the hydride intermediate, additional peaks appear on the spectrum. An intense peak is seen at 2250 cm^{-1}, which represents the Si-H stretching mode. This confirms the conversion of the surface silanols to the hydride monolayer.

2. Solid State Nuclear Magnetic Resonance Spectroscopy Results

a) ^{29}Si CP-MAS NMR

Figure 6 represents the ^{29}Si NMR spectrum of the silica hydride intermediate. The characteristic features are the peaks at -110 ppm and -100 ppm which are due to the silica matrix and a surface silicon atom with three siloxane linkages and silanol group respectively. Further, the two peaks at -85 and -75 ppm are due to a

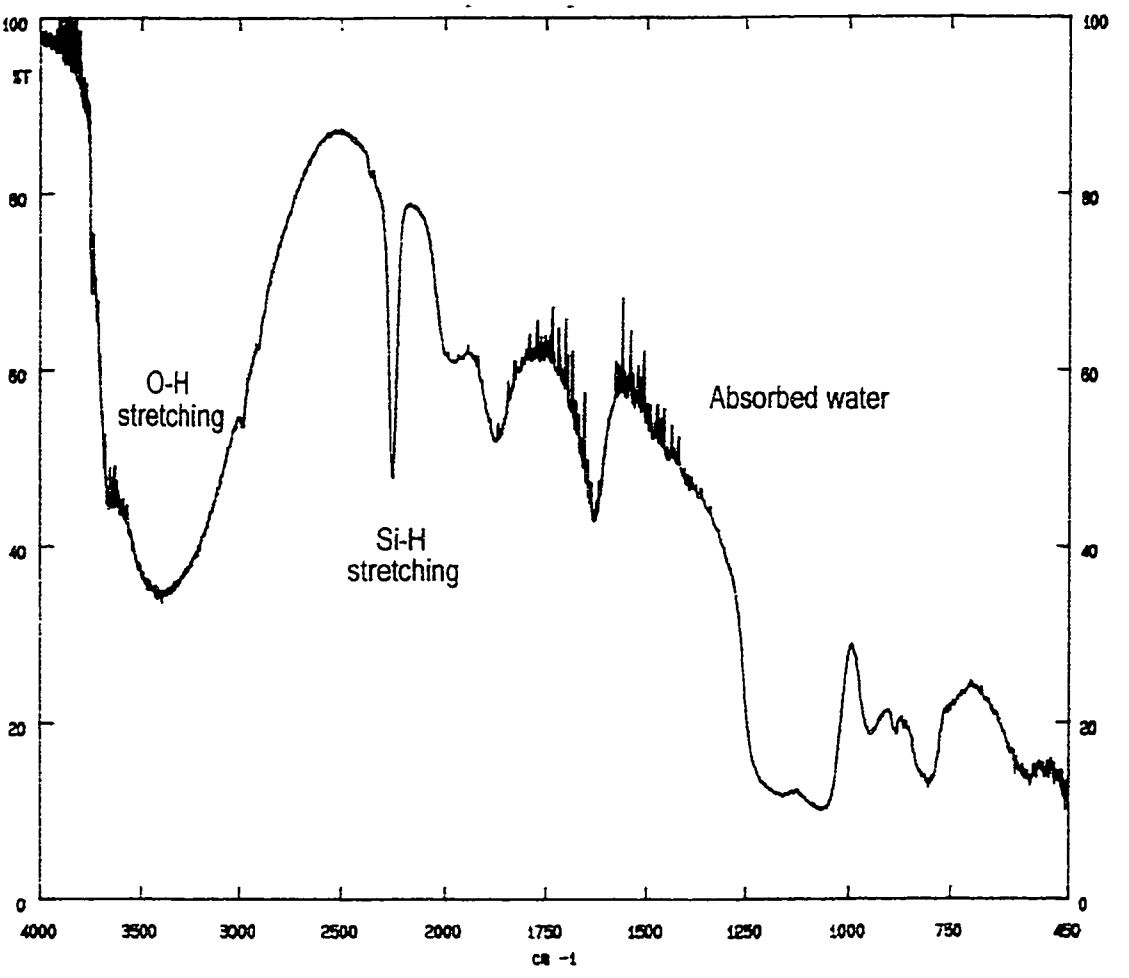


Figure 5 : The DRIFT spectrum of vydac silica hydride

surface silicon atom with three siloxane bonds and a hydride and a surface silicon atom with two siloxane bonds, a hydroxyl and a hydride respectively.

b) ^{13}C CP-MAS NMR

Figure 7 shows the ^{13}C NMR of Vydac silica hydride. The characteristic features of this spectrum are the two peaks at 16 and 60 ppm. These peaks can be assigned to the methyl and the methylene group of the ethoxy moiety remaining after the TES silanization process which results in the hydride surface that binds to the organic moiety [4]. The high intensity of the peaks can be attributed to the fact that the species are persistent on vydac silica bonded phases. Further, the presence of the ethoxy moiety on the surface could be a result of incomplete hydrolysis of TES or the bonding of ethanol released during TES silanization.

B. Confirmation of 10- Undecynoic bonded silica

1. Diffuse Reflectance Fourier Transform Infrared Spectroscopy results

Figure 8 is the DRIFT spectra of 10-undecynoic acid bonded silica. When compared to the spectrum for Vydac silica hydride, (Figure 5) the main difference is the decrease in the intensity of the Si-H band at ~2250 cm^{-1} and the appearance of carbon-hydrogen stretching bands between ~2850-3100 cm^{-1}. The band at 1600 cm^{-1} is due to the C=O stretching frequency. The later bands represent the bonding

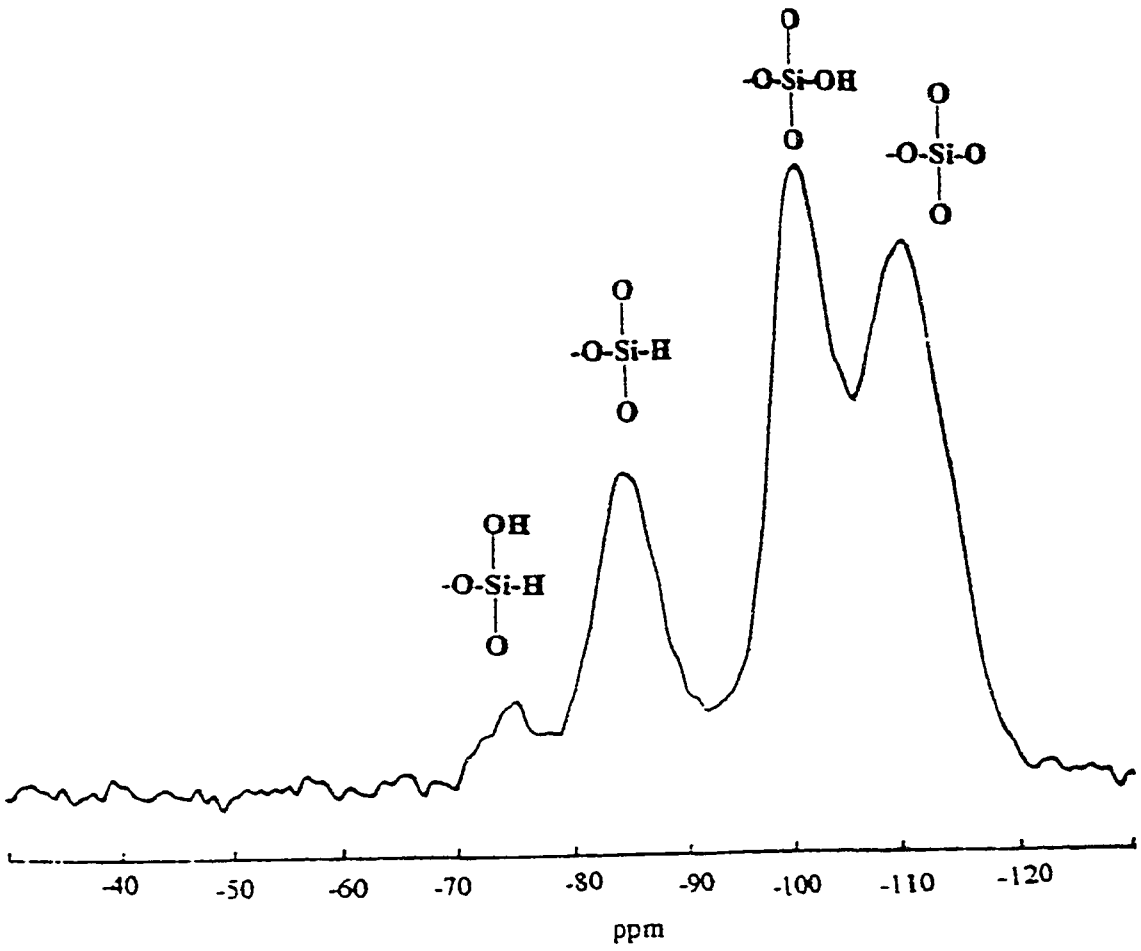


Figure 6 : The ^{29}Si CP-MAS NMR spectrum of vydac silica hydride

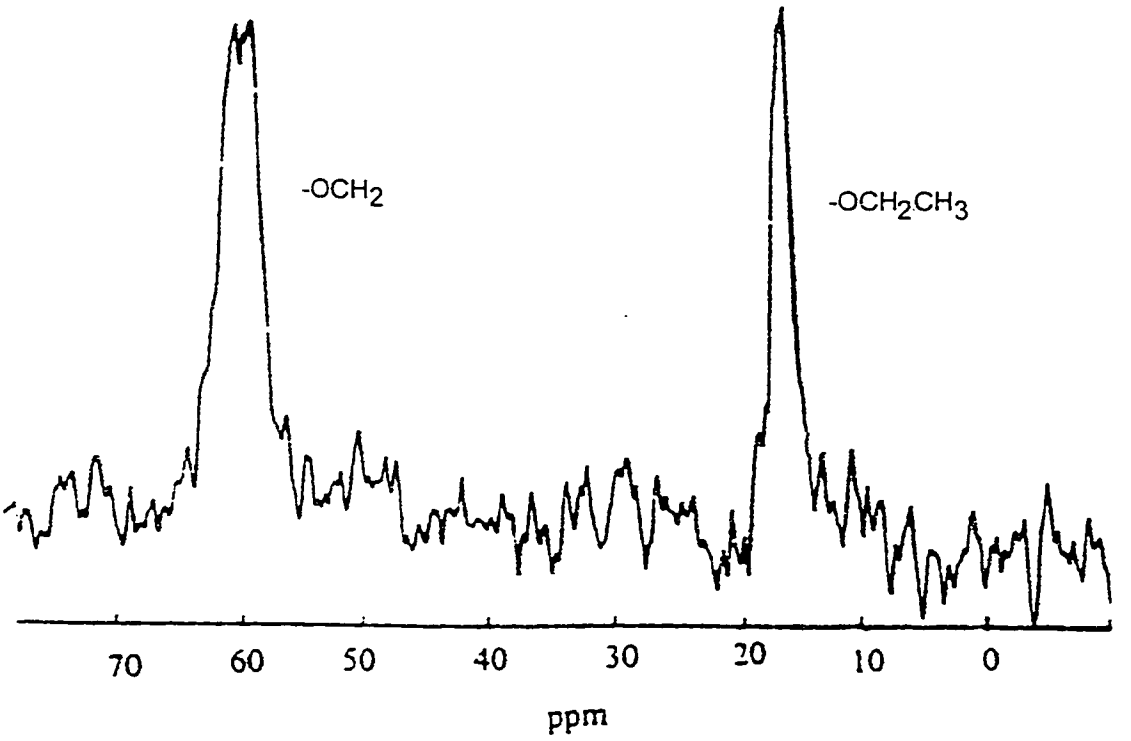


Figure 7 : The ^{13}C CP-MAS NMR spectrum of vydac silica hydride

of 10-Undecynoic acid to silica. Figure 9 is the DRIFT spectra of the acid hydrolyzed 10-undecynoic acid bonded silica. Hydrolysis was carried out with hydrochloric acid (pH 2.0). From the spectra, it can be said that the Si-H and C-H stretching peaks are still present after acid hydrolysis. However, there is a slight decrease in the intensity of the Si-H and C=O stretching bands. The DRIFT spectrum of 10-undecynoic acid bonded phase after acid hydrolysis at 50° C is shown in Figure 10. The Si-H stretching peak at ~2250 cm^{-1} and C-H stretching between 2850-3100 $cm^{-1}$are still present. Hence, it can be said that the 10-undecynoic acid bonded phase is tolerant to such harsh acidic conditions. Figure 11 is the DRIFT spectra of base hydrolyzed 10-undecynoic acid bonded phase. Base hydrolysis was carried out with NaOH (pH 9.0). Base hydrolysis did not have any effect on the 10-undecynoic acid bonded phase as the Si-H and C-H stretching bands are still prominent. The DRFIT spectrum of base hydrolysis at 50°C is shown in Figure 12. The Si-H, C-H and C=O stretching peaks are slightly reduced in intensity when compared to the DRIFT spectrum of 10-undecynoic acid bonded silica (Figure 8). This is due to the loss of the bonded phase from hydrolysis under basic conditions.

2. Solid State Nuclear Magnetic Resonance Spectroscopy Results

a) ^{29}Si CP-MAS NMR Spectra

^{29}Si NMR was used to confirm the bonding of 10-undecynoic acid to the silica surface. Figure 13 is the ^{29}Si NMR spectra of 10-undecynoic acid bonded to the

silica surface. The characteristic features of the spectrum are the peaks at -110 ppm and -100 ppm due to the silica matrix. The presence of the peak at -100 ppm indicates that there are a significant number of silanols as a part of the overall composition of the material. Because of the porous nature of silica all of the silanols are not converted into hydrides as they are inaccessible to the TES silanization reagent [4]. After the hydrosilation reaction has occurred, usually a new peak is observed at -65 ppm. This is due to a silicon atom with three siloxane linkages and a carbon atom. When large organic molecules are bonded on the surface, the hydrophobic environment excludes water near the silicon which facilitates cross-polarization. This may be the probable cause for the absence of the peak at -65 ppm [6].

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

Carbon-13 CP-MAS NMR is a very effective method for the characterization of chemically modified surfaces. In many cases, extensive structural information about the molecule bonded on the surface can be obtained facilitating positive identification. Figure 14 shows the ^{13}C NMR spectrum of 10-undecynoic acid bonded silica. The chemical shifts at 16 and 60 ppm can be attributed to the methyl and the methylene group of the ethoxy moiety. The high intensity of the peaks can be attributed to the fact that the species are persistent on Vydac silica bonded phases. The peaks at 23 ppm are due to a methylene resonance. This supports structure I which consists of a double Si-C linkage where the carbon triple bond is

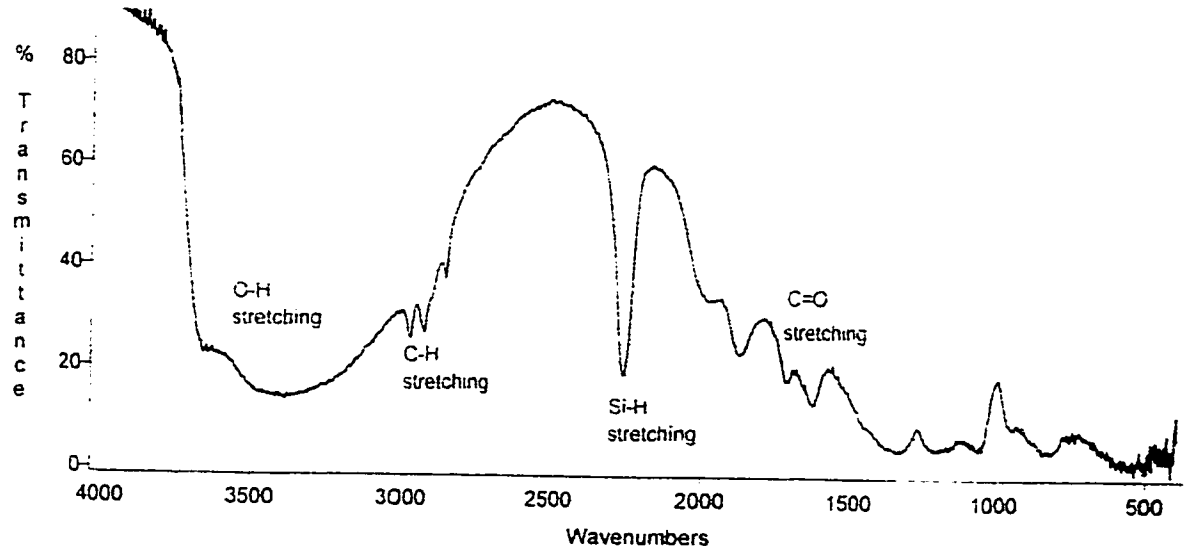


Figure 8 : The DRIFT spectrum of 10 - undecynoic acid bonded silica

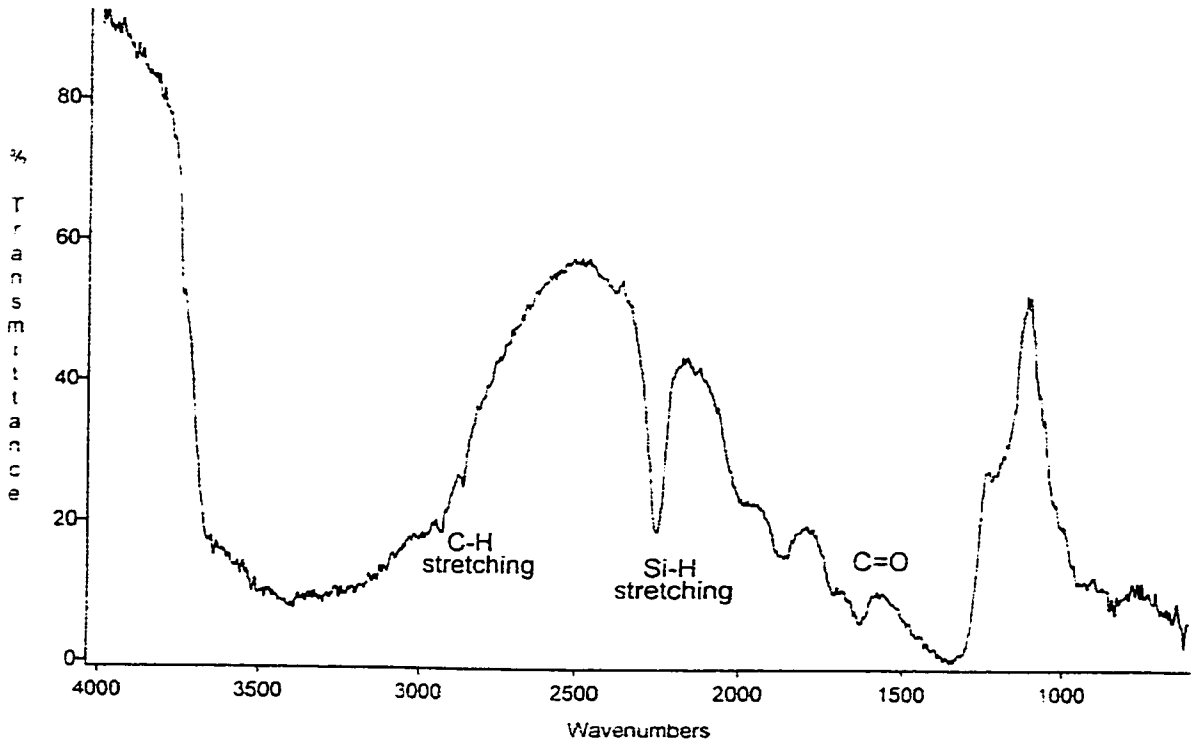


Figure 9 : The DRIFT spectrum of 10 - undecynoic bonded silica after HCl hydrolysis

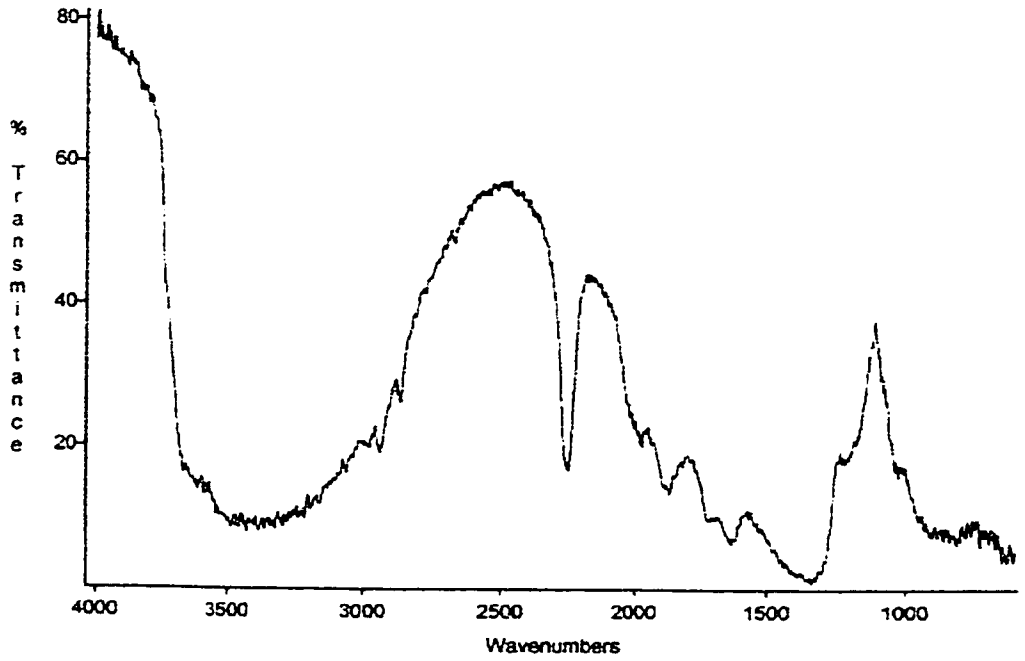


Figure 10 : The DRIFT spectrum of 10 - undecynoic acid bonded silica after HCl hydrolysis at 50°C

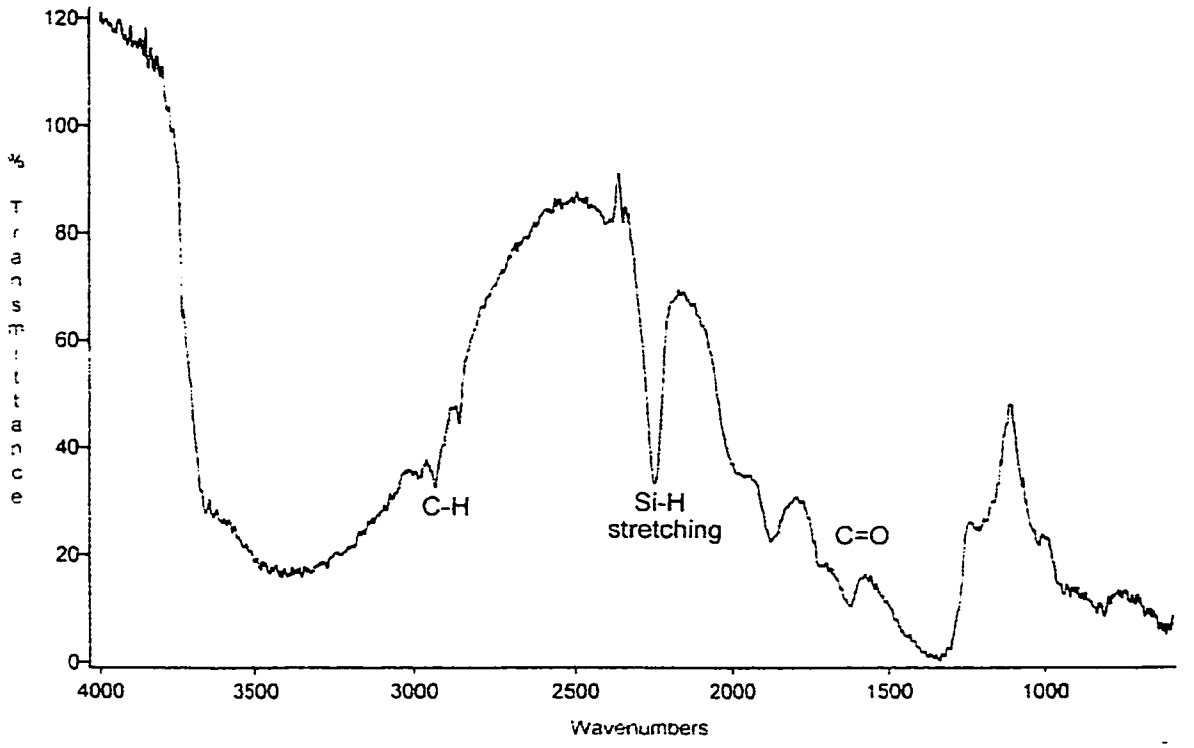


Figure 11 : The DRIFT spectrum of 10 - undecynoic acid bonded silica after NaOH hydrolysis

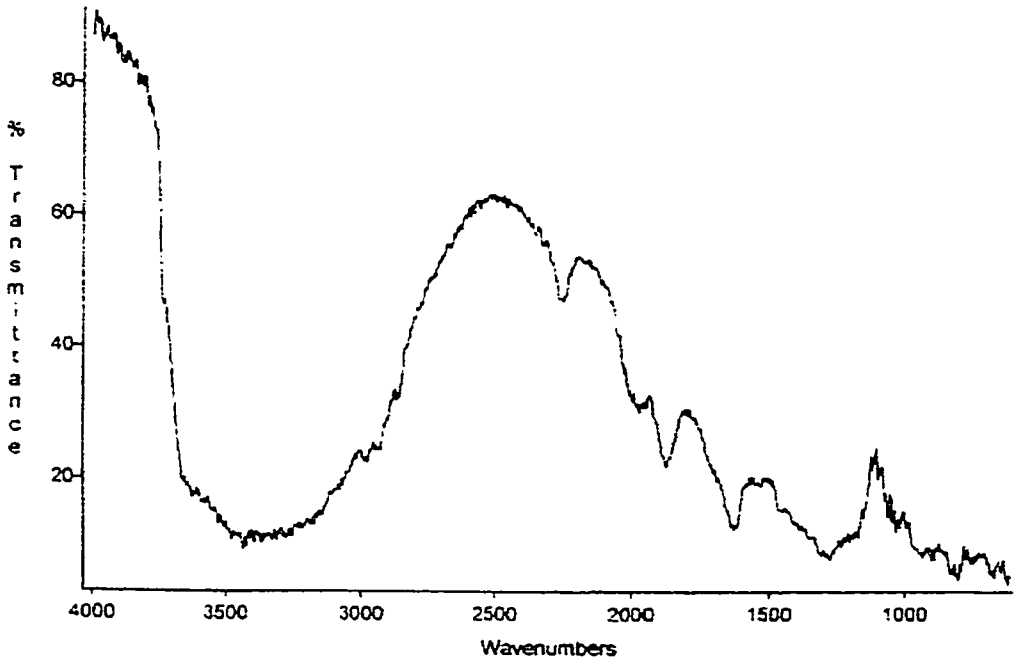


Figure 12 : The DRFIT spectrum of 10 - undecynoic acid bonded silica after NaOH hydrolysis at 50°C

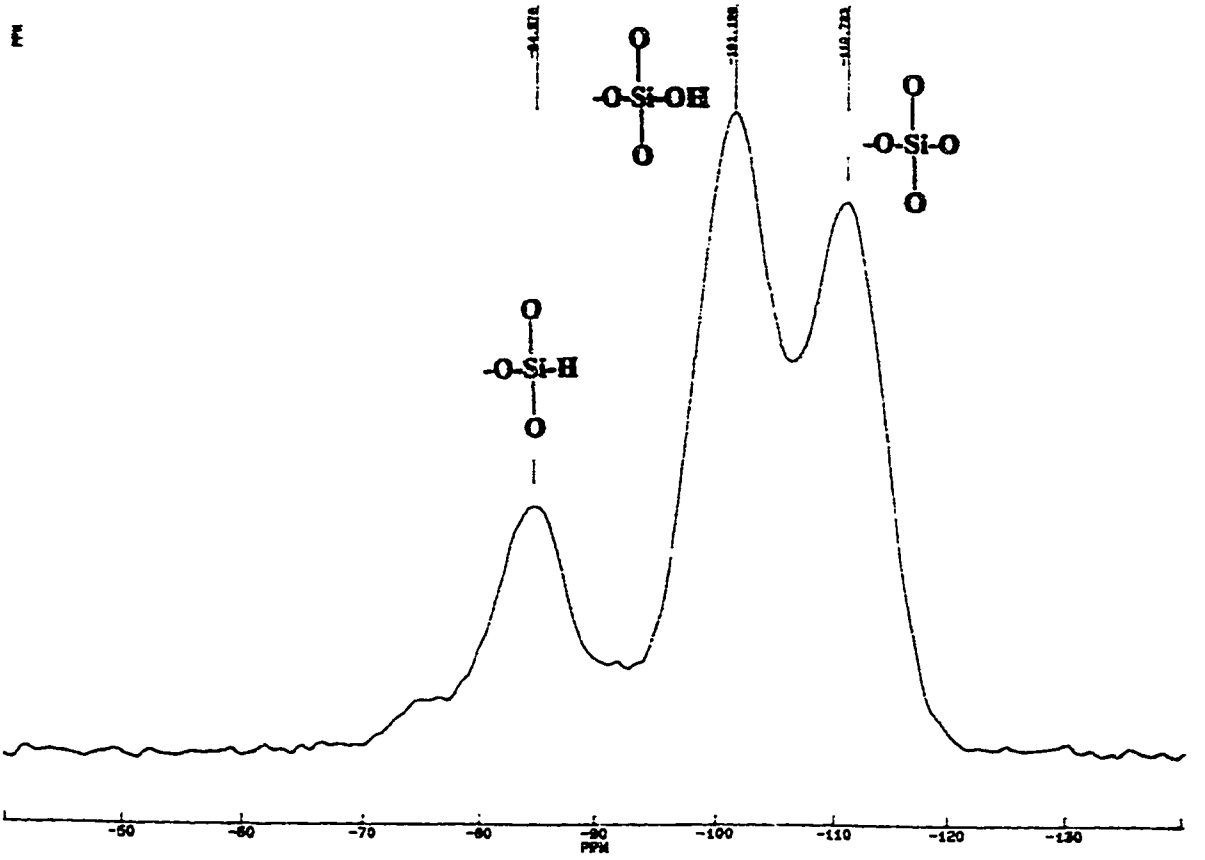


Figure 13 : The ^{29}Si CP-MAS NMR spectrum of 10 - undecynoic acid bonded silica

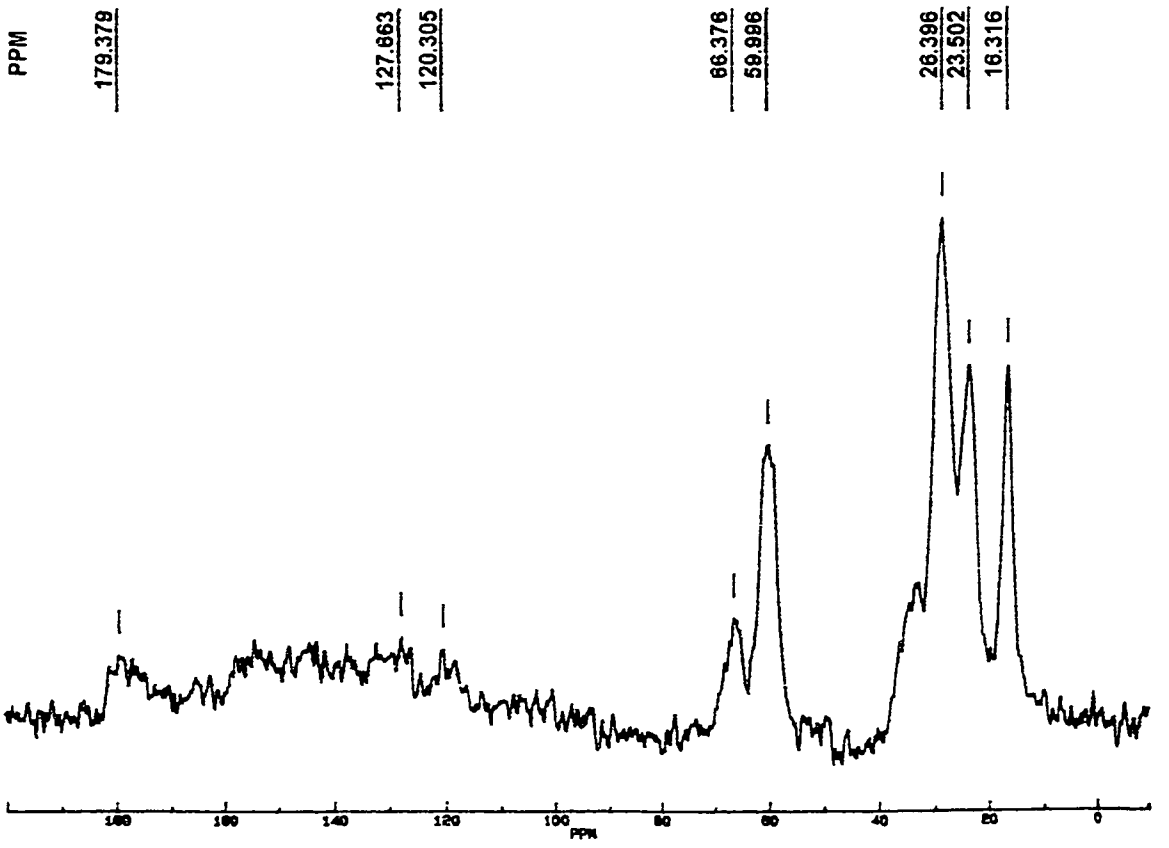


Figure 14 : The ^{13}C CP-MAS NMR spectrum of 10 - undecynoic acid bonded silica

completely broken and the terminal carbon is bonded to two silicon atoms. The peak at 28 ppm could be due to methylene carbons of structures I, II, and III. The chemical shifts at 120, 127 ppm can be attributed to the cis and trans isomers of olefin carbons (C=C) [8]. These two shifts account for the presence of structures II, III. The chemical shift at 179 ppm is due to -C=O resonance. A peak at 12 ppm is due to the methylene carbon attached to the silica surface (Si-C), is not observed. The rigidity and steric constraints of the Si-C bond may be a probable cause that this resonance was not detected by NMR.

C. Confirmation of Undecylenic acid Bonded Silica

1. DRIFT Spectra

Figure 15 shows the DRIFT spectrum of undecylenic acid bonded to vydac silica hydride. A decrease in the Si-H stretching frequency at ~2250 cm^{-1} and the appearance of C-H stretching bands between ~2800 - 3000 cm^{-1} and the C=O band at ~1700 cm^{-1}, indicate the success of the hydrosilation reaction. The DRIFT spectrum of undecylenic acid bonded silica after acid hydrolysis is shown in Figure 16. The Si-H stretch at 2250 cm^{-1} is still prominent. However, the C-H stretching at ~2800 cm^{-1} has decreased in intensity. Figure 17 shows the undecylenic acid bonded silica after acid hydrolysis at 50°C. Both the Si-H and C-H stretching bands are present but diminished in intensity.

The DRIFT spectra after NaOH hydrolysis at room temperature and at 50° C are shown in Figures 18 and 19 respectively . The bonded phase was affected by base hydrolysis because, the Si-H and C-H stretching frequencies are seen at

~2250 cm^{-1} and 2800 - 3000 cm^{-1}, respectively but are diminished in intensity. Overall, the undecylenic acid column is somewhat tolerant to harsh acidic and basic conditions.

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

Figure 20 shows the ^{29}Si NMR of undecylenic acid bonded to silica hydride. The spectrum contains the Si-H peak at -85 ppm, the silanol peak at -100 ppm and, the siloxane peak at -110 ppm. When compared to the ^{29}Si spectrum of vydac silica hydride (Figure 6) it is seen that the Si-H peak at -75 ppm is gone and the peak at -85 ppm is reduced in intensity. These changes confirm the success of the hydrosilation reaction. The Si-C peak that should have appeared at -65 ppm is probably reduced to a low intensity by the absence of nearby hydrogen.

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

The ^{13}C NMR spectrum of undecylenic acid bonded silica is shown in Figure 21. The small peaks at 16 and 60 ppm can be attributed to the methyl and the methylene group of the ethoxy moiety [4]. The huge peak at 28 ppm is due to the methylene groups of structures I and III. The small peak at 176 ppm is due to the carbonyl group of the carboxylic acid moiety [11]. The peak at 127 ppm could be due to the olefin carbons of structure III. Thus, from the ^{13}C CP-MAS NMR

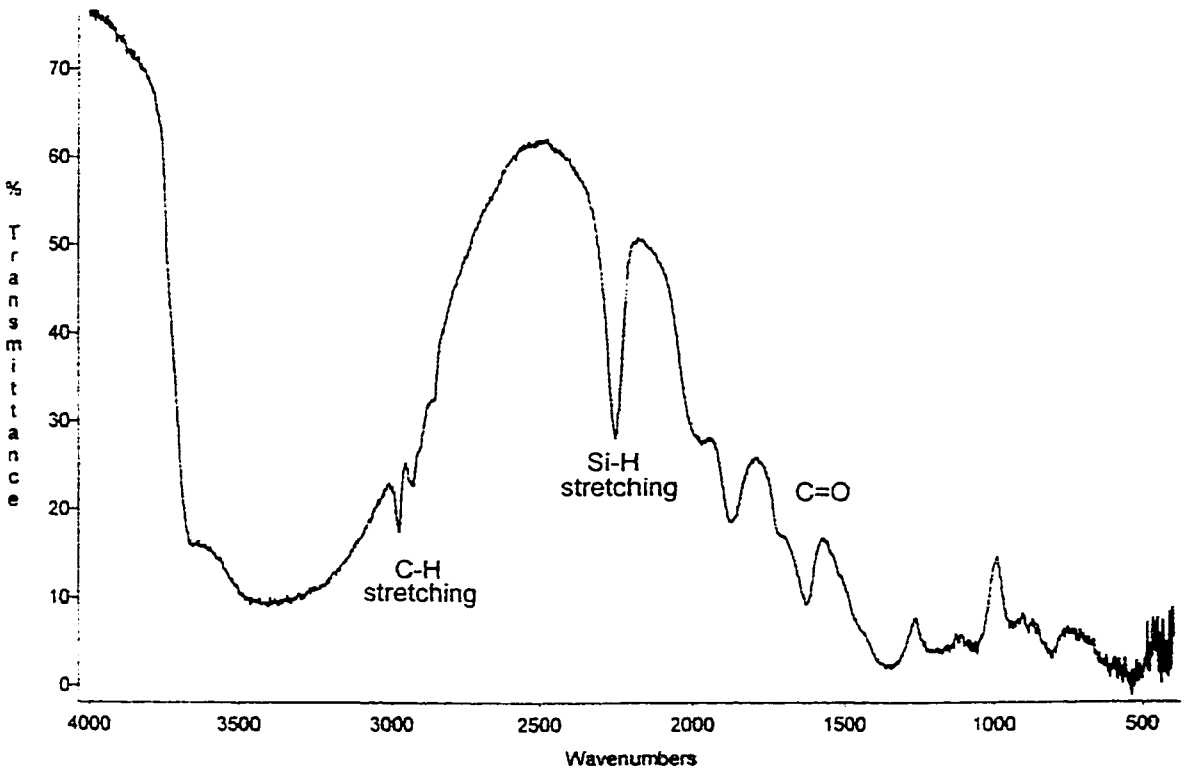


Figure 15 : The DRIFT spectrum of undecylenic acid bonded silica

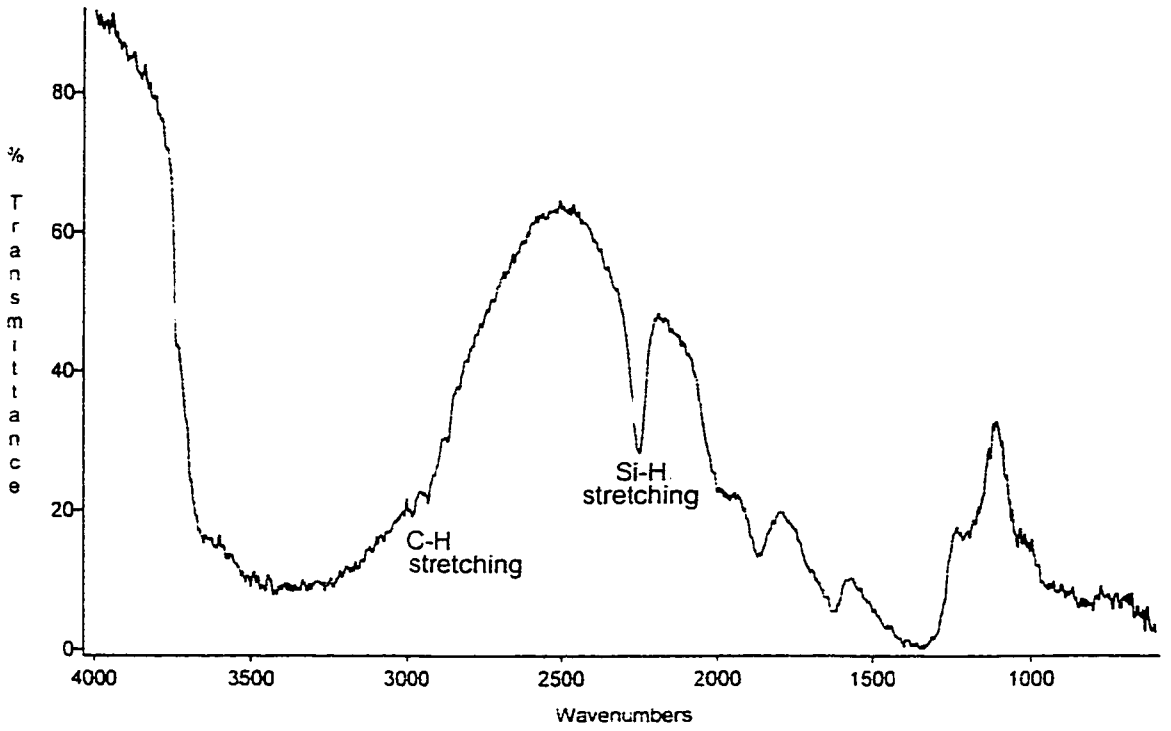


Figure 16 : The DRIFT spectrum of undecylenic acid bonded silica after HCl hydrolysis

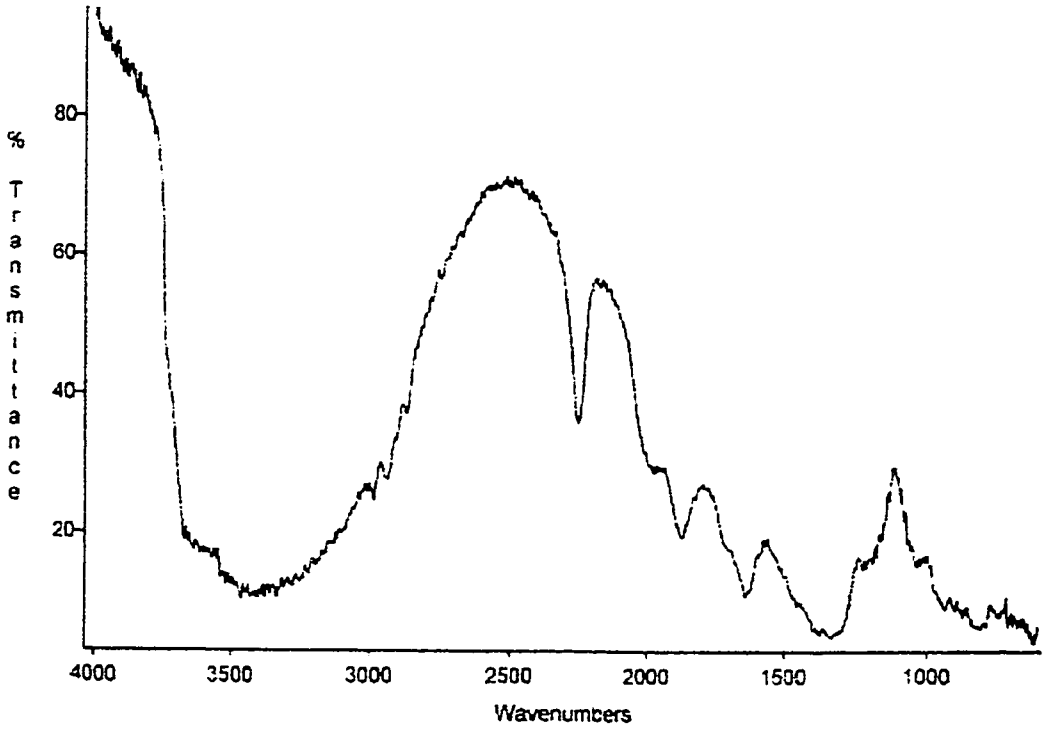


Figure 17 : The DRIFT spectrum of undecylenic acid bonded silica after HCl hydrolysis at 50° C

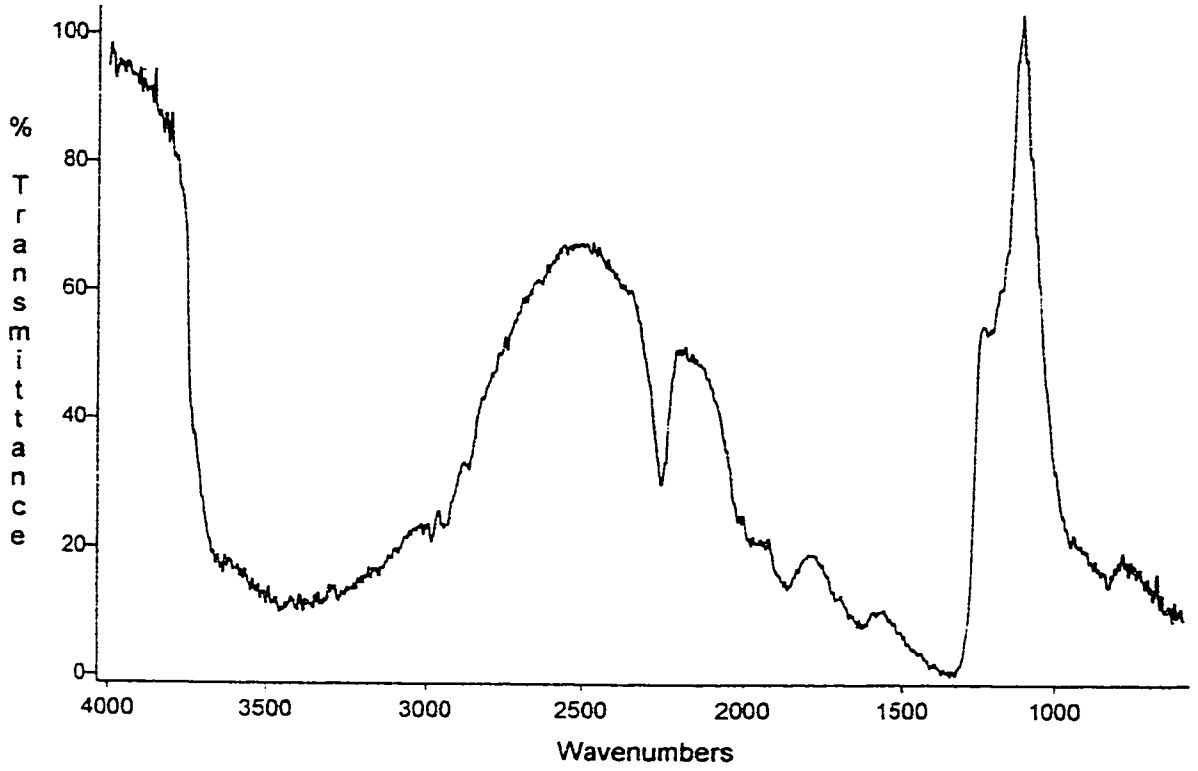


Figure 18 : The DRIFT spectrum of undecylenic acid bonded silica after NaOH hydrolysis

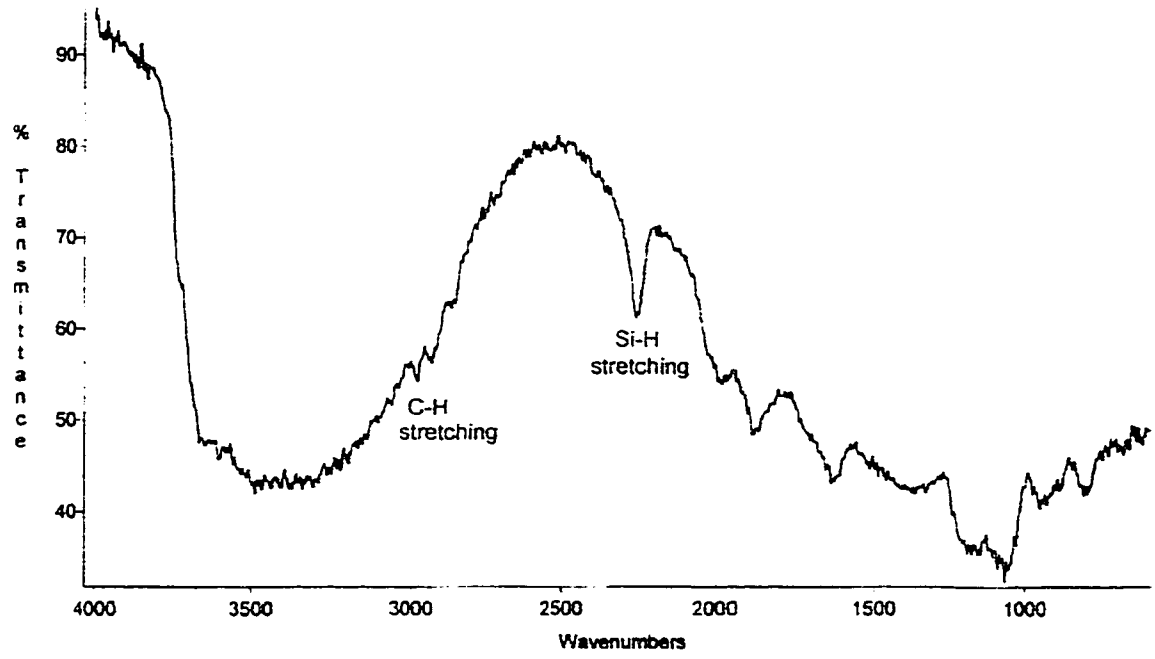


Figure 19 : The DRIFT spectrum of undecylenic acid bonded silica after NaOH hydrolysis at 50° C

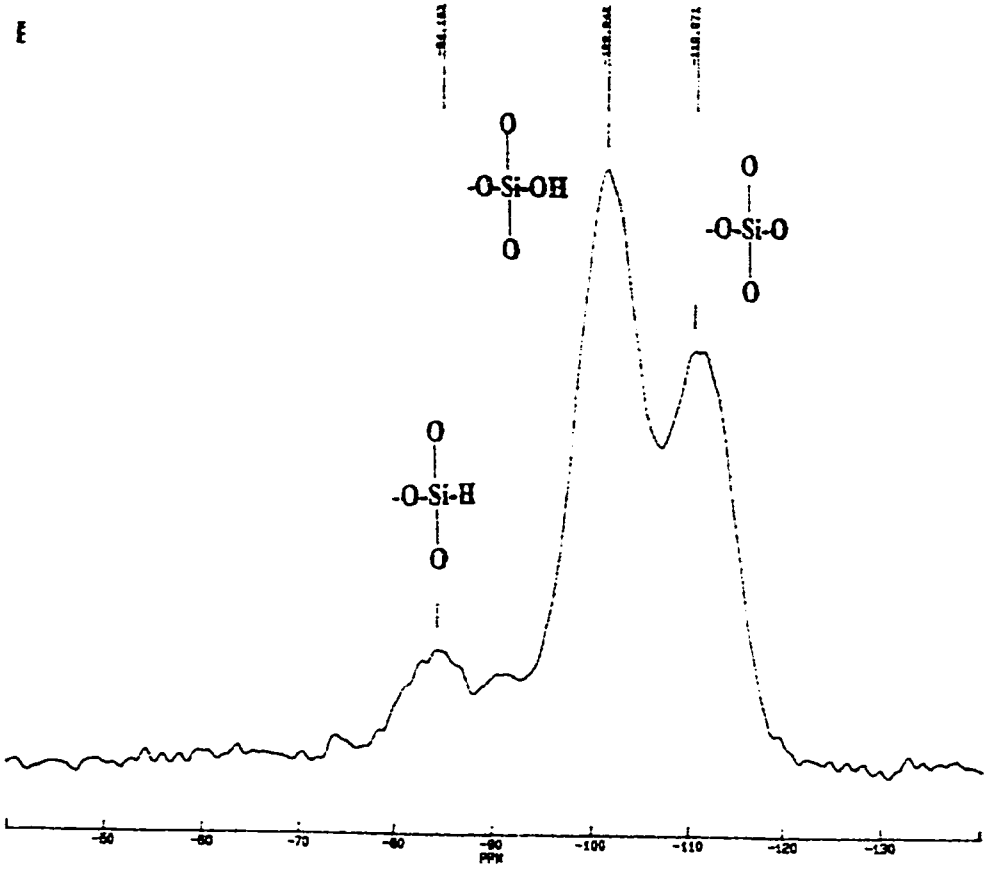


Figure 20 : The ^{29}Si CP-MAS NMR spectrum of undecylenic acid bonded silica

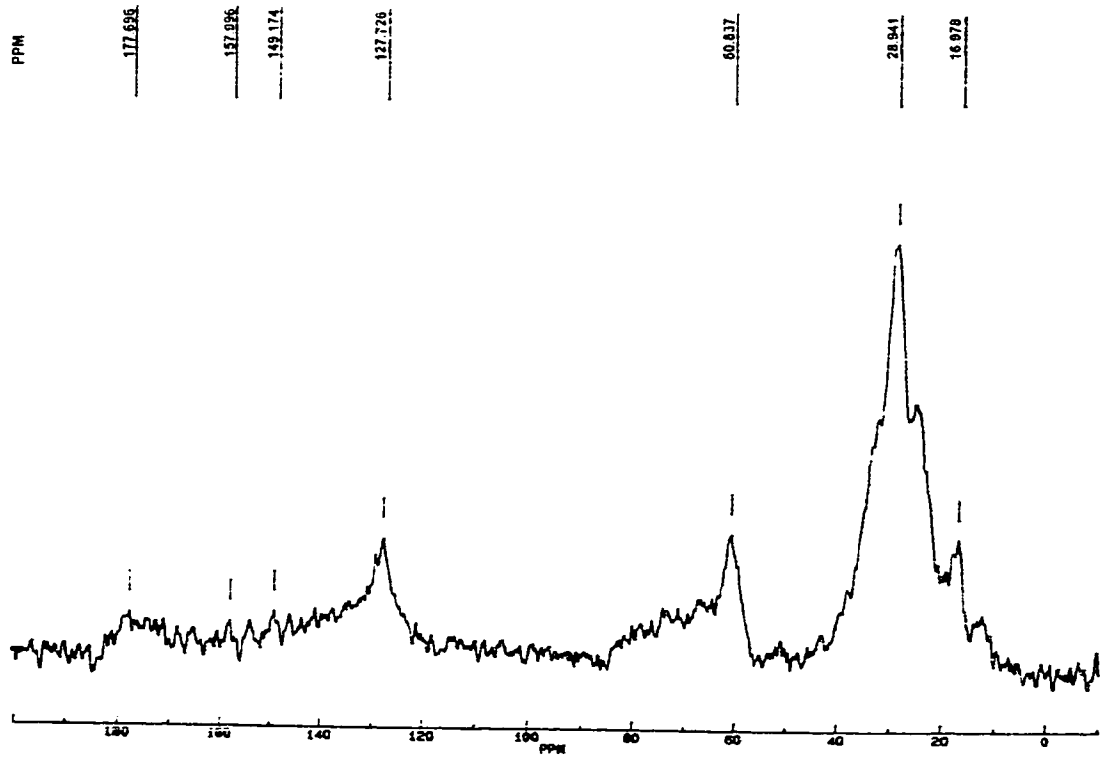


Figure 21 : The ^{13}C CP-MAS NMR spectrum of undecylenic acid bonded silica

spectra of undecylenic acid, it can be said that in the hydrosilation of carboxylic acids more than one type of bonding is possible [8].

D. Confirmation of 4-Pentenoic acid bonded silica

1. DRIFT Spectra

The DRIFT spectrum of 4-pentenoic acid bonded to vydac silica hydride is shown in Figure 22. The characteristic features of the spectrum are the C-H stretching at ~2850 cm^{-1} and the C=O resonance at 1700 cm^{-1}. When compared to the DRIFT spectrum of vydac silica hydride (Figure 5) it can be said that there is a decrease in the intensity of the Si-H stretching frequency at ~2250 cm^{-1}. The DRIFT spectra after acid hydrolysis at room temperature and at 50° C are shown in Figures 23 and 24, respectively. From the spectra, it can be said that the Si-H and C=O stretching peaks are still present after acid hydrolysis but are greatly reduced in intensity. The DRIFT spectra of base hydrolyzed 4-pentenoic acid bonded phase at room temperature and at 50°C are shown in Figures 25 and 26, respectively. In both the spectra, there is a reduction in the intensity of the Si-H, C-H and C=O stretching frequency. These results indicate moderate stability of the bonded phase under acidic and basic conditions.

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

^{29}Si NMR spectrum of 4-pentenoic acid bonded silica is shown in Figure 27. The main features of the spectrum are the siloxane peak at -110 ppm, the silanol peak at -100 ppm, a small Si-H peak at -85 ppm and a Si-C peak at -66 ppm. When compared to the silica hydride spectrum (Figure 6), the main differences are the appearance of the Si-C peak and the reduction of the Si-H peak at -75ppm. These results confirm the success of the hydrosilation reaction.

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

Figure 28 shows the ^{13}C NMR spectrum of 4-pentenoic acid bonded silica. The peaks at 16 and 60 ppm can be attributed to the methyl and the methylene group of the ethoxy moiety. These species are persistent on silica bonded phases accounting for the intensity of the peaks. The small peak at 10 ppm is due to the methylene carbon attached to the silica surface (structure I). The huge peak at 22 ppm is due to the methylene group next to the surface bonded carbon. The chemical shift at 66 ppm represents the methylene group adjacent to the carboxylic acid moiety. The small peak at 176 ppm is due to the carbonyl group carbon.

E. Analysis 4-Styrene sulfonic acid bonded silica

1. DRIFT Spectra

The DRIFT spectrum of 4-styrene sulfonic acid bonded to vydac silica hydride is shown in Figure 29. When compared to the DRIFT spectrum of vydac silica hydride (Figure 5), there is a decrease in the intensity of the Si-H peak at ~2250 cm^{-1}. A C-H bond stretch at ~3000 cm^{-1} is not seen.

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

The ^{13}C NMR spectrum of 4-styrene sulfonic acid bonded silica is shown in Figure 30. The peaks at 17 and 60 ppm represent the ethoxy groups present on silica. The peaks at 19 and 29 ppm are caused by the methylene resonances in the molecule. The peaks at 40 and 52 ppm are caused by the adsorption of methanol through hydrogen bonds or methoxy groups [12]. The peaks at 125 - 145 ppm are due to the aromatic ring structure. Since, satisfactory results were not obtained when the synthesis was repeated, 4-styrene sulfonic acid bonded silica was not chosen for further chromatographic evaluation.

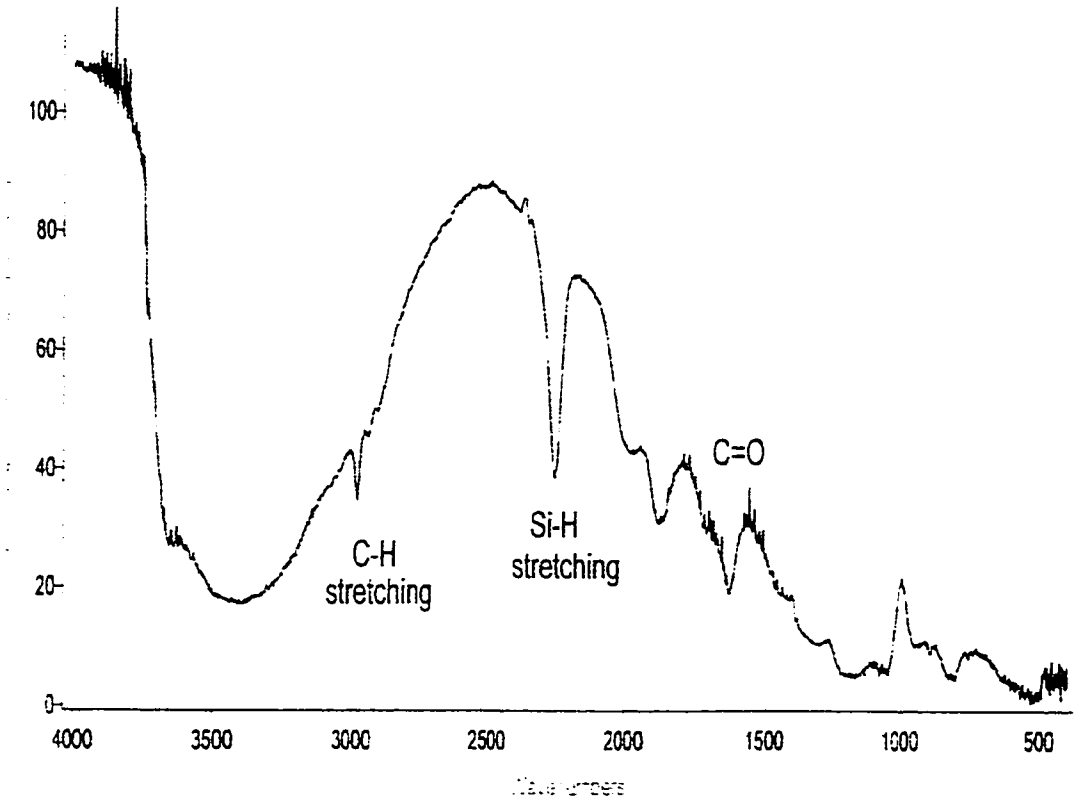


Figure 22 : The DRIFT spectrum of 4 - pentenoic acid bonded silica

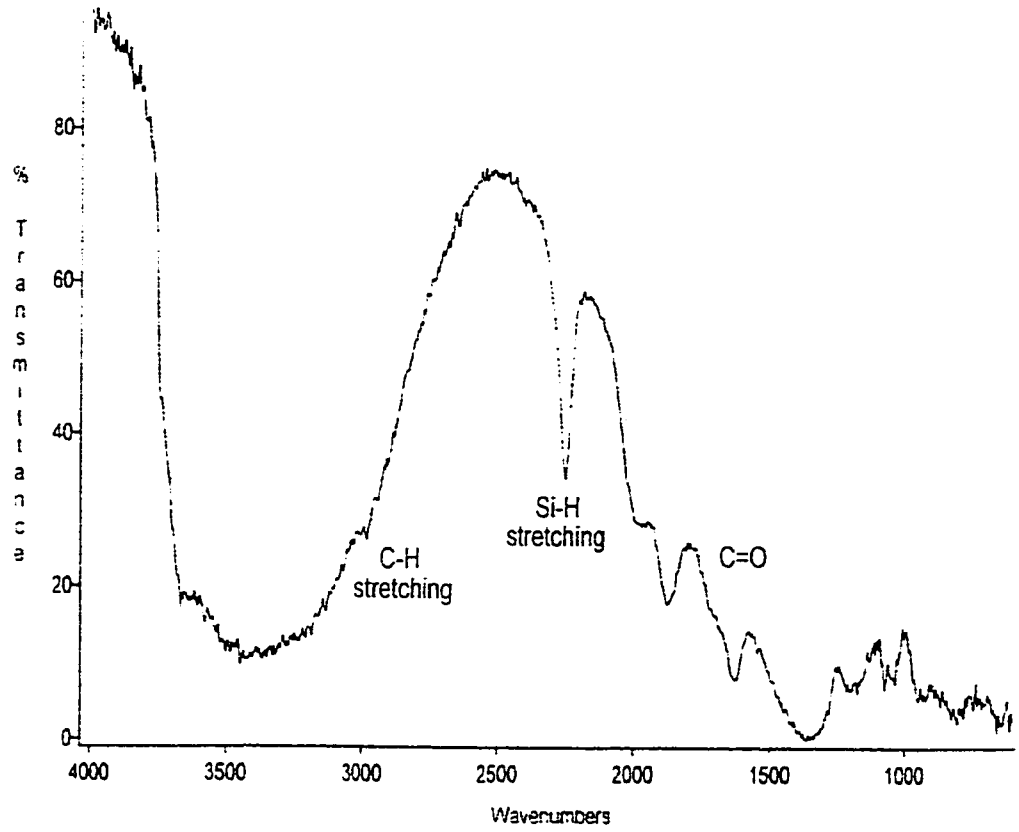


Figure 23 : The DRIFT Spectrum of 4-Pentenoic acid bonded Silica after HCl Hydrolysis

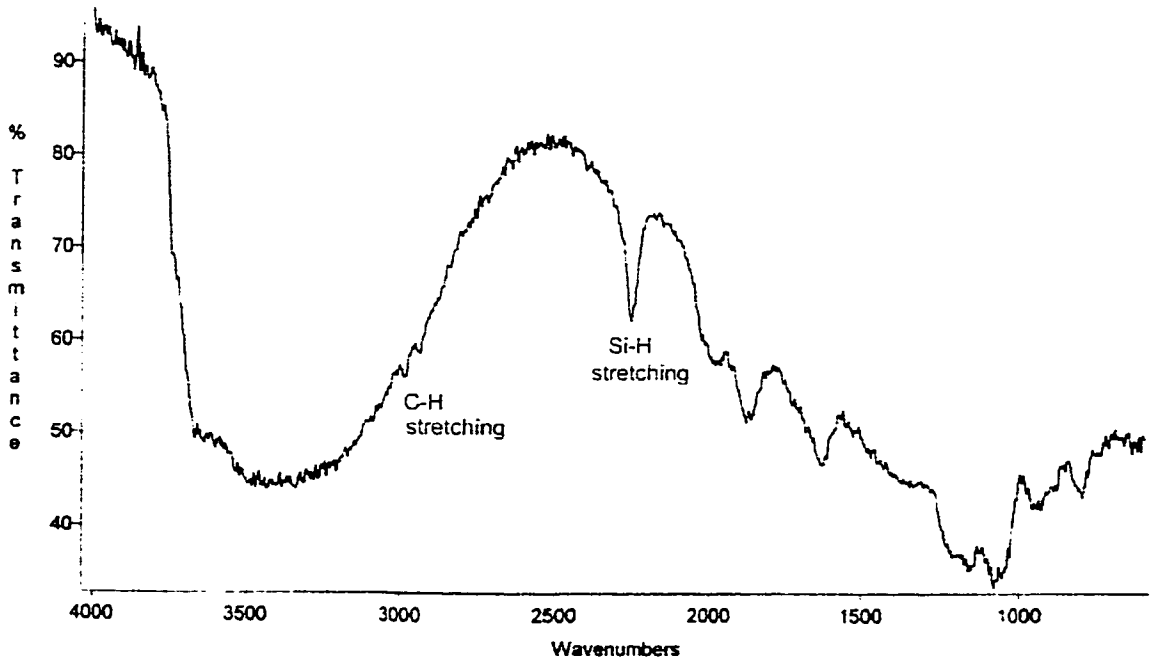


Figure 24 : The DRIFT spectrum of 4 - pentenoic acid bonded silica after HCl hydrolysis at 50° C

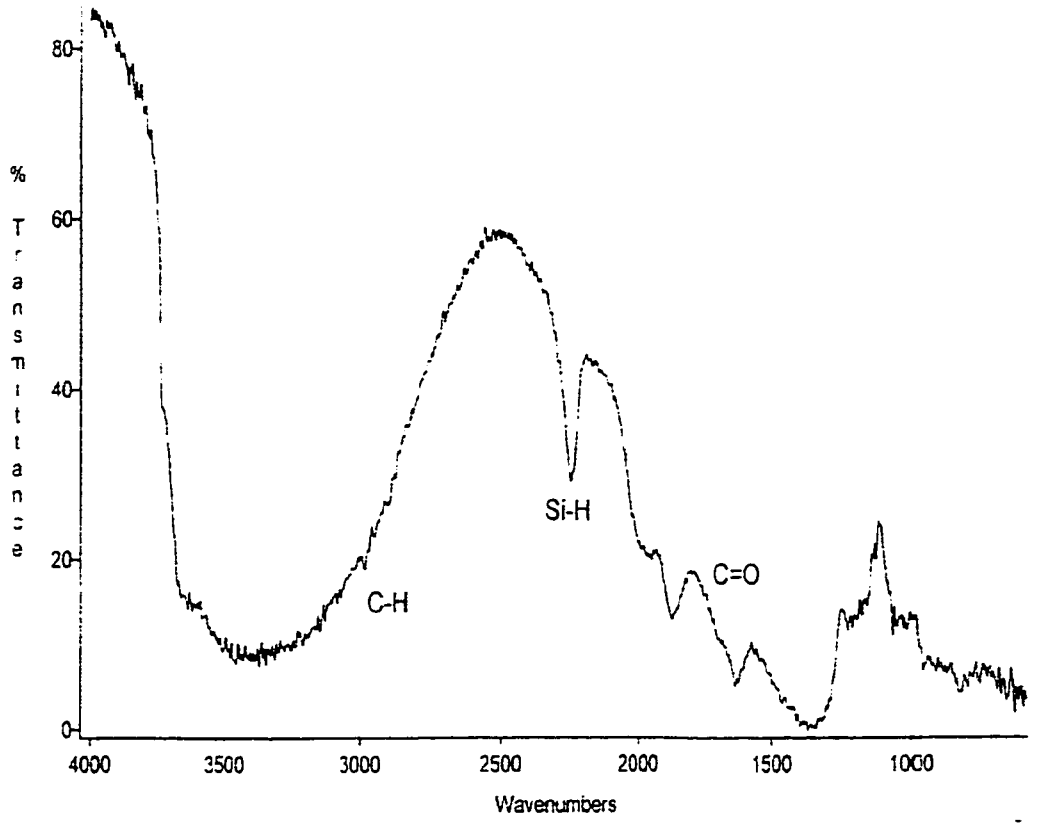


Figure 25 : The DRIFT spectrum of 4 - pentenoic acid bonded silica after NaOH hydrolysis

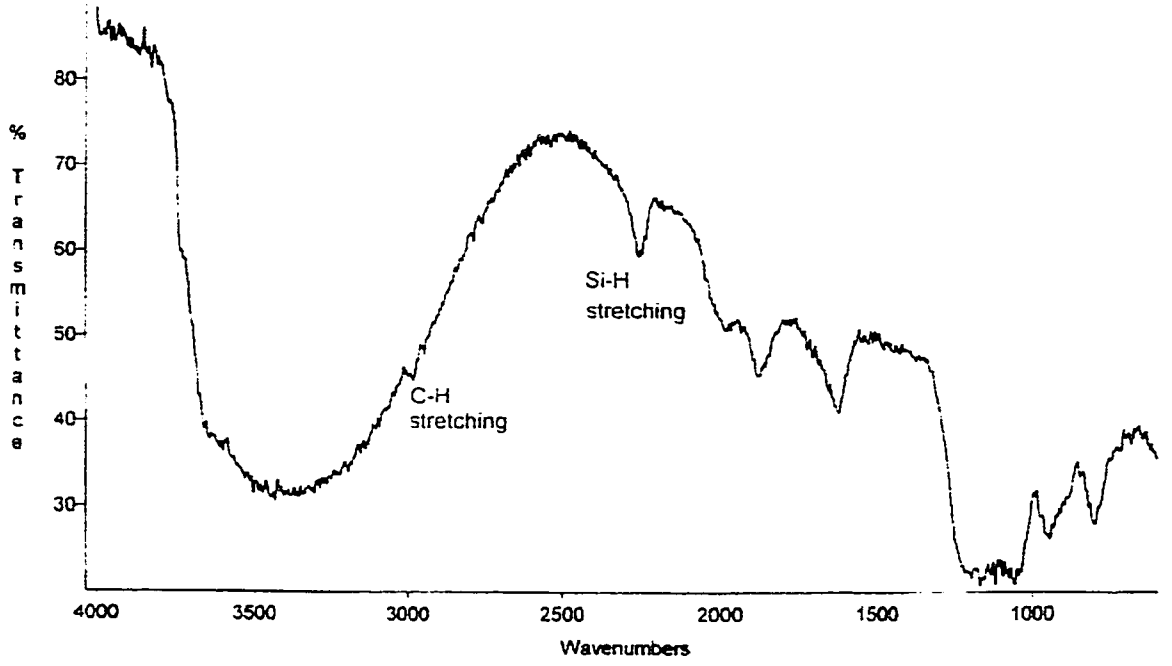


Figure 26 : The DRIFT Spectrum of 4-Pentenoic acid bonded Silica after NaOH Hydrolysis at 50° C

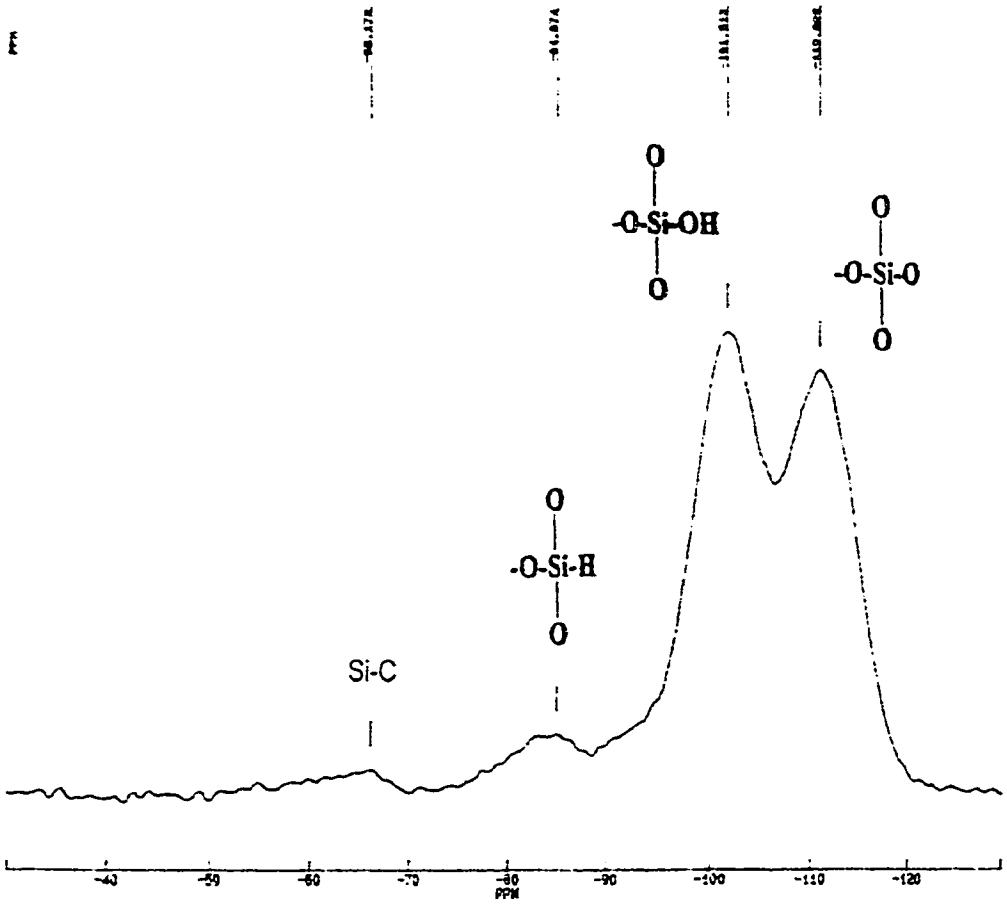


Figure 27 : The ^{29}Si CP-MAS NMR spectrum of 4 - pentenoic acid bonded silica

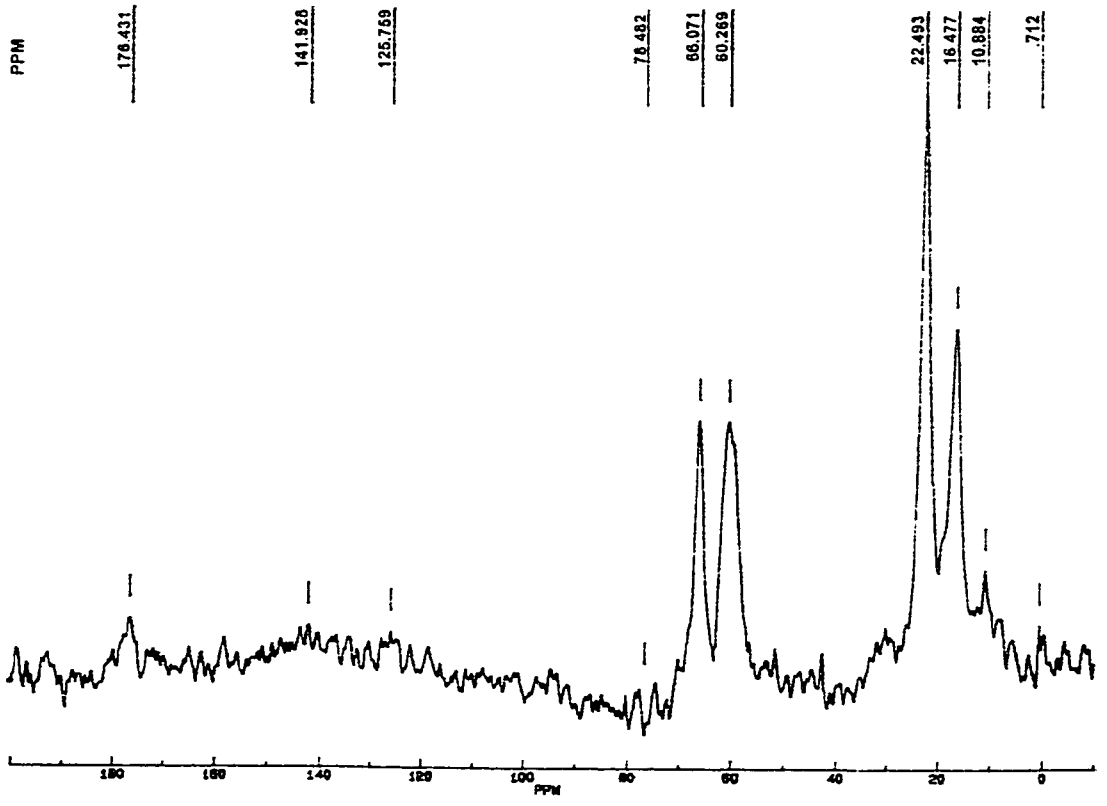


Figure 28 : The ^{13}C CP-MAS NMR spectrum of 4 - pentenoic acid bonded silica

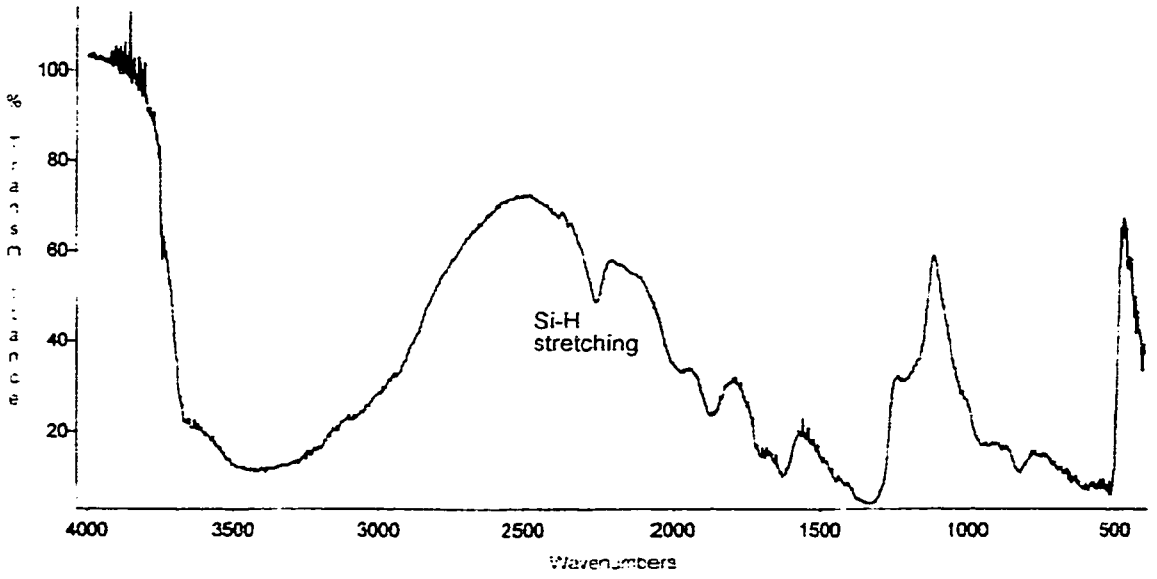


Figure 29 : The DRIFT spectrum of 4 - styrene sulfonic acid bonded silica

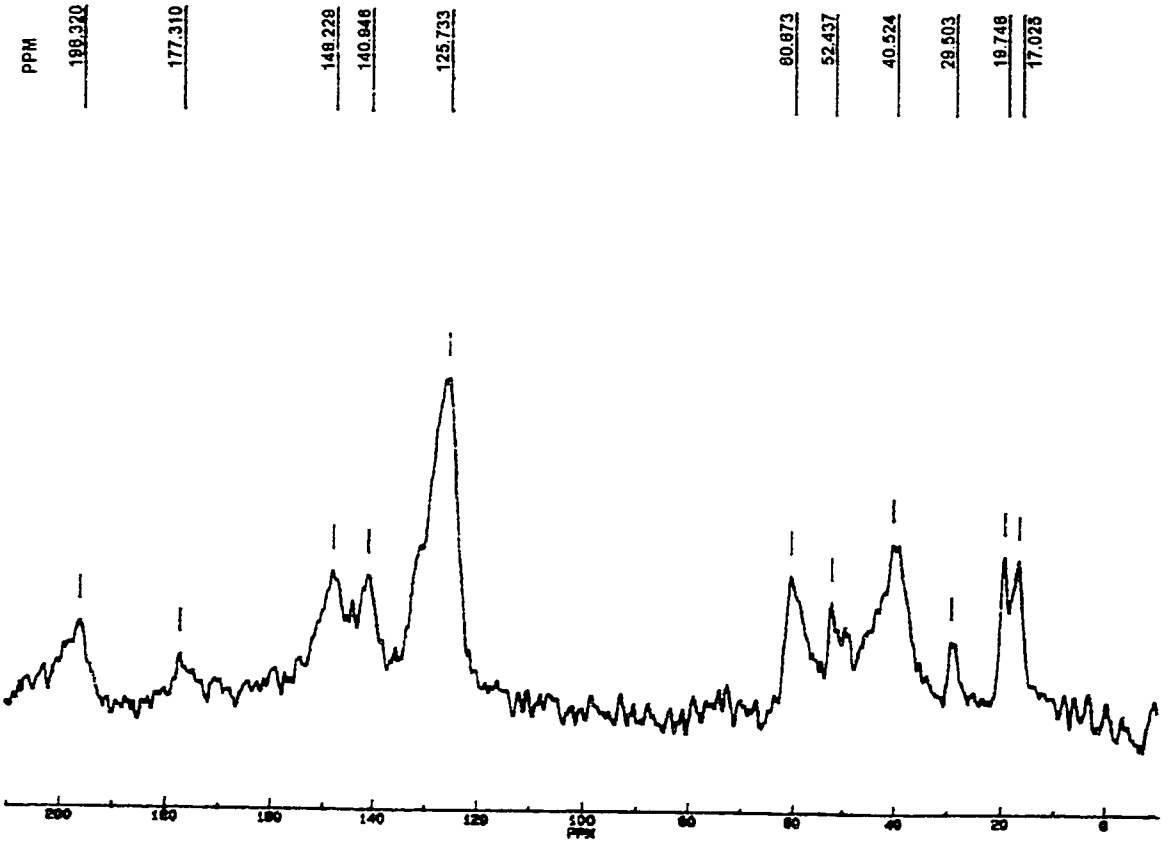


Figure 30 : The ^{13}C CP-MAS NMR spectrum of 4 - styrene sulfonic acid bonded silica

F. Elemental Analysis

Table 5 : Percent Carbon and Surface Coverage of Synthesized Bonded Phases

Compound	Percent Carbon (%C)	Surface Coverage (α) $\mu mol/m^2$
10-Undecynoic acid	2.14	1.57
Undecylenic acid	1.70	1.24
4-Pentenoic acid	1.07	1.71
4-Styrene sulfonic acid	1.40	1.41

Percent carbon values are an indication of the alkyl coverage of silica. From the results obtained it is seen that the surface coverage decreased slightly with increasing chain length. The attachment of the olefin to the hydride surface during hydrosilation reaction involves a transition step where the catalyst forms a complex with the olefin. In cases where the transition metal catalysts are used (e.g. hexachloroplatinic acid), the intermediate complex can be of considerable size. This affects the accessibility of the catalyst-olefin complex to the surface active sites (Si-H) [13]. Thus resulting in a lower surface coverage of silica.

G. Chromatographic Studies

1. 10-Undecynoic acid Column

a) PTH - amino acids

PTH-Amino acids dissolved in water : methanol (1:1) were injected into the

10-undecynoic acid column separately. The mobile phase was 0.001 M $NH_4H_2PO_4$ (pH 3.5) at a flow rate of 0.2 ml/min. The retention values (k') of the PTH-amino acids were calculated. The results are shown in Table 6. The capacity factor, k' of a column is a direct measure of the strength of the interaction of the sample with the packing material and is defined by the expression $k' = (t_R - t_o) / t_o$, where t_R is the time taken for a specific solute to reach the detector and t_o is the time taken for a non-retained species to reach the detector. Optimum values for k' range from 1 - 10. If the k' values are too low, it is likely that the solutes may not be adequately resolved, and for high k' values the analysis time may be too long. The general order of elution for the PTH-amino acids was acidic amino acids < neutral < basic amino acids. The greater retention of the non polar amino acids can be attributed to the hydrophobic interaction with the long chain stationary phase. Further, the increased retention of the polar amino acid (serine) could be due to the adsorption of polar molecules to the unreacted silanols on the silica surface. A mixture of four PTH-amino acids was made and injected onto the column. The chromatogram is shown in Figure 31. The four amino acids are well resolved. As expected, cysteic acid (acidic amino acid) eluted first followed by the neutral amino acids (serine and methionine sulfone) followed by glutamine (basic amino acid). Peak tailing and excessive retention could be caused by silanols (Si-OH) or its acidic sites on the bonded phase.

b) Theophylline and its derivatives

Caffeine, theophylline, aminophylline are xanthines most commonly used in

Table 6 : Retention data of PTH-Amino acids on 10-Undecynoic acid Column

Number	Solute	k'
1	PTH - Cysteic acid	0.04
2	PTH - Aspartic acid	0.57
3	PTH - Isoleucine	0.30
4	PTH - Leucine	0.31
5	PTH - Hydroxyproline	0.57
6	PTH - Methionine sulfone	0.63
7	PTH - Serine	0.96
8	PTH - Proline	0.79
9	PTH - Tryptophan	0.79
10	PTH - Asparagine	0.86
11	PTH - Glutamine	1.72

Table 7 : Dissociation Constants of Amino acids

Constants are expressed in terms of negative logarithms (pK values) at 25°C

	Amino acid	**pK_1** α - COOH	**pK_2** α - NH_3^+	**pK_R** **Side chain**
1	Cysteine	1.86	10.25	8.37
2	Aspartic acid	2.10	9.82	3.90
3	Isoleucine	2.32	9.76	
4	Leucine	2.33	9.74	
5	Hydroxyproline	1.92	9.73	
6	Methionine	2.28	9.20	
7	Serine	2.21	9.15	
8	Proline	2.00	10.60	
9	Tryptophan	2.38	9.39	
10	Asparagine	2.02	8.80	
11	Glutamine	2.17	9.13	

medicine. Theophylline and aminophylline are used for their bronchodilating action in the management of obstructive airways diseases such as asthma and caffeine is the most active of the xanthines in stimulating the central nervous system. Theophylline, 7-(2,3 dihydroxypropytheophylline, aminophylline and caffeine were analyzed on the 10-undecynoic acid column. Samples were prepared by dissolving the solids in deionized water. The mobile phase used was 0.01 M $NH_4H_2PO_4$ (pH 4.5) at a flow rate of 0.2 ml/min. The compounds were first injected separately on the column. The retention values of these compounds were calculated. The results are shown in Table 8. The k' values of the compounds are in the order theophylline < aminophylline < 7-2,2 dihydroxytheophylline < caffeine. A mixture of the four compounds was made and injected on the column. Mobile phase conditions were the same as mentioned above. The chromatogram of this separation is shown in Figure 32. The acidic theophylline elutes first. The high retention of caffeine can be explained by the hydrophobic interactions with the non polar components of the stationary phase. Aminophylline and 7-(2,3 dihydroxypropyltheophylline were not resolved and eluted as a single peak.

c) Nucleic acids

The retention of five nucleic acid monomers was observed on the 10-undecynoic acid column. The nucleic acids and nucleosides analyzed were: adenine, adenosine, uracil, cytosine, and thymine. Samples were prepared by dissolving the solids in isopropanol : water mixture. The mobile phase conditions were 0.01 M $NH_4H_2PO_4$ (pH 4.5) at a flow rate of 0.2 ml/min. The retention data of these

compounds is given in Table 9. The order of elution of these compounds is as expected on cation-exchange columns. The order is uracil < thymine < cytosine < adenine < adenosine. This follows the postulation that the group and position of the substituent on the aromatic ring affect the capacity factors with the order of the effects being OH < H < NH < NHR [14]. A mixture of three nucleic acids was made and injected on the column. The mobile phase conditions were the same as mentioned above. The chromatogram of the separation is shown in Figure 33. Three well resolved peaks (base line separation) are seen. The peak shapes are also good.

2. Undecylenic acid Column

a) PTH - Amino acids analysis

PTH - amino acids were injected into the undecylenic acid column using 0.001 M $NH_4H_2PO_4$ (pH 3.5) as the mobile phase at a flow rate of 0.2 ml/min. The retention data of the various amino acids on the undecylenic acid column is shown in Table 10. The general order of elution of the PTH - amino acids follows the order, acidic < neutral < basic. Exceptions to the rule are the neutral amino acids PTH - proline and PTH - hydroxyproline which are strongly retained. This can be attributed to the strong non polar interactions between the cyclic side chains of these amino acids with the long alkyl chain of the stationary phase. The lower bonded phase coverage when compared to the 10-undecynoic acid column could also be a factor in the decreased retention. A mixture of four PTH - amino acids

Table 8 : Retention data of theophyilines and its derivatives on 10 - undecynoic acid column

	Compound	k'
1	Theophylline	0.06
2	Aminophylline	0.66
3	7-2,3 dihydroxytheophylline	0.69
4	Caffeine	2.47

Table 9 : Retention data of nucleic acid monomers on 10 - undecynoic acid column

	Compound	k'
1	Adenine	0.37
2	Adenosine	0.72
3	Cytosine	0.2
4	Thymine	0.13
5	Uracil	0.12

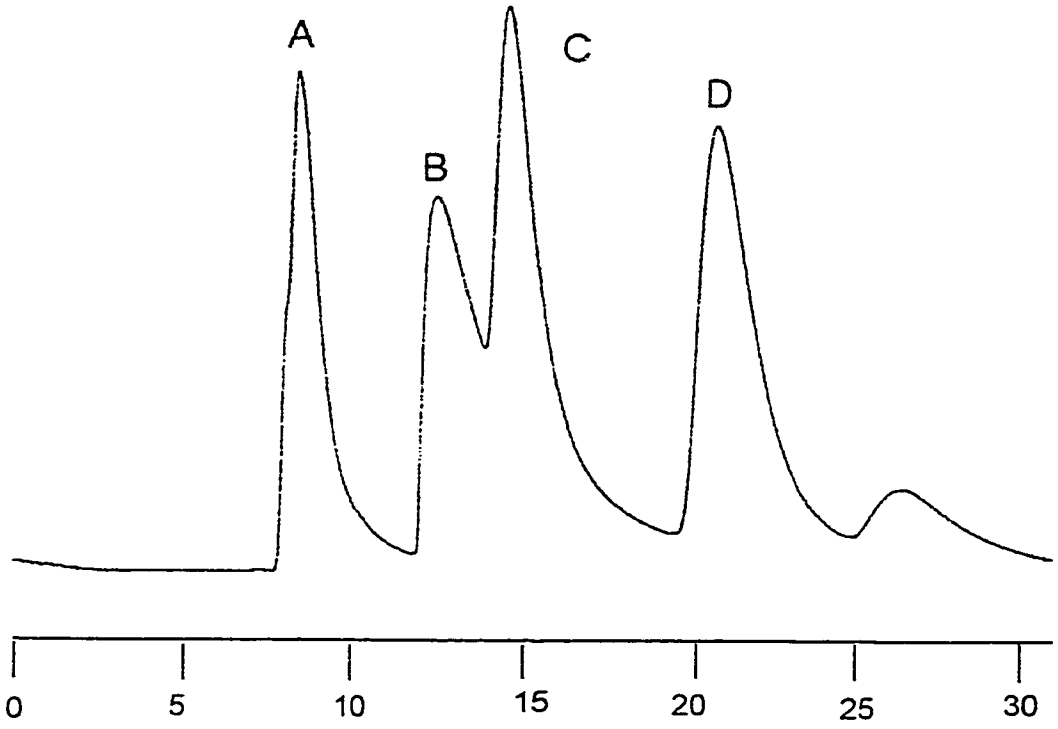


Figure 31 : Separation of mixture of PTH - amino acids on 10 - undecynoic acid column. Mobile phase - 0.001 M $NH_4H_2PO_4$ (pH 3.5) at 0.2 ml/min. Detection - UV at 254 nm. Peaks - A = PTH - Cysteic acid, B = PTH - Methionine sulfone, C = PTH - Serine, D = PTH - Glutamine.

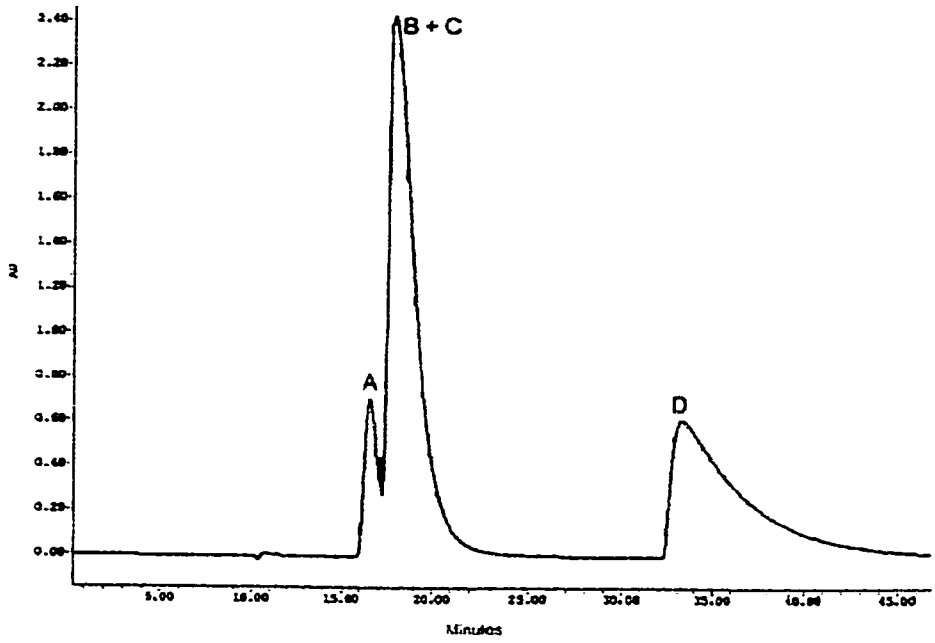


Figure 32 : Separation of caffeine & theophylline compounds on 10-undecynoic acid column. Mobile phase - 0.01 M $NH_4H_2PO_4$ (pH 4.5) at 0.2 ml/min. Detection - UV at 254 nm. Pressure - 207 psi. Peaks- A = Theophylline, B = Aminophylline, C = 7-2,3 dihydroxytheophylline, D = Caffeine.

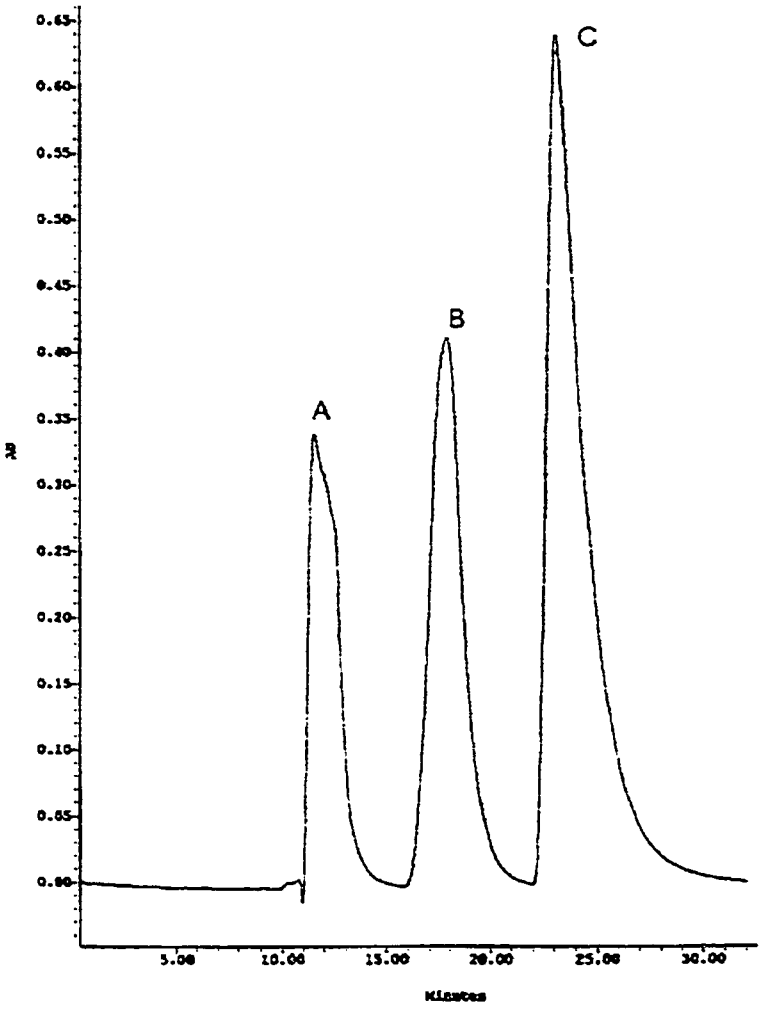


Figure 33 : Separation of Nucleic acids on 10-Undecynoic acid column. Mobile phase - 0.01 M $NH_4H_2PO_4$ (pH 4.5) at 0.2 ml/min. Detection - UV at 254 nm. Pressure - 204 psi. Peaks - A = Thymine, B = Adenine, C = Adenosine

was made and injected on the column. The chromatogram is shown in Figure 34. Adequate separation of the four amino acids is obtained. The peak shapes are also good.

Table 10 : Retention data of PTH-Amino acids on Undecylenic acid Column

Number	Solute	k'
1	PTH - Cysteic acid	0.05
2	PTH - Aspartic acid	0.55
3	PTH - Isoleucine	0.48
4	PTH - Leucine	0.38
5	PTH - Hydroxyproline	0.8
6	PTH - Methionine sulfone	0.73
7	PTH - Serine	-
8	PTH - Proline	2.83
9	PTH - Tryptophan	0.62
10	PTH - Asparagine	0.86
11	PTH - Glutamine	0.88

b) Theophylline and its derivatives

Theophylline, 7-(2,3 dihydroxypropytheophylline, aminophylline and caffeine were analyzed on the undecylenic acid column. The compounds were injected on the column with a mobile phase of 0.01 M $NH_4H_2PO_4$ (pH 4.5) at a flow rate of 0.2 ml/min. Table 11 shows the retention values of these compounds. The k' values of the solutes follow the order theophylline < aminophylline < 7-2,3 dihydroxypropyltheophylline < caffeine. A slightly lower surface coverage of the undecylenic acid column could be the cause for lower retention values for these

solutes as compared to those obtained on the 10-undecynoic acid column. A mixture of the four compounds was made and injected on the column. The mobile phase conditions were the same as mentioned above. The chromatogram of this separation is shown in Figure 35. Theophylline and aminophylline were not resolved and eluted as a single peak.

c) Nucleic acid monomers

The capacity factors of nucleic acid monomers and nucleosides on the undecylenic column is shown in Table 12. The mobile phase conditions were 0.01 M $NH_2H_2PO_4$ pH 4.5 at a flow rate of 0.2 ml/min. The solutes were injected separately and their retention times noted. The k' of the solutes is uracil < thymine < adenine < adenosine < cytosine. The retention data of the solutes is comparable to those obtained from the 10-undecynoic acid column. Increased retention of cytosine with the stationary phase could be due to interaction with residual silanols. A mixture of three nucleic acids was made and injected. Figure 36 shows the chromatogram of this separation.

3. 4 - Pentenoic acid column

a) PTH - amino acids

PTH - amino acids were injected separately into the 4-pentenoic acid column with a mobile phase of 0.001 M $NH_4H_2PO_4$ (pH 3.5) at a flow rate of 0.2 ml/min. Detection

Table 11 : Retention data of theophyllines and its derivatives on undecylenic acid column

	Compound	k'
1	Theophylline	0.26
2	Aminophylline	0.36
3	7-2,3 dihydroxytheophylline	1.00
4	Caffeine	1.31

Table 12 : Retention data of nucleic acids on undecylenic acid column

	Compound	k'
1	Adenine	0.36
2	Adenosine	0.57
3	Cytosine	0.86
4	Thymine	0.14
5	Uracil	0.11

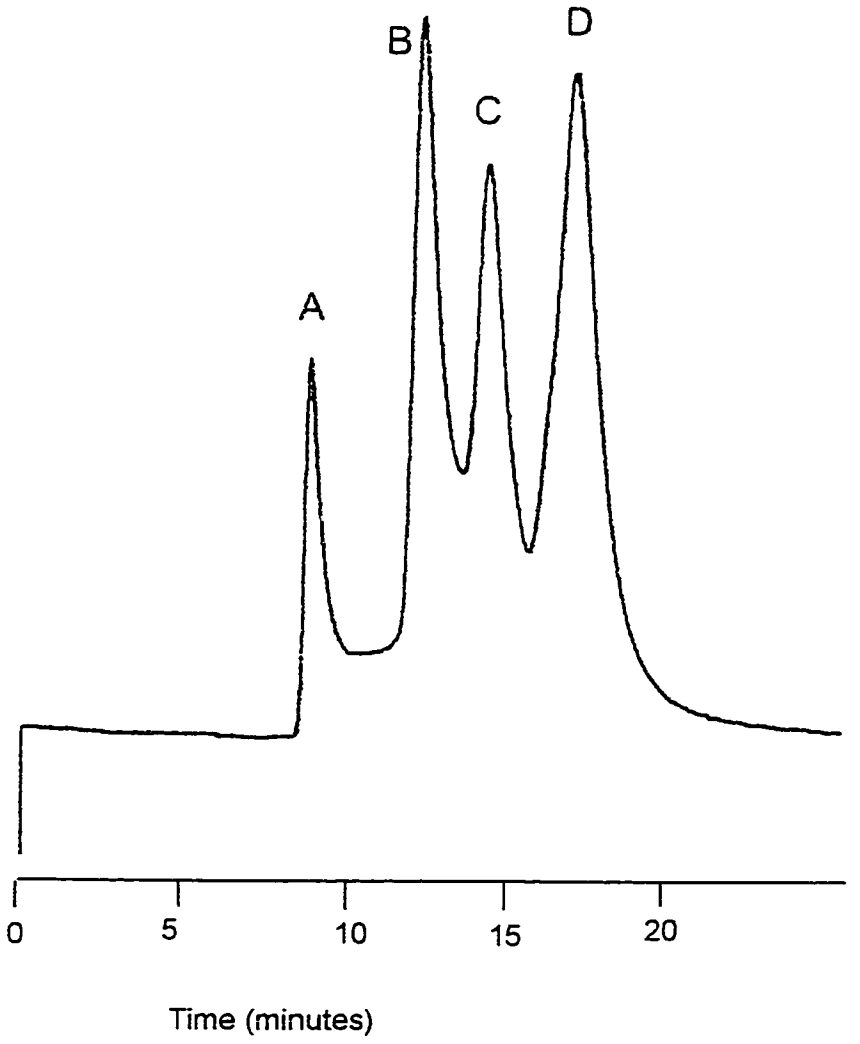


Figure 34 : Separation of PTH - amino acids on undecylenic acid column. Mobile phase - 0.001 M $NH_4H_2PO_4$ (pH 3.5) at 0.2 ml/min. Detection - UV at 254 nm. Peaks - A = PTH-Cysteic acid, B = PTH- Hydroxyproline, C = PTH - Asparagine, D = PTH - Glutamine.

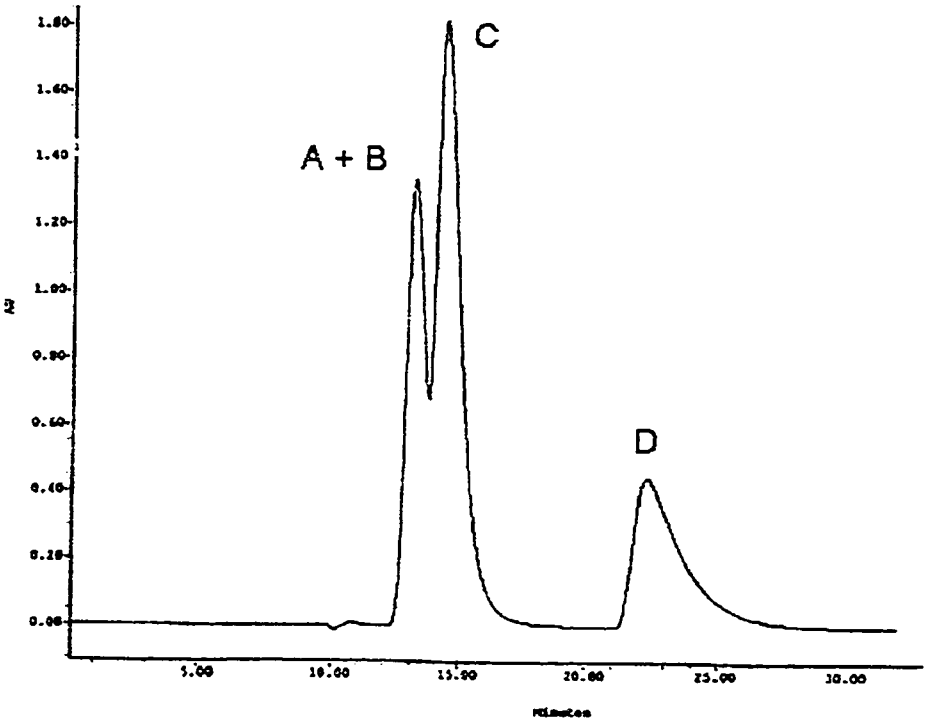


Figure 35 : Separation of caffeine & theophylline compounds on undecylenic acid column. Mobile phase - 0.01 M $NH_4H_2PO_4$ (pH 4.5) at 0.2 ml/min. Detection - UV at 254 nm. Pressure - 670 psi. Peaks - A = Theophylline, B = Aminophylline, C = 7-2,3 dihydroxytheophylline, D = Caffeine.

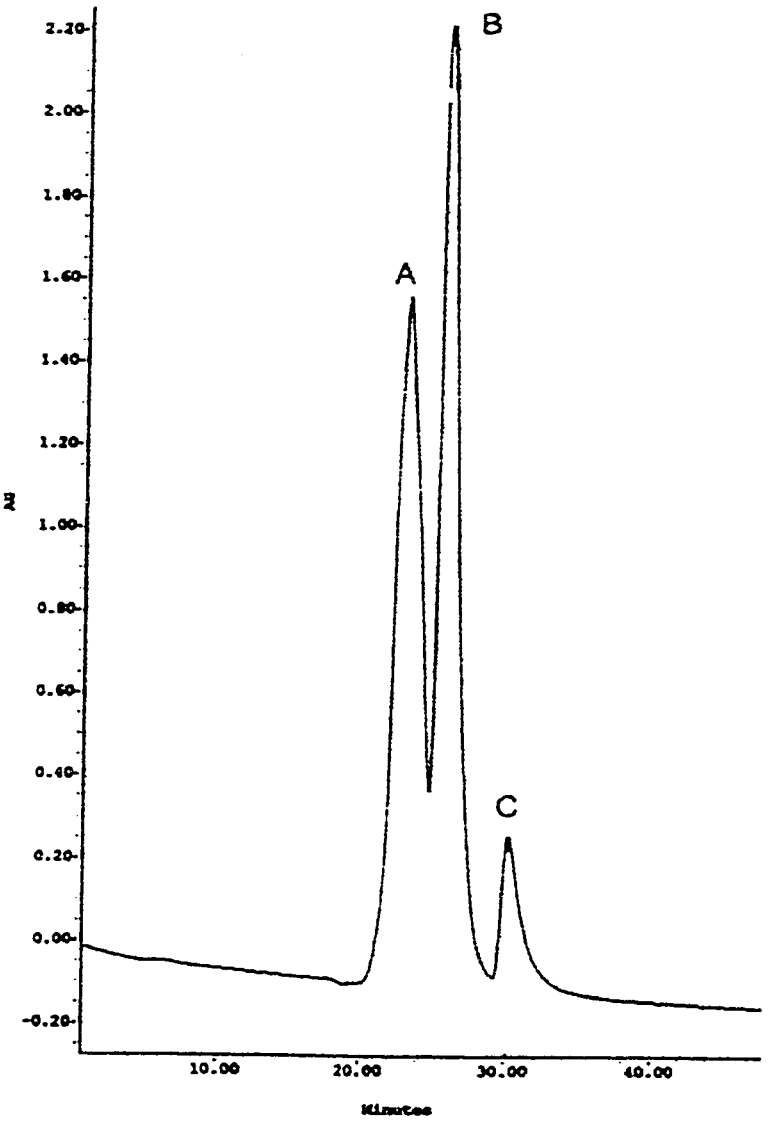


Figure 36 : Separation of nucleic acids on undecylenic acid column. Mobile phase - 0.01 M $NH_4H_2PO_4$ (pH 4.5) at 0.2 ml/min. Detection - UV at 254 nm. Pressure - 636 psi. Peaks - A = Adenine, B = Adenosine, C = Cytosine.

of the eluting compounds was carried out with UV detection at 254 nm. The retention values of these compounds were calculated. The results are shown in Table 13.

Table 13 : Retention data of PTH - amino acids on 4 - pentenoic acid column

Number	Solute	k'
1	PTH - Cysteic acid	0.00
2	PTH - Aspartic acid	0.05
3	PTH - Isoleucine	0.25
4	PTH - Leucine	0.24
5	PTH - Hydroxyproline	0.16
6	PTH - Methionine sulfone	0.13
7	PTH - Serine	0.10
8	PTH - Proline	0.53
9	PTH - Tryptophan	0.65
10	PTH - Asparagine	0.54
11	PTH - Glutamine	0.68

The order of elution of the PTH - amino acids on the 4 - pentenoic acid column is typical of a weak cation-exchange column with the acidic amino acids eluting first followed by the neutral amino acids and finally the basic amino acids. A mixture of four PTH - Amino acids was made and injected on the column. The conditions were the same as mentioned above. The chromatogram is shown in Figure 37. All four amino acids are partially resolved. The peak shapes are generally good. The one exception is the peak tailing seen for PTH - tryptophan. This can be due to the secondary interaction of basic solutes with residual acidic silanol groups on the silica surface. From a comparision to the retention values of these amino acids with the k' values on 10 - undecynoic acid and undecylenic acid columns, it can be seen

that there is an increase in retention with increasing alkyl chain length of the bonded material. This can be explained by the hydrophobic or partition type interactions because as the chain length is doubled, the amount of stationary phase is doubled and hence the retention time increases [15].

b) Theophylline and its derivatives

Table 14 shows the retention values of these compounds on the 4-pentenoic acid column. The k' values of theophylline, aminophylline and 7-2,3 dihydroxytheophylline are close together. Caffeine is well retained because of strong non polar interactions with the stationary phase. A mixture of the four compounds was made and injected on the column. The mobile phase used was 0.01 M $NH_4H_2PO_4$ (pH 4.5) . The chromatogram of this separation is shown in Figure 38. Only two peaks are seen. Theophylline and 7-2,3 dihydroxytheophylline co-eluted. Separations of these compounds on the 10-undecynoic acid and undecylenic acid columns can be explained by the increased hydrophobic interactions between the solutes and stationary phase. The increased hydrophobic interactions can be attributed to the longer alkyl chain lengths of those bonded phases.

c) Nucleic acids

The k' values of nucleic acids on the 4-pentenoic acid column are shown in Table 14. 0.01 M $NH_4H_2PO_4$ (pH 4.5) was used as the mobile phase. The flow rate was set at 0.2 ml/min. The order of elution is uracil < thymine < cytosine <

adenosine < adenine. The retention values of the solutes on this column are less than those obtained from the C11 columns. Thus, it can be said that the retention times increase as the alkyl chain length of the stationary phase increases. A mixture of three nucleic acid bases was made and injected. Figure 39 shows the chromatogram of this separation. Two peaks are seen. Adenine and Adenosine co-eluted as a single peak.

Table 14 : Retention data of theophyllines and its derivatives on 4 - pentenoic acid column

	Compound	k'
1	Theophylline	0.16
2	Aminophylline	0.17
3	7-2,3 dihydroxytheophylline	0.2
4	Caffeine	0.85

Table 15 : Retention data of nucleotides on 4 - pentenoic acid column

	Compound	k'
1	Adenine	0.16
2	Adenosine	0.11
3	Cytosine	0.09
4	Thymine	0.07
5	Uracil	0.05

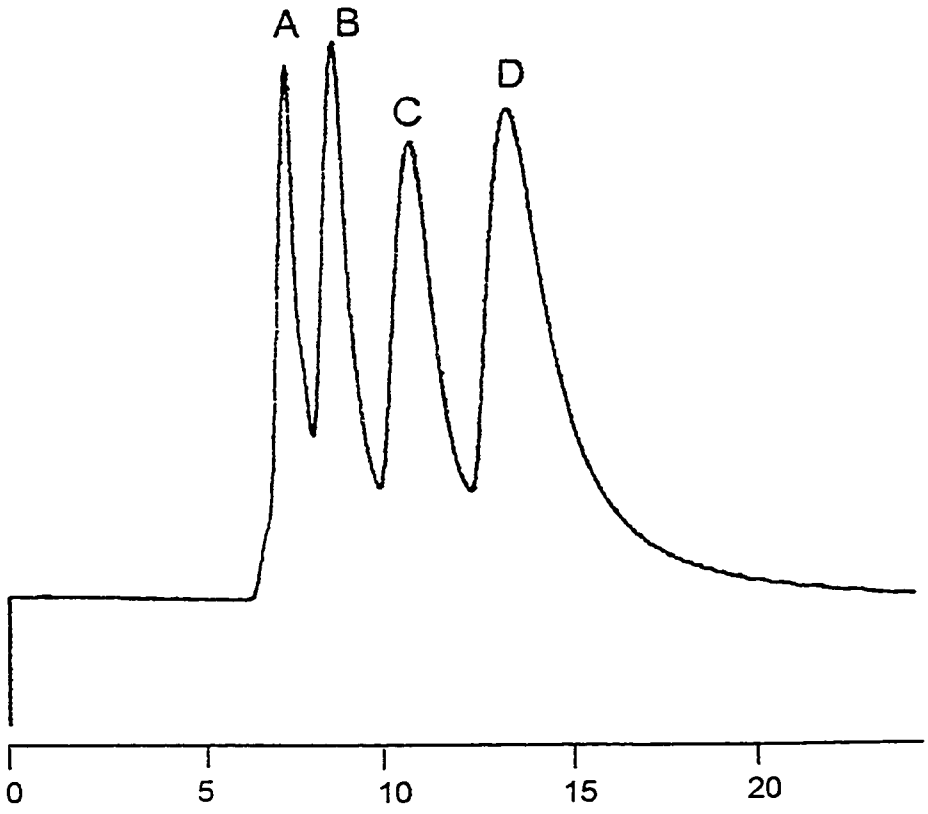


Figure 37 : Separation of PTH - amino acids on 4 - pentenoic acid column. Mobile phase - 0.001 M $NH_4H_2PO_4$ (pH 3.5) at 0.2 ml/min. Detection - UV at 254 nm. Peaks - A = PTH - Cysteic acid, B = PTH - Aspartic acid, C = PTH - Hydroxyproline, D = PTH - Tryptophan.

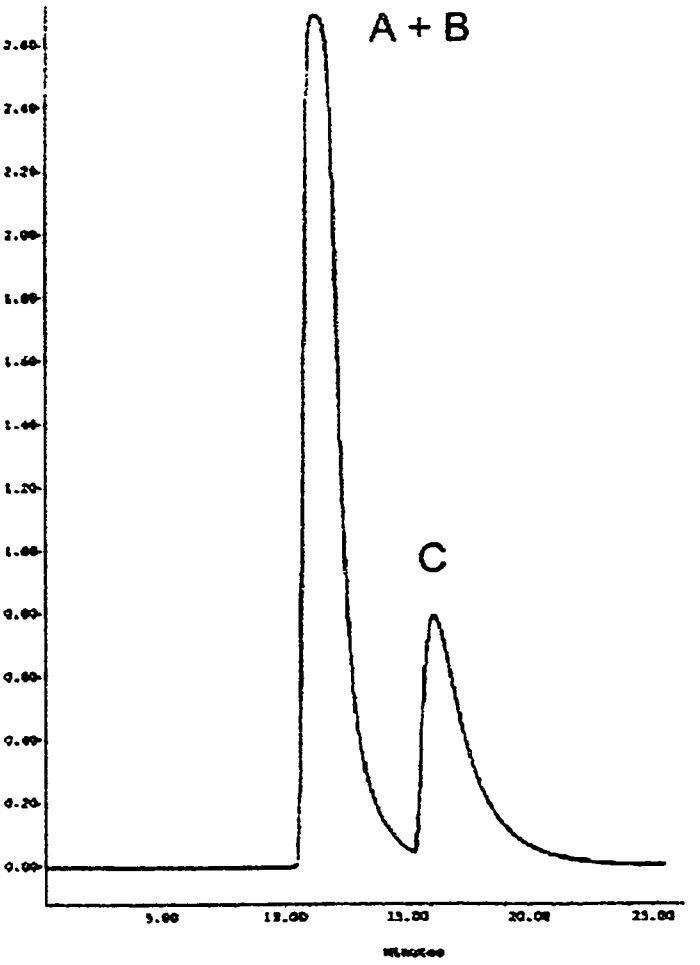


Figure 38 : Separation of caffeine & theophylline compounds on 4 - pentenoic acid column. Mobile phase - 0.01 M $NH_4H_2PO_4$ (pH 4.5) at 0.2 ml/min. Detection - UV at 254 nm. Pressure - 350 psi. Peaks - A = Theophylline, B = 7-2,3 dihydroxytheophylline, C = Caffeine.

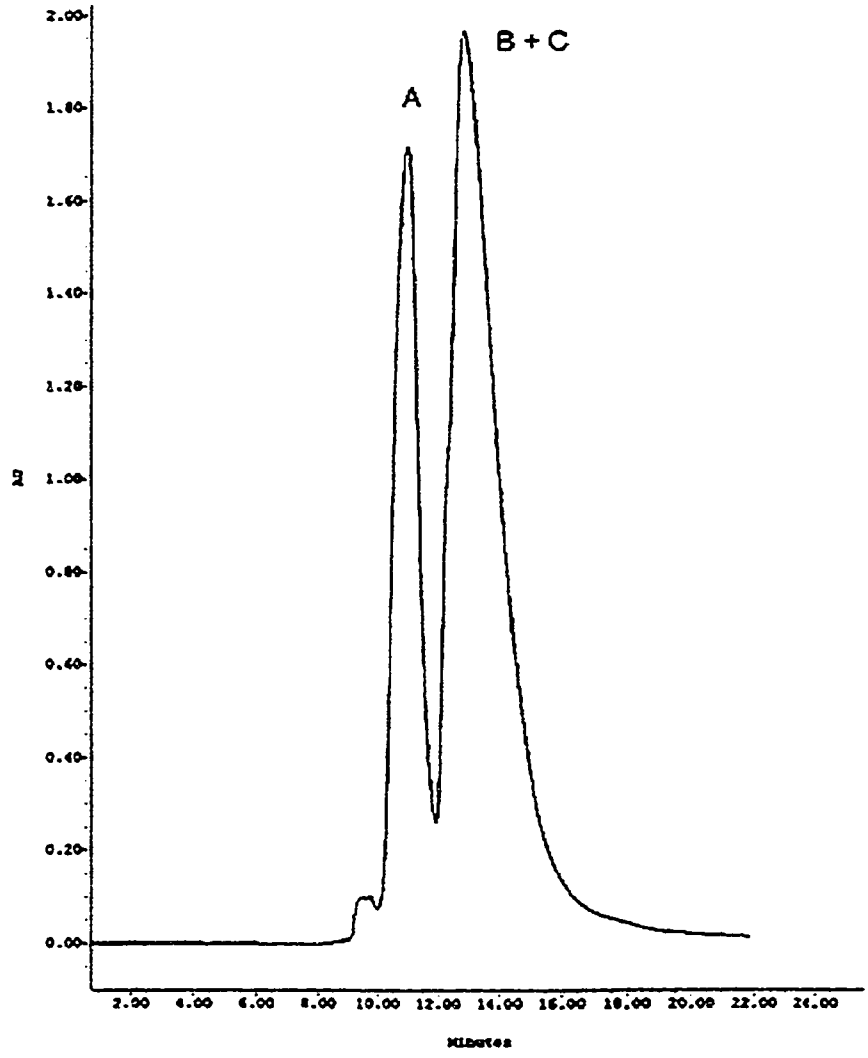


Figure 39 : Separation of nucleic acids on 4 - pentenoic acid column. Mobile phase - 0.01 M $NH_4H_2PO_4$ (pH 4.5) at 0.2 ml/min. Detection - UV at 254 nm. Pressure - 401 psi. Peaks - A = Cytosine, B = Adenine, C = Adenosine.

IV. CONCLUSION

Weak Cation-exchange stationary phases for high performance liquid chromatography were synthesized via the hydride intermediate route and hydrosilation. Hydrosilation is the addition of silicon hydrides to compounds with multiple bonds. The techniques used to evaluate the synthesized stationary phases were diffuse reflectance infrared fourier transform spectroscopy (DRIFT), cross-polarization magic angle spinning nuclear magnetic resonance spectroscopy (^{29}Si and ^{13}C), carbon analysis, and HPLC phases. On the basis of DRIFT and NMR results, 10-undecynoic acid, undecylenic acid and 4-pentenoic acid bonded silica were packed into columns. Elemental analysis showed a surface coverage, α_R (μ mol / m^2) of 1.57, 1.24 and 1.7, respectively. The 4-styrene sulfonic acid bonded silica was not chosen for further chromatographic evaluation because reproducibility in its synthesis was not obtained. In the case of unsaturated carboxylic acids, hexachloroplatinic acid catalyses the reaction of the hydroxyl groups of the carboxylic acids with Si-H bonds. This is supported by the occurrence of C=C peaks in the ^{13}C NMR of undecylenic and 4-pentenoic acid bonded phases. Thus, the hydrosilation reaction can be used to bond olefin and acetylene compounds containing carboxylic acid groups to the silica surface.

Chromatographic evaluation of the three columns was carried out by the separations of PTH-amino acids, theophylline compounds, nucleosides and other purine and pyrimidine compounds. Mixtures of PTH-amino acids were successfully

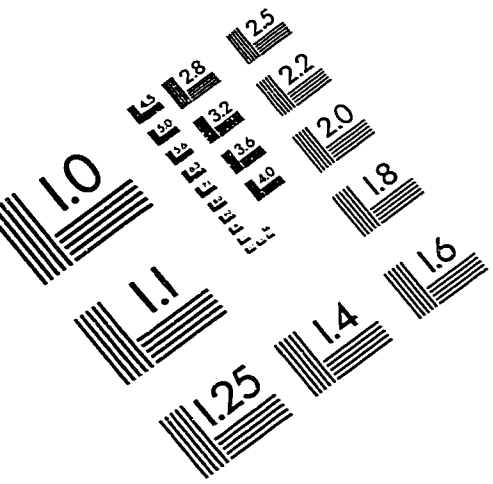
resolved on the three columns. The hydrophobic character of the stationary phases facilitates separation of non-ionic compounds as well. An increase in retention with increasing alkyl chain length is seen which can be attributed to the increased hydrophobic interactions. In many cases, peak tailing and excessive retention of basic solutes can occur due to increased interactions with the exposed acidic silanols (-Si-OH) on the silica surface.

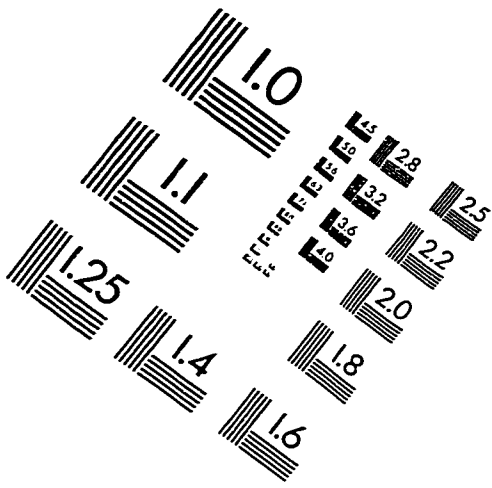
Future work includes the influence of organic modifiers in the mobile phase on the retention of the above mentioned compounds, long term stability tests of these columns under chromatographic conditions. Batch-to-batch reproducibility of the synthesized stationary phases should be investigated. The effect of varying experimental conditions such as reaction temperature, reaction time, type of catalyst (free radical initiation) on the surface coverage should be examined.

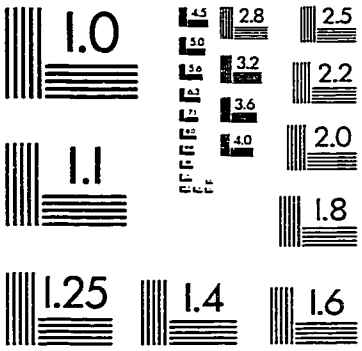
V. REFERENCES

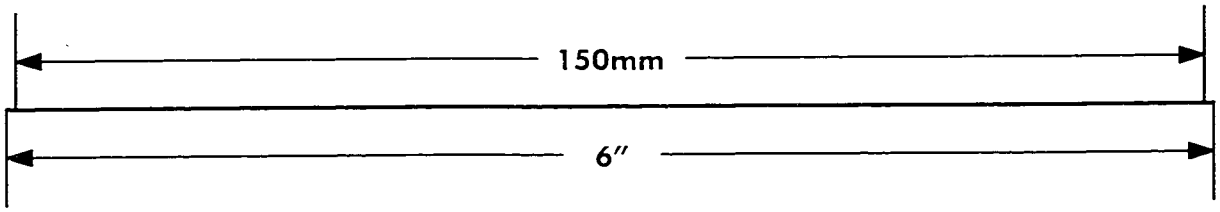
1. HPLC and CE: Principles and Practice; Weston, A., Brown, P.; Academic Press., CA, 1997; p1.
2. Nawrocki, J.; *J. of Chromatography A.*, **1997**, 779, 29-71.
3. Majors, R. E.; *LC-GC, Current Issues in HPLC Technology.*, May **1997**, S8-S19.
4. Pesek, J. J.; Sandoval, J. E.; Matyska, M.; *J. Liq. Chrom. & Rel. Technol.*, **1996**, 19, 2843-2865.
5. Dorsey, J.; Cooper, W.; *Analytical Chemistry*, **1994**, 66, 17, 857-865.
6. Pesek, J. J.; Matyska, M. T.; *Interface Science*, **1997**, 5, 103-117.
7. Characterization and Chemical modification of the silica surfaces; Vansant, E. F.; Van Der Voort, P.; Vrancken, K. C.; Elsevier science, 1995; p183.
8. Comprehensive Handbook on Hydrosilation; Marciniec. B.; Pegamon Press., Oxford, 1992.
9. High Performance Liquid Chromatography, 2nd ed.; Lindsay, S.; John Wiley and Sons, Chichester, England, 1992; Chapters 1,7.
10. Introduction to Spectroscopy; Pavia, D. L.; Saunders College Publishing, PA., 1979.
11. Berendsen, G. E.; DeGalan, L.; *J. Liq. Chromatogr.*, **1978**, 1, 561-568.
12. Bayer, E. A.; Reiners, J.; Nieder, M.; *J. Chromatogr.*, **1983**, 264, 197-213.
13. Sandoval, J. E.; Pesek, J. J.; *Analytical Chemistry*, **1991**, 63, 2634-2641.
14. Brown, P. E.; Grushka, E.; *Analytical Chemistry*, **1980**, 52, 1210-1215.
15. Barrett, D. A.; Brown, P.; Ross, P.; *J. Chromatographic Science*, **1996**, 34, 146-156.

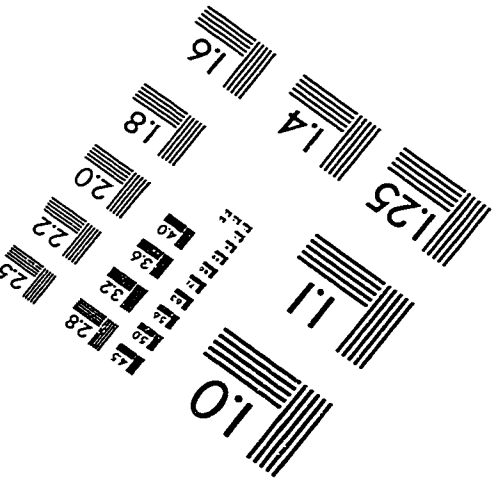
IMAGE EVALUATION
TEST TARGET (QA-3)

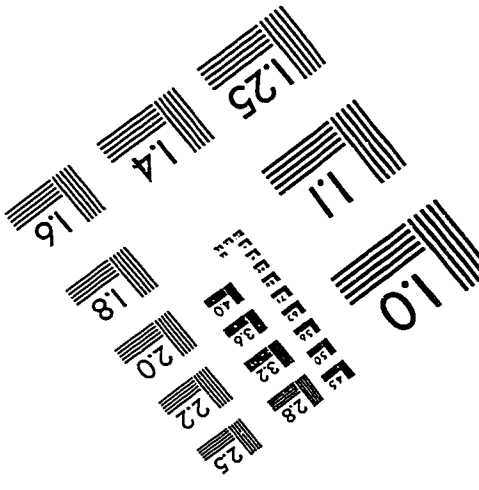












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