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Charge transfer-like stationary phase for high performance liquid chromatography

Valli Grandhi
San Jose State University

CHARGE TRANSFER-LIKE STATIONARY PHASE FOR HIGH PERFORMANCE LIQUID CHROMATOGRAPHY

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Valli Grandhi
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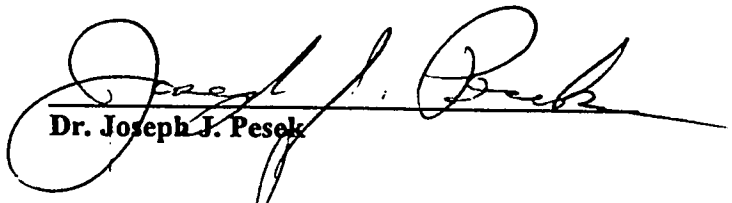

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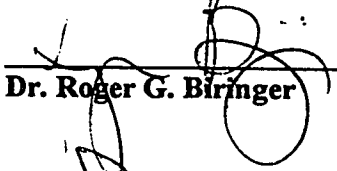

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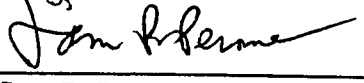

Dr. Sam P. Perone

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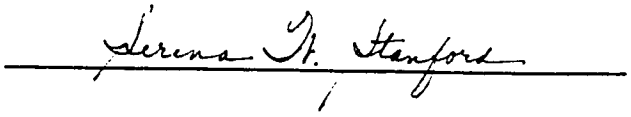

ABSTRACT

CHARGE TRANSFER-LIKE STATIONARY PHASE FOR HIGH PERFORMANCE LIQUID CHROMATOGRAPHY

by Valli Grandhi

A new approach to produce a charge-transfer type stationary phase for the separation of biochemical compounds is described and discussed. The procedure involves the attempted formation of a 7-(2,3-dihydroxypropyl) theophylline derivative by an esterification reaction, followed by the catalytic addition of the derivative to the silica hydride intermediate. Spectroscopic techniques such as diffuse reflectance infrared Fourier transform spectroscopy (DRIFT), nuclear magnetic resonance spectroscopy (NMR), and thermal analysis such as CHN analysis and differential scanning calorimetry (DSC) are employed to characterize the bonded phase. The bonded material, but not the expected derivative, shows promise as a useful stationary phase in high-performance liquid chromatography (HPLC).

ACKNOWLEDGMENTS

I would like to thank Dr. Joseph J. Pesek for his support and guidance throughout this project. I am especially thankful to Dr. Eric Williamsen for his advice and suggestions that helped me to achieve my goal. Also my sincere thanks to Dr. Roger Biringer and Dr. Sam Perone for acting as graduate committee members. A special note of thanks to Dr. Roger Biringer for his time and valuable comments on my thesis.

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I. INTRODUCTION

A. CHROMATOGRAPHY

Chromatography is one of the most widely used analytical techniques in separation science. It was originally developed by the Russian botanist Michael Tswett in 1906.[1] The separation of a mixture is brought about by the differential migration of the various compounds passing through a column (stationary phase) under the influence of a moving fluid (mobile phase) at high pressure (~ 6000 psi).

The mobile phase is can be either a liquid or a gas. The stationary phase can be either a solid or liquid which is fixed in place on a column or on a solid surface. Depending on the relative polarities of both the stationary and mobile phases, the chromatography is of two types: (1) normal phase, where the mobile phase is non-polar and stationary phase is polar and (2) reverse phase, where the mobile phase is polar and stationary phase is non-polar.

Unlike gas chromatography (GC), liquid chromatography (LC) provides separations of non-volatile samples (without derivatization) and ionic solutes. The instruments developed in the 1960's used smaller-diameter silica particles and columns to achieve high-efficiency separations, hence the name high-performance liquid chromatography (HPLC).

Separation efficiency in high-performance liquid chromatography is influenced by the stationary phase as well as the mobile phase. In general, stationary phases are of three different types: adsorptive, liquid-coated and bonded. The bonded stationary phase is more stable because it can withstand a shear force better than either adsorptive or liquid-coated materials under varying chromatographic conditions. Hence, in this study, the stationary phase is developed by chemically bonding the organic moiety to the silica.

In order to increase the range of interactions between the solutes and the stationary phase, a variety of functional groups have been bonded to the silica surface. The most commonly used stationary phase for liquid chromatographic separations is the C_{18} column where the stationary phase is a hydrocarbon chain containing 18 carbon atoms.

One of the major advantages of LC over other separation techniques is its ability to function in several different modes. Depending on the retention of the sample molecules by the stationary phase, LC is classified into four basic methods: (1) liquid-liquid chromatography (LLC) where the separation is based on the partitioning coefficient of the various analytes; (2) liquid-solid chromatography (LSC) where the retention occurs by the attraction of the sample molecules onto the surface of the particle; (3) ion-exchange chromatography which involves the substitution of one ionic species for another where the stationary phase is a rigid matrix, the surface of which carries a net positive or negative charge; and (4) size-exclusion chromatography where the separation is based on the molecular size and shape of the sample molecules.[2]

B. SILICA SURFACE

Although alumina and zirconia are used as inert supports, silica is the most commonly used base material in both normal- and reverse-phase chromatography. Silica used for these chromatographic purposes is porous and amorphous in nature with the general formula $SiO_2 . xH_2O$ and it is prepared by the polymerization of silicic acid ($Si(OH)_4$). Porous silica provides more surface area so that there is an increased interaction between the analyte and the surface-active silanols. Commercially available silicas can vary in their physical properties such as pore size, pore diameter and particle size from lot to lot depending on the manufacturing procedures. Amorphous silica has a

three dimensional network of siloxane moieties (-Si-O-Si) with silanol groups (-Si-OH) on the surface as shown in Figure 1.[3]

These silanols serve as attachment points that anchor bonded phases to the silica support. Figure 1 shows the possible types of silanols present on the surface. They are (1) vicinal, (2) geminal, and (3) isolated silanols. Each different surface group exhibits its own unique properties that affect both the bonding reactions and adsorptive character.

The density of silanols on chromatographic-grade silica is ~ 8 ± 1 $\mu mol/m^2$. Because of steric hindrance, the maximum possible concentration of alkyl groups in a bonded phase is ~ 4.5 $\mu mol/m^2$ for C_{18} ligands. Hence, after silica surface modification, numerous unreacted silanol groups are left within the bonded phase. These residual silanols are weakly acidic (pK_a between 5 and 7) and can interact with polar compounds through strong hydrogen bonding and dipole-dipole interactions resulting in peak tailing and loss of chromatographic resolution.

C. SURFACE MODIFICATION

1. Reaction between Silica and Chlorodimethylalkylsilane

Various synthetic approaches have been developed to covalently bond the organic moieties onto the silica surface. In the 1970's, commercial bonded phases were prepared by reacting chlorodimethylalkylsilane with surface silanols to produce Si-O-Si-C linkages as shown in equation 1.[4]

$$\equiv Si\text{-}OH + Cl\text{-}Si(CH_3)_2\text{-}R \text{ ----------> } \equiv Si\text{-}O\text{-}Si\text{-}(CH_3)_2\text{-}R + HCl \quad (1)$$

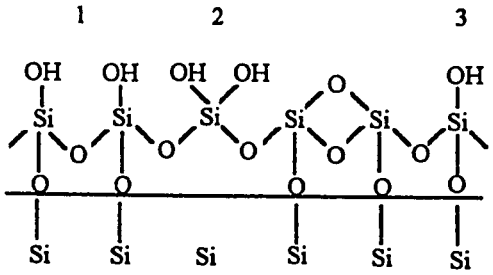


Figure 1. Three different types of hydroxyl groups on the silica surface: (1) vicinal silanols; (2) geminal silanols; (3) isolated silanol.

The bonded phase developed via a siloxane (Si-O-Si-R) linkage has some limitations. The steric hindrance of the triorganosilyl group causes a relatively limited coverage of the organic moieties on the silica surface. Consequently, a significant fraction of silanol (SiOH) groups remain after the reaction. These residual silanols may interact with the species under separation, particularly with basic solutes. Additionally, the bonded phase displays limited long-term hydrolytic stability in the presence of certain aqueous solutions.

The derivatized reaction shown above is a monomeric reaction, in which only one leaving group is present on the reactive silane and the bonding can occur only at that point. Bonded phases can also be produced where two or three leaving groups are present on the silane. These polymeric phases result in a molecular network extending away from the surface of the silica. Unlike polymeric phases, monomeric bonded phases produce a well-defined surface coverage and is easily reproducible.

2. Reaction between Silica and Lithium Aluminum Hydride

Alternative methods have been developed for producing bonded phases bearing direct Si-C linkages on the silica support. One such method is the chlorination and reduction of the silica surface.[5] The reaction is carried out in two steps: The first step is the chlorination of silica by reacting the surface with a reagent such as thionyl chloride (equation 2).

$$\equiv \text{Si-OH} + \text{SOCl}_2 \xrightarrow{\text{toluene}} \equiv\text{Si-Cl} + \text{HCl} + \text{SO}_2 \qquad (2)$$

The second step involves the reduction of the chlorinated surface with an appropriate alkylating reagent such as lithium aluminum hydride to yield the desired silica hydride intermediate (equation 3). The silica hydride intermediate is further reacted via a hydrosilation reaction with a terminal olefin in the presence of a suitable catalyst to produce bonded material with a direct silica-carbon linkage (equation 4).

$$4 \equiv Si\text{-}Cl + LiAlH_4 \xrightarrow{\text{ether}} 4 \equiv Si\text{-}H + LiCl + AlCl_3 \quad (3)$$

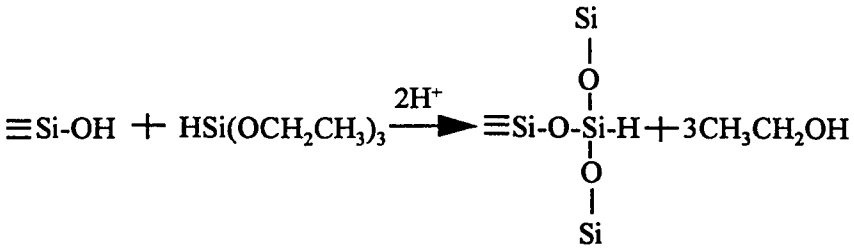
$$\equiv Si\text{-}H + CH_2{=}CH\text{-}R \xrightarrow{\text{catalyst}} \equiv Si\text{-}CH_2\text{-}CH_2\text{-}R \quad (4)$$

The bonded phase resulting from the chlorination/reduction method is hydrolytically more stable than that obtained by the corresponding organosiloxane type bearing Si-O-Si-C linkages. However, the chlorination/reduction method has some limitations. The most severe limitation is that both the chlorination and reduction steps are extremely sensitive to moisture which require time-consuming and labor-intensive procedures. This method also produces undersirable side-products such as LiCl and $AlCl_3$. These salts are often entrapped in the silica matrix and produce non-uniform retention characteristics in the final product. Hence, the clean-up procedures required to remove these residual salts further complicates the synthesis.

3. Hydrosilation after Silanization with TES

Hydrosilation is an addition reaction between an organic moiety and an inorganic silicon hydride to multiple bonds such as =C-C=, -C≡C-, =C=O, =C=N-, -C≡N, -N=N-, -N=O or ≡C-OH to produce a bonded phase. Sandoval et al. developed a bonded phase

via a hydrosilation reaction as shown in equations 5 and 6.[6] The procedure involves the reaction between an organic moiety containing a terminal double bond and silica hydride.



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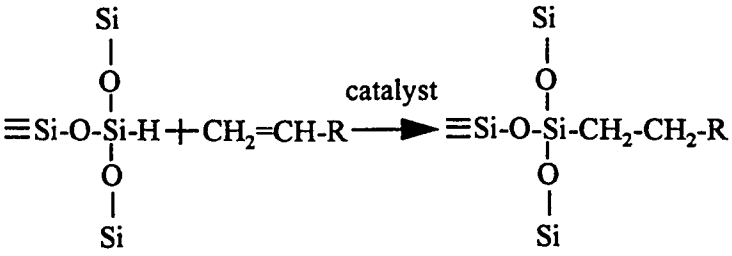


(6)

Equation 5 involves the preparation of silica hydride intermediate by reacting the surface silanols with triethoxysilane (TES) in the presence of water, an acid catalyst, and an appropriate solvent. The procedure, referred to as "silanization," produces the controlled deposition of the hydrolysis product of TES to preferentially form a Si-H monolayer. With this approach, the time-consuming processes associated with the previously mentioned chlorination/reduction procedure are eliminated.

This silica hydride intermediate is then reacted with an olefin in the presence of a suitable catalyst (equation 6). The addition of the olefin to the silica-hydride intermediate is known as hydrosilation. The hydrosilation reaction is carried out in the presence of a transition metal complex such as platinum, rhodium, palladium, nickel and ruthenium (group VIII). The most commonly used complex is "Speier's" catalyst.[7] It is a solution of

chloroplatinic acid hexahydrate ($H_2PtCl_6 \cdot 6H_20$) dissolved in 2-propanol. An induction period is often required before the addition of silica hydride. During the induction period, the platinum forms a complex with the olefin. Normally, hydrosilation is carried out with an excess of olefin (usually 10-fold molar excess or more) with respect to the silica hydride in order to obtain maximum surface coverage. The extent of the hydrocarbonaceous moiety bound to silica surface depends upon many factors, such as the olefin concentration, reaction temperature and solvent.

D. CHARGE TRANSFER-LIKE STATIONARY PHASE

Peak asymmetry and low separation efficiency are problems that are often encountered in the separation of basic compounds. One can improve the selectivity by using different mobile phases, by altering the mobile phase composition during the analysis, by changing the mobile phase pH, by altering the column temperature, by adding other modifiers to inhibit the reaction between the silanols and the analyte, or by changing the stationary phase. However, not all bonded phase silicas are equal in their ability to separate a particular group of compounds.

C_{18} columns have poor selectivity towards certain aromatic, nitrogen-containing compounds. These compounds have been implicated as direct-acting mutagens and have been found in airborne particulates,[8] in fly-ash from coal fired power plants,[9] and in diesel exhaust particulates.[10] In the past, GC/MS (gas chromatography/mass spectrometry) was used for the separation and identification of these environmental pollutants. However, it was shown that some aromatic nitrated-compounds are thermally labile at the temperatures required for GC/MS analysis.[10] Charge transfer-like stationary phases have been used to enhance the separation of these aromatic, nitrogen-containing compounds.[11]

The charge- transfer stationary phases are also employed in the separation of other aromatic hydrocarbons.[12]

Stationary phases based upon charge transfer interaction were first introduced by Pirkle and co-workers for the chromatographic resolution of enatiomeric sulfoxides.[13] Charge transfer-type stationary phases have been used for several types of separation problems, for example, separation of enatiomeric helicenes and broad range of polynuclear aromatics.[14,15] In these charge transfer-like interactions, no complete transfer of charge between an electron-rich stationary phase and an electron-deficient solute occurs. It is simply an interaction which occurs between the solute and the stationary phase during the separation process.

E. INSTRUMENTATION USED IN THIS WORK

Diffuse-reflectance infrared Fourier transform spectrophotometry (DRIFT), Nuclear Magnetic Resonance Spectrophotometry (NMR), differential scanning calorimetry (DSC), and CHN elemental analysis were the techniques used to evaluate the hydride-modified material and the bonded silica. A general description of these techniques is given below:

1. DRIFT Background

Fourier Transform Infrared Spectroscopy (FTIR) provides qualitative information about the structure of molecular species. The mid-infrared spectrum for organic compounds, 4000 - 400 cm^{-1}, provides a unique absorption pattern for each compound, and is therefore useful for identification purposes.[16] The most widespread technique that is used for the analysis of solids is called diffused reflectance infrared spectroscopy (DRIFT). In this technique, the finely ground solid sample is irradiated with radiation

ranging in wavelength from 10,000 to 4000 cm^{-1}. The radiation penetrates the surface layer of the particles and excites vibrational modes of the analyte molecule. As a result, diffused reflectance occurs in all directions. The reflectance spectrum produced is dependent upon the composition of the sample.

2. NMR Background

Nuclear magnetic resonance spectroscopy (NMR) is one the most powerful tools which is useful for the chemist to elucidate the structure of a species. The technique involves the measurement of absorption of electromagnetic radiation in the radio-frequency region of 4 - 600 MHz. In pulse NMR measurements, the nuclei in a strong magnetic field are irradiated periodically with highly intense pulses of radio-frequency radiation. The pulse length is usually less than 10 μs and the delay between pulses is approximately one second. In between the pulses, a time-domain, radio-frequency signal, called the free induction decay (FID), is emitted as the nuclei begin to relax and return to their equilibrium position. FID represents some of the frequencies emitted by the nuclei present in the sample that are characterisitic of the nuclei and environment.

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 the line broadening due to chemical shift anisotropy. MAS involves rotating solid sample rapidly (> 2 kHz) in an air-driven rotor at an angle of 57.4 °C with respect to the applied field. Cross polarization (CP) is used to improve sensitivity. CP technique causes the energy absorbed by the ^{1}H nuclei to be transferred to the nuclei of low natural abundance (such as ^{13}C and ^{29}Si). This increases the signal intensity. Transfer of energy also helps to relax the ^{13}C and ^{29}Si nuclei faster allowing the

spectrum to be taken more frequently. Thus more data sets can be obtained resulting in a higher signal to noise ratio.

3. DSC Background

Differential scanning calorimetry provides a simple and accurate way of determining the thermal properties of compounds. In this technique, the sample and a reference are subjected to a continuously increasing temperature (at a constant rate). Heat flows into both the sample and a reference as necessary to maintain the two at identical temperatures. When a transition such as melting, boiling, dehydration, or crystallization occurs in the sample, an endothermic or exothermic reaction occurs. The change in heat required to maintain the sample temperature the same as the reference temperature during the transition is recorded as a peak. This information is useful in determining the purity of the substance, or information about a surface.

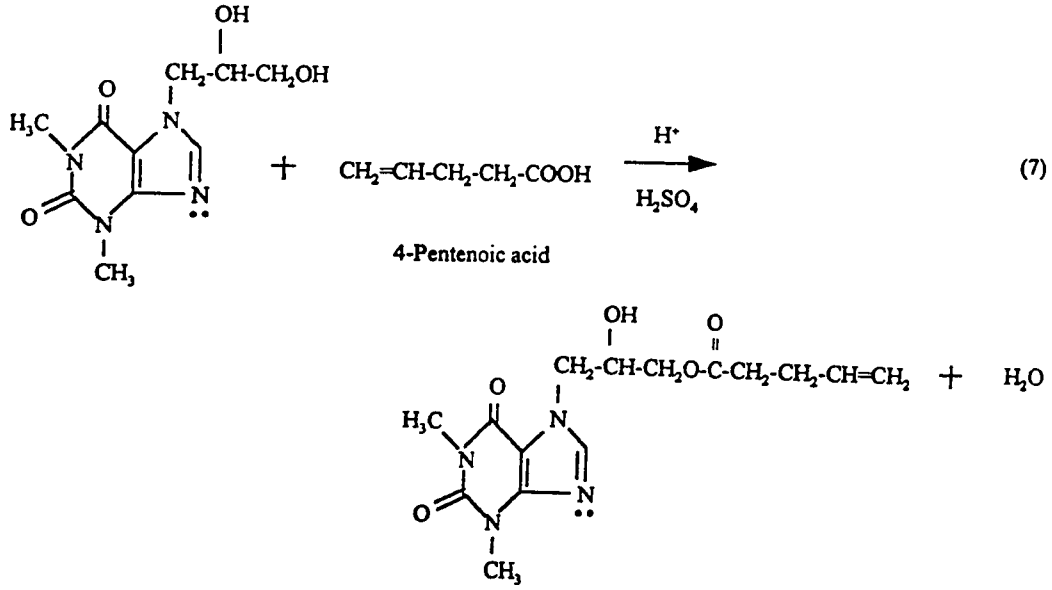
4. CHN Elemental Analysis Background

The CHN elemental analyzer determines the carbon, hydrogen, and nitrogen content of organic compounds (CO_2, H_2O, and nitrogen). Combustion occurs in pure oxygen under static conditions. The combustion products are then analyzed automatically in a self-integrating, thermal conductivity analyzer. Results are recorded in bar graph form on a 0-1 mV recorder.

F. AIMS OF THIS WORK

The present research has several fundamental objectives. The main goal is to develop a chemically bonded, charge transfer-like stationary phase via a silanization/ hydrosilation processes and to test the applicability of this stationary phase for the

separation of biochemical compounds. This research includes the preparation of a theophylline derivative (equation 7) and its chemical bonding to a porous silica substrate through a hydride intermediate.



(7)

The hydride intermediate is prepared by reacting the silica with triethoxysilane as mentioned earlier (section IB). In order to obtain the maximum amount of hydrocarbon onto the silica substrate, reaction variables such as temperature, reaction time, amount of solvent, type of catalyst, and the catalyst to olefin ratio were altered. The bonded stationary phase was characterized using various analytical techniques such as DRIFT, DSC, CHN elemental analysis, and ^{13}C and ^{29}Si CP-MAS NMR.

II. EXPERIMENTAL

A. MATERIALS

All the materials used for this research are listed in Tables 1, 2, and 3. Most chemicals were used as received. Exceptions are noted in the text.

B. INSTRUMENTAL PROCEDURES

1. Diffused Reflectance Infrared Spectroscopy

DRIFT spectra were taken using a Perkin-Elmer (P-E) Model 1800 Infrared spectrometer (Norwalk, Connecticut) equipped with a deuterated triglycine sulfate (DTGS) detector and controlled by a Perkin-Elmer Model 7500 computer with CDS-3 software. A diffuse-reflectance accessory (Spectra Tech., Stamford, CT) with a 2-mm deep sampling cup was used. The KBr was ground using a mortor and pestle (~ 10 minutes) and stored in an oven at 110 °C until use. The samples were prepared by diluting the bonded silica with finely ground KBr (1:3 wt/wt). The instrument was purged (~ 20 minutes) with nitrogen gas from a liquid nitrogen tank to provide a moisture-free sample atmosphere. Additional moisture protection was provided by placing a drying tube between the gas inlet and the sample atmosphere. Spectra were collected at 2 cm^{-1} resolution in the region of 4000 - 450 cm^{-1} and averaged over 100 scans. All sample scans were referenced to KBr.

Table 1. Chemicals used in Synthesis.

Name	Manufacturer	CAS NUMBER
7-(2,3-dihydroxypropyl) theophylline (99%)	Aldrich Chemical Co. Milwaukee, WI	479-18-5
4-Pentenoic Acid (97%)	Aldrich Chemical Co. Milwaukee, WI	591-80-0
N,N-Dimethylformamide (DMF)	Burdick and Jackson Laboratories Muskegon, MI	68-12-2
Chloroplatinic Acid hexahydrate (38 - 40% Pt)	Strem Chemicals Newburyport, MA	16941-12-1
Petroleum Ether	Spectrum Chemical Mfg. Group San Pedro Street, CA	8032-32-4
Isopentanol (Reagent Grade)	J. T. Baker Phillipsburg, NJ	123-513
Triethoxysilane (TES) (95%)	Aldrich Chemical Co. Milwaukee, WI	998-30-1
Methylene Chloride (99.9%) HPLC grade	Fisher Scientific Fair Lawn, NJ	75-09-2
Ethyl Ether (99.9%) HPLC grade	Fisher Scientific Fair Lawn, NJ	60-29-7
Tetrahydrofuran (THF) anhydrous (99%)	Aldrich Chemical Co. Milwaukee, WI	109-99-99
Chlorotis (triphenyl-phosphine) rhodium (99%) Wilkinson's catalyst	Strem Chemicals, Inc. Newburyport, MA	14694-95-2
1,3-Bis(diphenylphosphino) propane nickel (II) Chloride	Strem Chemicals, Inc. Newburyport, MA	15629-92-2

Table 2. Chemicals used in Characterization.

7-(2,3-dihydroxypropyl) theophylline (99%)	Aldrich Chemical Co. Milwaukee, WI	479-18-5
Acetanilide	Perkin Elmer Norwalk, Connecticut	103-84-4
Cyclohexanone-2,4-dinitro-phenyl hydrazone	Perkin Elmer Norwalk, Connecticut	1589-62-4
Potassium Bromide (KBr) IR grade	J & Baker Chemical Co. Phillipsburg, NJ	7758-02-3

Table 3. Chemicals used in HPLC Separation.

Prednisone	Aldrich Chemical Co. Milwaukee, WI	53-03-2
Corticosterone	Aldrich Chemical Co. Milwaukee, WI	1173-26-8
4-Androstene-3,17-dione	Aldrich Chemical Co. Milwaukee, WI	63-05-8
Adrenosterone	Aldrich Chemical Co. Milwaukee, WI	382-45-6
Methylene Chloride HPLC grade	B & J Laboratories Muskegon, MI	75-09-2
Hexane HPLC grade	B & J Laboratories Muskegon, MI	110-54-3
Tetrahydrofuran (THF) HPLC grade	B & J Laboratories Muskegon, MI	109-99-99

2. Nuclear Magnetic Resonance Spectroscopy

The ^{13}C and ^{29}Si CP-MAS NMR spectra were taken on a Bruker MSL 300 spectrophotometer. Solid samples were placed in ZrO_2, double-bearing rotor spun at 4700-5200 Hz. External glycine and polyhydridosiloxane samples served as references for the ^{13}C and ^{29}Si analyses, respectively. A pulse width of 6.5 and 5.0 μs were used for the ^{13}C and ^{29}Si spectra, respectively. A 5 μs contact time and a 5 s repetition rate were used for both analyses. The probe temperature was 20 ± 2 °C.

3. Differential Scanning Calorimetry

DSC was performed using a P-E DSC 7 Differential Scanning Calorimeter equipped with P-E TAC 7 instrument controller using TAS 7 software. 5 to 10 mg samples were loaded into a sealed, aluminum pan for heat measurement below 550 °C. The heat absorbed or given off was measured as the temperature was increased from 50 °C to 500 °C at a rate of 20 °C/min. The temperature scale was calibrated with an indium standard.

4. CHN Elemental Analysis

CHN analysis was done using a P-E 240-C Elemental Analyzer in the presence of helium and oxygen (both high purity grade). The helium and oxygen pressure settings were 17.5- and 32.5 psi, respectively. The combustion and reduction temperature settings of the oven were ~1000 °C and 700 °C, respectively. The analyzer was calibrated with a two-point method using acetanilide and cyclohexanone-2,4-dinitrophenylhydrazone. 7-(2,3-dihydroxy-propyl) theophylline was used as a known standard and was run each time before the sample. 1 to 3 mg samples were weighed using a Cahn Model 4700 - electrobalance (2 - 200 mg range). The sample runs were made in triplicate.

The surface coverage was calculated using the equation proposed by Berendsen and De Galan[17]

$$\alpha_R\,(\mu mol/m^2) = \frac{10^6 P_c}{(10^2 M_c n_c - P_c M_R) S_{BET}} \qquad (8)$$

where P_c is the difference in carbon percentage of the modified silica and the silica hydride, n_c is the number of carbon atoms in the theophylline derivative, M_R is the molecular weight of theophylline derivative, M_c is the atomic weight of carbon, and $S_{BET}\,(m^2/g)$ is the specific surface area of the silica hydride as determined by the Brunauer, Emmet and Teller (BET) nitrogen adsorption method performed at Chevron Research Center, Richmond, CA.

5. High Performance Liquid Chromatography

The liquid chromatograph used in this work was a Hewlett-Packard system (Model 1050) equipped with quaternary gradient pump, automatic injector, variable wavelength UV detector and a computer data station. The organic solvents and water were degassed using high purity grade helium gas for ~ 10 minutes prior to use. The back pressure was maintained below 100 bar and the ripple function within ± 2%.

Bonded material was packed into 1/4-in O.D., 4.6-mm I.D. x 150-mm stainless steel tubes (Altech Co., Deerfield, IL) using a Haskel (Burbank, CA) pneumatic pump. Approximately 1.85 g of modified silica was mixed with 20 mL of 10% (v/v) methanol-carbon tetrachloride in a 50-mL beaker followed by 10 minutes sonication. This slurry was added slowly to the reservoir and the remaining volume of the reservoir was filled with filtered methanol (~ 20 mL). Methanol was filtered through a 4.0-μm nylon

membrane. The mixture in the reservoir was packed into the column in the presence of nitrogen under ~ 6000 psi. After completion, the column remained attached to the packing apparatus for about 30 minutes before removal.

The separations were performed under normal-phase conditions as defined in Section IA. After separations were finished, the column was flushed with dichloromethane. The mobile phase composition was altered in increments of 10% by volume.

C. SYNTHETIC PROCEDURES

1. Synthesis of 7-(2,3-dihydroxypropyl) theophylline derivative

The organic phase was prepared by the esterification reaction of 7,(2,3-dihydroxy-propyl)theophylline with 4-pentenoic acid[18,19] (equation 7). 19.06 g of 7-(2,3-dihydroxy-propyl) theophylline was placed in a 250-mL, three-necked, round-bottom flask equipped with a magnetic stirrer. The center neck of the three-necked flask was equipped with a distilling head containing a 200 °C thermometer and in one opening of the distilling head a West condenser followed by a vacuum adapter and a collection flask. One of the remaining necks was equipped with a 200 °C thermometer that was immersed in the solution and the other was stoppered. 100 mL of DMF was added to the flask and the reaction mixture was heated until 7-(2,3-dihydroxypropyl)- theophylline was dissolved completely (~ 15 minutes). Then, 40 μL of concentrated sulfuric acid was added along with 5,100 μL of 4-pentenoic acid. The reaction mixture was heated at 145 °C for six hours.

The DMF solvent was removed from the product obtained in step 1 by vacuum distillation at 40 mm/Hg and 125 °C. About 90 mL of DMF was distilled and discarded. The remaining product was transferred into a 1000-mL conical flask. About 500 mL of

$CHCl_3$ (undistilled) was heated on a hot plate. About 100 mL of boiling $CHCl_3$ was added to the conical flask and heated for 5 minutes. Next, another 100 mL of $CHCl_3$ was added and heated for 5 more minutes. This procedure was repeated until the product was completely dissolved. Then, hot petroleum ether was added to this mixture dropwise until the solution turned cloudy. Additional hot $CHCl_3$ was added dropwise until the cloudiness disappeared and then left overnight in an icebath. Product crystals were filtered by gravity filtration and air dried for several hours at room temperature. The crystals were off-white in color.

2. Preparation of Speier's Catalyst

Within a glove box that had been previously flushed by vacuum and nitrogen alternatively three times, 0.04 g of chloroplatinic acid hexahydrate was dissolved in 100 mL of distilled isopentanol. The catalyst was then stored in the refrigerator.

3. Preparation of Silica Hydride

In a N_2-filled glove box, 9.51 mL of triethoxysilane (TES) (1.00 M) was added to a 50-mL volumetric flask and distilled 1,4-dioxane was added up to the mark. TES was transferred to a 125-mL pressure-equalizing addition funnel in the glove-box. 11.1 g of silica (Vydac 101 TP #900201) with a particle diameter of 6.583 μm, an average pore diameter of 380 Å and a specific surface area (S_{BET}) of 106.5 m^2/g) was transferred to a 500-mL, 3-necked, round-bottomed flask equipped with a West condenser, addition funnel, a stopper, and a magnetic stir bar. 273.0 mL of distilled dioxane was added followed by 10.8 mL of 2.3 M HCl solution. The joints were greased with lubriseal (Thomas Scientific). The reaction mixture was heated to reflux at 93 °C until a clear solution was observed. Then, the TES solution was added dropwise to the flask from the

additional funnel over 25 to 30 minutes. After complete addition of the TES solution, the reaction mixture was heated for one hour at reflux.

After the joints were degreased with xylene, the p-dioxane was poured off from the flask without losing silica. The contents of the flask were evenly poured into four centrifuge tubes equipped with a stir bar. Using an IEC HN-S centrifuge, the tubes were centrifuged for 10 minutes at 1500 rpm. The p-dioxane was poured off and replaced with 1:1 (v/v) THF/water until the centrifuge tube was 55% full. The stirring procedure was continued for 10 minutes. The same washing procedure was repeated three more times with a fresh 1:1 (v/v) THF/water mixture, twice with THF, and lastly twice with ether.

After the washing procedure was complete, the silica hydride was transferred into a recrystallization dish, covered with a Speedy Vac watch glass, and placed overnight in the hood to evaporate the solvents. The next day the silica hydride was placed in the drying oven at 110 °C for at least one day.

4. Hydrosilation of Theophylline Derivative on Silica Hydride

4 g of dried silica hydride was transferred to a bent glass tube. A 50-mL, 3-necked round-bottomed flask was equipped with a thermometer, West condenser, and a nitrogen venting system. 0.16 g of the theophylline derivative was transferred to the flask followed by 20 mL of distilled chloroform and 59 μL of Speier's catalyst solution. The mixture was heated at 52 °C until a clear solution was observed (~ 10 minutes). At this point, silica hydride was added to the reaction mixture by slowly rotating the bent tube. This procedure usually took ~ 10 minutes. After the complete addition of silica hydride, the reaction flask was flushed with nitrogen for 1-2 seconds. The reaction was allowed to proceed at 52 °C for 72 hours. The product was washed four times with 5-mL portions

of dry chloroform[1], two times with 5-mL portions of methylene chloride and two times with 5-mL portions of ethyl ether. The product was dried under vacuum at room temperature for 24 hours.

[1] (Baxter, HPLC grade) was dried by refluxing over calcium chloride ($CaCl_2$) for one day and distilled as needed[20]

III. RESULTS AND DISCUSSION

The principal goal of this work was to explore the feasibility of bonding the theophylline derivative to a silica support by means of hydrosilation reaction and also to investigate whether it is chromatographically useful for the separation of biochemical compounds such as steroids and vitamins.

Figure 2 shows the structure of the 7-(2,3-dihydroxypropyl) theophylline. The numbers on the various atoms are used in some of the spectroscopic analyses in subsequent sections.

A. DRIFT Spectroscopic Evaluation

As mentioned in Section IE-2, DRIFT spectroscopy provides a qualitative means for identifying the surface and monitoring the chemical changes.

1. Silica

The DRIFT spectrum of native silica (Curve A) and hydride intermediate (Curve B) are shown in Figure 3. Native silica refers to the silica before hydride modification. The band at 3750 cm^{-1} is due to the isolated Si-OH groups (i.e. not hydrogen bonded). The broad band centered between 3500 - 3200 cm^{-1} region is attributed to H-bonded OH groups and/or adsorbed water. Also, the band near 1630 cm^{-1} is assigned to water.[6] For the hydride intermediate (Curve B), the strong band at 2260 cm^{-1} represents the Si-H stretching. After hydrosilation, the Si-H peak is reduced in intensity relative to the adsorbed water peak between 3500 - 3200 cm^{-1}.

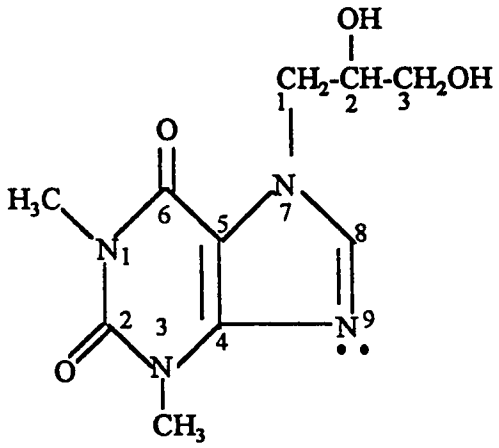


Figure 2. Structure of 7-(2,3-dihydroxypropyl) theophylline.

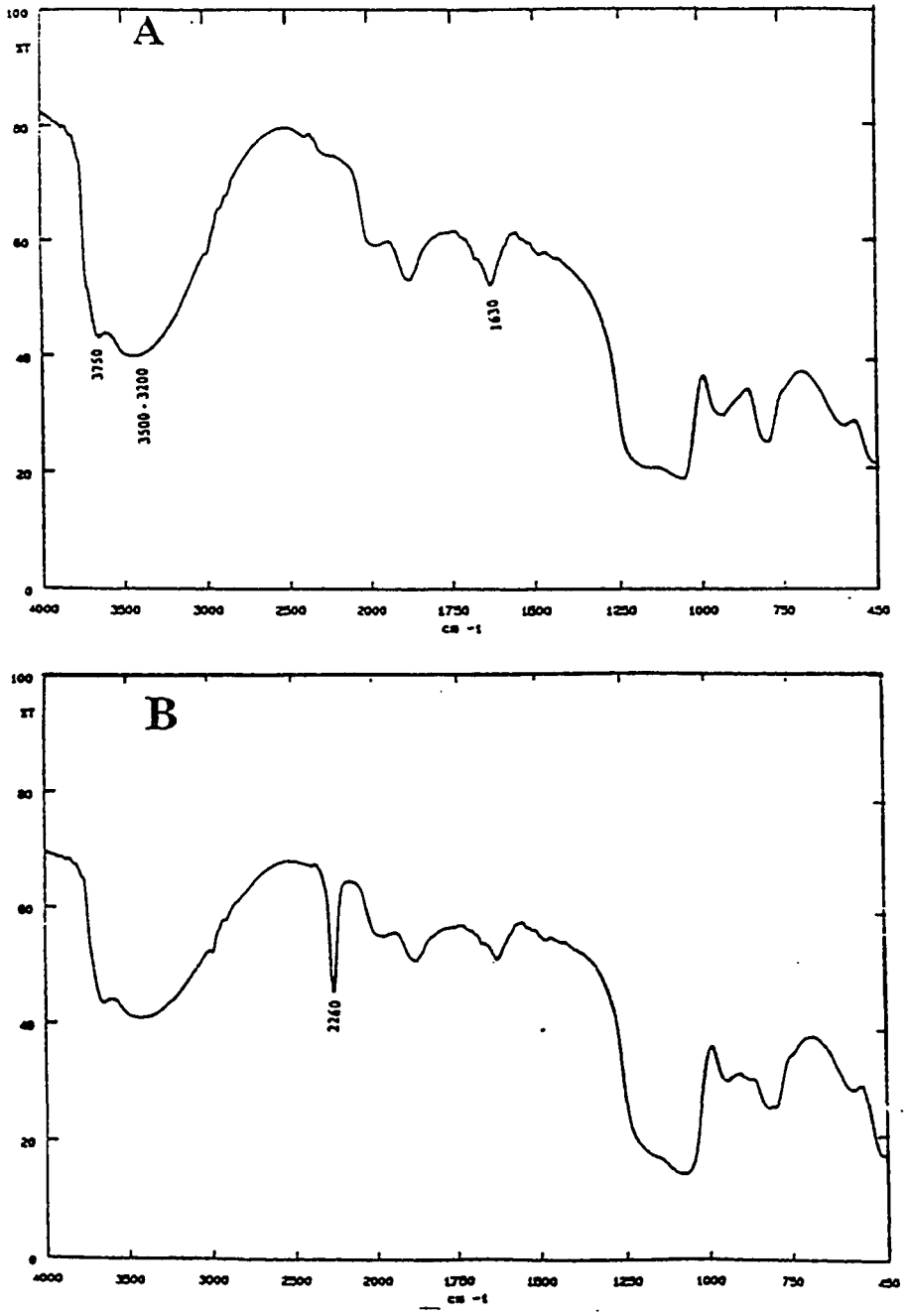


Figure 3. DRIFT spectra of native silica (Curve A) and silica hydride intermediate (Curve B).

2. 7-(2,3-dihydroxypropyl) theophylline and 7-(2,3-dihydroxypropyl) theophylline derivative

The drift spectrum of the 7-(2,3-dihydroxypropyl) theophylline (Curve A) and the derivative (Curve B) are shown in Figure 4. The two strong bands between 3200-3400 cm^{-1} represent O-H stretching probably from water. The peaks between 2853-2962 cm^{-1} are the C-H alkane stretches. The aromatic C-H stretching is around 3030 cm^{-1}. The C-N aromatic vibration is present at 1310-1360 cm^{-1}. The two strong peaks between 1705-1725 cm^{-1} and 1665-1685 cm^{-1} correspond to C=O (C2 and C6 positions) stretching vibrations.[21] A peak at 1550 cm^{-1} is the C=N (C8 position) stretching vibration. These are the characteristic features of the 7-(2,3-dihydroxypropyl) theophylline before the esterification process.

To prove conclusively that the esterification reaction has occurred, the 7-(2,3-dihydroxypropyl) theophylline derivative spectrum should show the following: terminal olefin C-H stretching at 3095-3075 cm^{-1} and C-H bending at 915, 995 and 1420 cm^{-1}. Even though the 7-(2,3-dihydroxypropyl) theophylline derivative spectrum shows peaks around 915, 995 and 1420 cm^{-1}, it is hard to confirm these peaks due to the presence several small peaks in this region. But, the spectrum does not show terminal olefin C-H stretching at 3095 cm^{-1} which is a key feature for the product of the esterification reaction. This result indicates that the esterification reaction did not occur.

3. Hydrocarbonaceous Bonded Phase

The DRIFT spectrum of the bonded silica is shown in Figure 5. Because most of the bonded material contains more silica than the theophylline material (w/w), the bonded phase spectrum shows only the strongest peaks of the 7-(2,3-dihydroxypropyl)

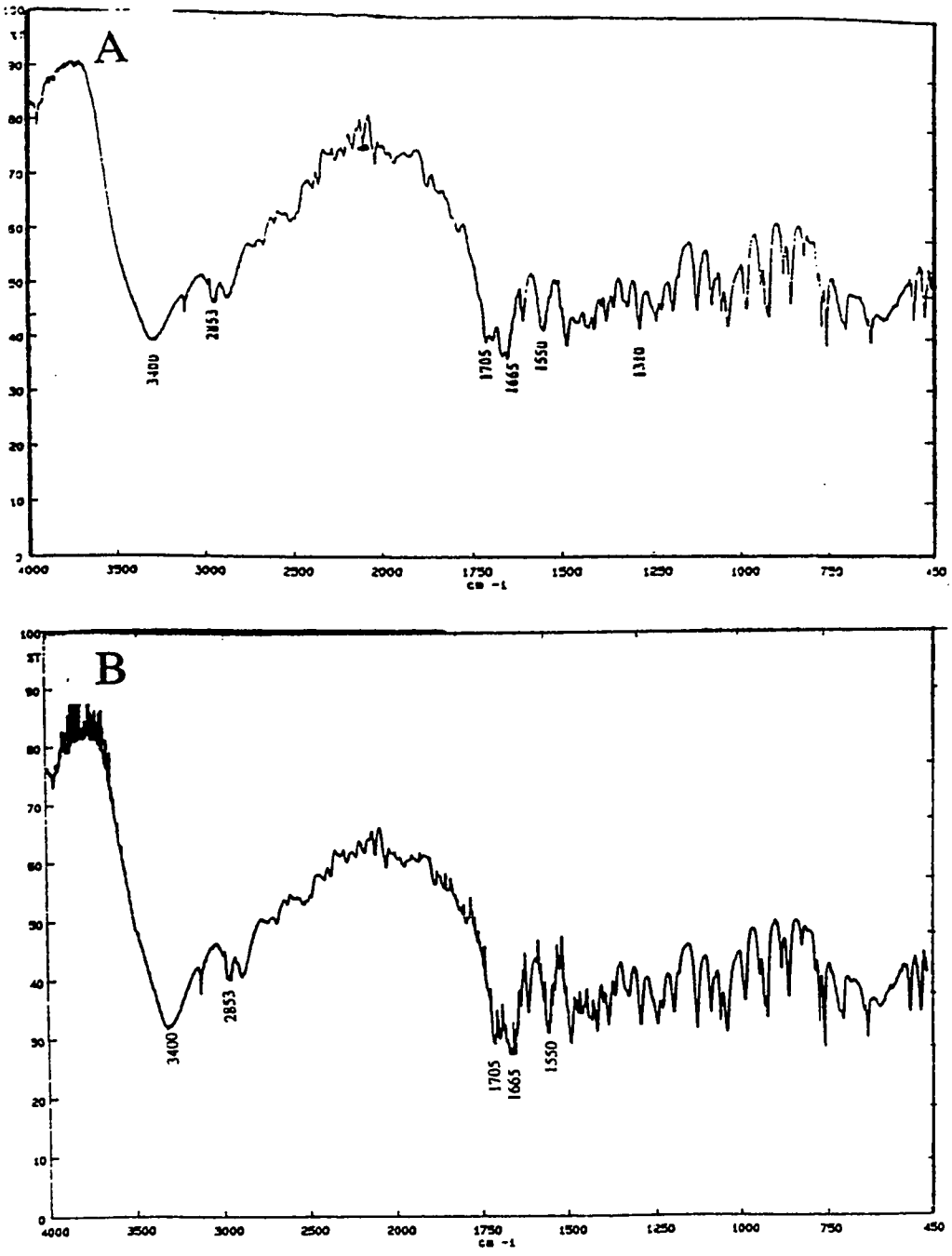


Figure 4. DRIFT spectra of 7-(2,3-dihydroxypropyl) theophylline (Curve A) and 7-(2,3-dihydroxypropyl) theophylline derivative (Curve B).

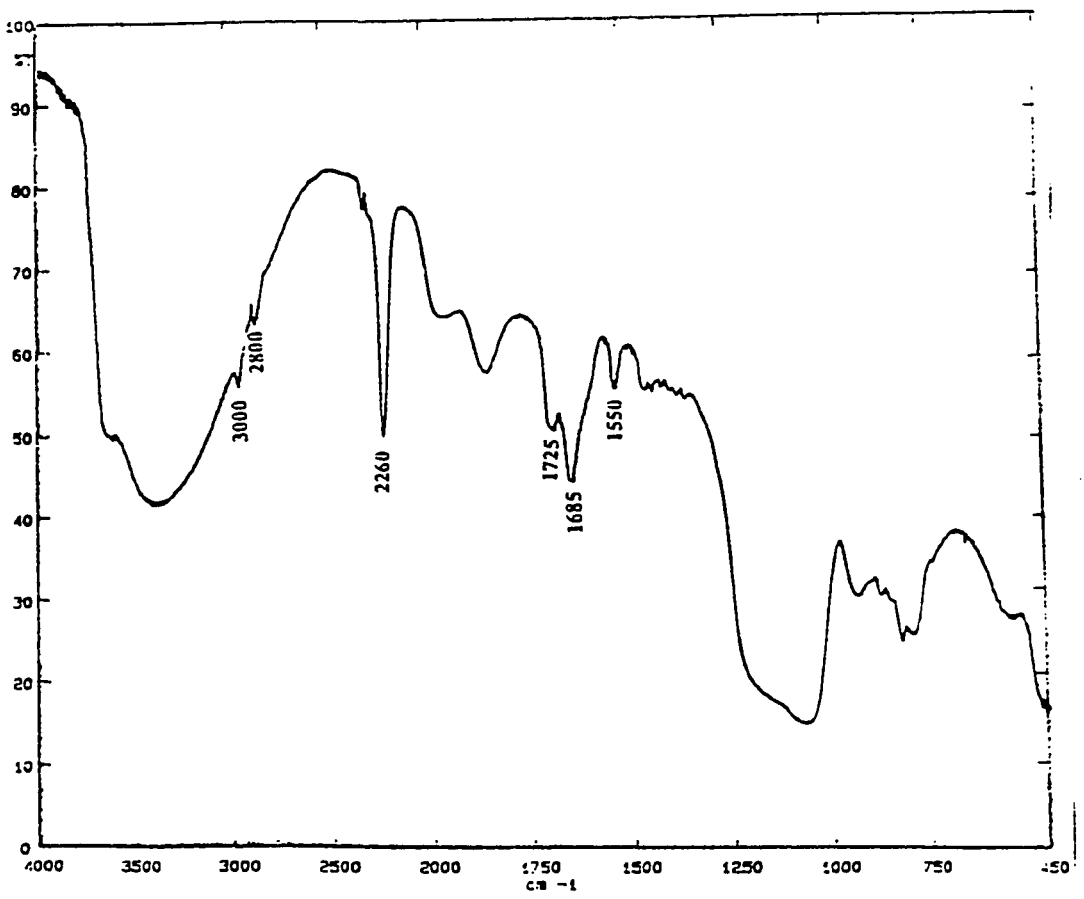


Figure 5. DRIFT spectrum of 7-(2,3-dihydroxypropyl) theophylline material bonded to silica hydride.

theophylline material. Upon reaction with this compound, the Si-H stretching vibration at 2260 cm^{-1} diminishes in intensity (relative to the water adsorbed peak around 3500 - 3200 cm^{-1}) and the aromatic C-H stretching bands appear in the 3000 - 2800 cm^{-1} region. In addition, the theophylline material key features such as two C=O (C2 and C6 positions) vibration stretches at 1685 and 1725 cm^{-1} and C=N (C8 position) at 1550 cm^{-1} stretching vibration indicate that the hydrosilation has occurred.

B. CP- MAS NMR Spectroscopic Evaluation

1. ^{29}Si Spectrum of Silica and Silica Hydride

With ^{29}Si and ^{13}C CP-MAS NMR spectrometry it is possible to obtain further qualitative information about the hydride intermediate and the bonded phase. Curve A of Figure 6 is the spectrum of the native silica and Curve B is the hydride intermediate. The ^{29}Si CP-MAS NMR spectrum of native silica shows two distinct peaks at -110 ppm and -100 ppm. The peak at -110 ppm represents the Si-$(OSi\equiv)_4$ framework and the peak around -100 ppm is assigned to surface single silanols ($HOSi(OSi\equiv)_3$. The shoulder at -89 ppm represents surface geminal silanols, $(HO)_2Si-(OSi\equiv)_2$. The new peak at -85 ppm (Curve B) represents the hydride ($H-Si(OSi\equiv)_3$ species. These peaks are the characteristic features of silica and are the same as reported by Bayer et al.[22]

2. ^{13}C Spectrum of Silica and Silica Hydride

In the native silica, the peaks at 16 and 61 ppm (Figure 7A) correspond to the methyl and methylene resonances of the ethoxy group which is adsorbed onto the silica surface.[23] The peak at 49 ppm corresponds to a surface-adsorbed methoxy group. These peaks can be attributed to organic materials that have been used in making Vydac silica.

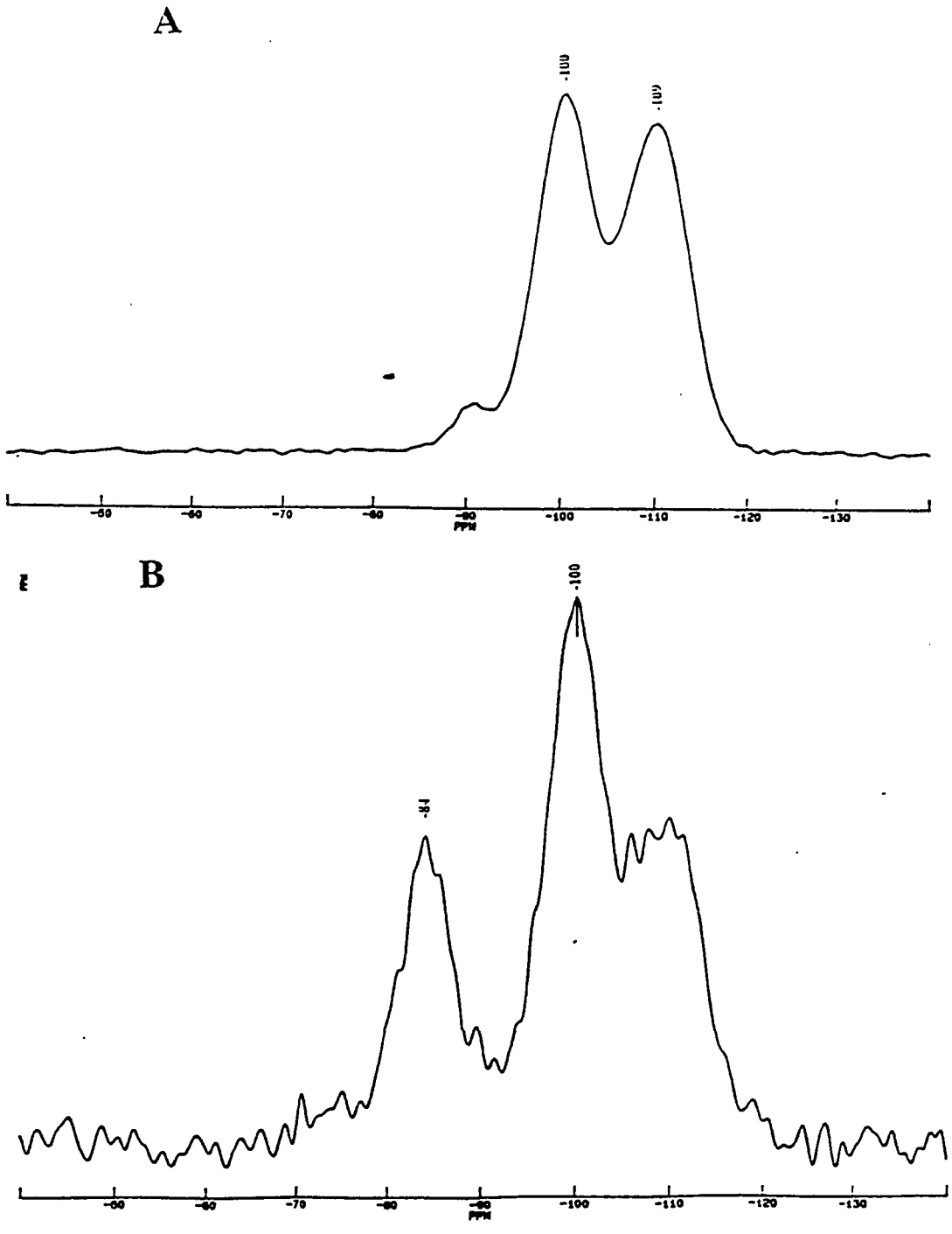


Figure 6. ^{29}Si CP-MAS NMR spectrum of native silica (Curve A) and silica hydride intermediate (Curve B).

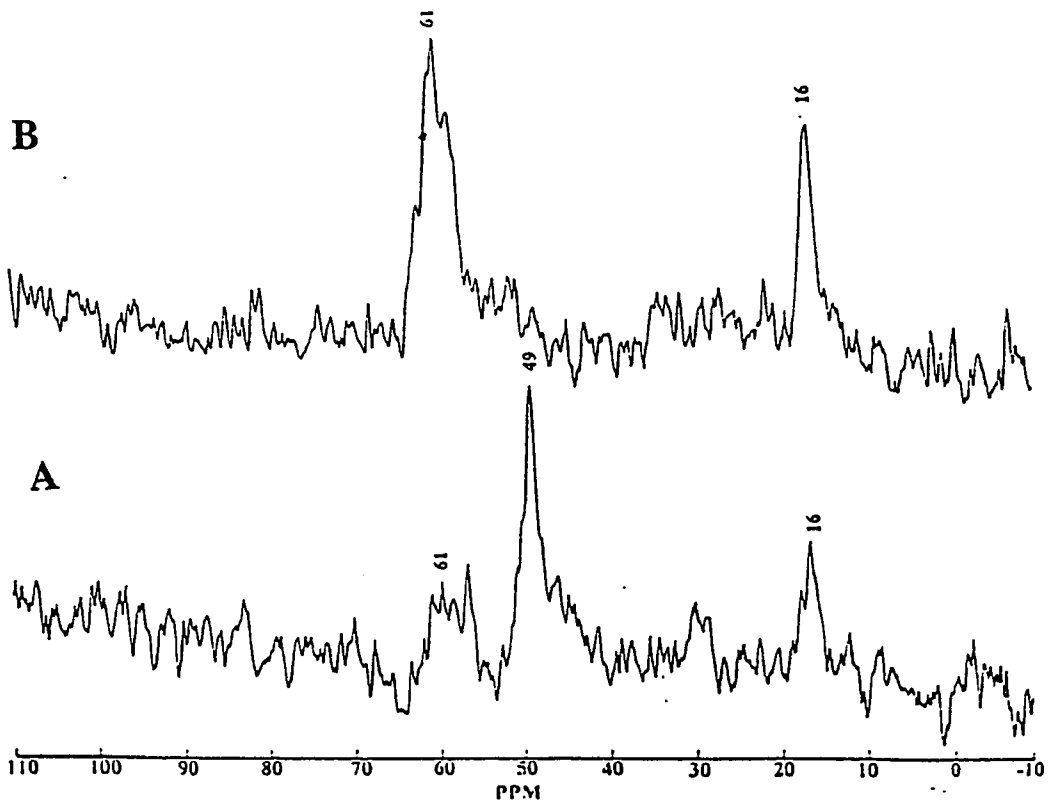


Figure 7. ^{13}C spectrum of native silica (Curve A) and silica hydride intermediate (Curve B).

In the silica hydride spectrum (Figure 7B), the methyl and methylene resonances (16 and 61 ppm) of the ethoxy group shows higher intensity than in native silica (Figure 7A). The presence of the ethoxy moiety on the surface may be due to the incomplete hydrolysis of TES or by subsequent bonding of the ethanol released during TES hydrolysis to the silica surface.

3. ^{13}C Spectrum of 7-(2,3-dihydroxypropyl) theophylline

The ^{13}C CP-MAS NMR spectrum of 7-(2,3-dihydroxypropyl) theophylline is shown in Figure 8. The two peaks around 154 and 152 ppm represents the two C=O groups (C2 and C6 positions). The two peaks near 147 and 144 ppm are assigned to the carbons (C4 and C8 positions) that are present between the two nitrogen atoms. The peak at 107 ppm represents the carbon at the C5 position. The peak at 29 ppm correspond to the two methyl groups (at N1 and N3 positions). The peak at 64 ppm belongs to the methylene carbon at the C1 position. These resonances are the same as those reported by Nicolan and Hildenbrand.[24] The peaks at 48 and 67 ppm are due to the primary and secondary alcohols, respectively.

4. ^{13}C Spectrum of 7-(2,3-dihydroxypropyl) theophylline Material

The ^{13}C CP-MAS NMR spectrum of the theophylline material (Figure 9) is similar to that of the spectrum of theophylline before esterification (reaction between theophylline and 4-pentenoic acid). Normally, the sp^2 carbon atoms of alkenes absorb in the range of ~ 110-150 ppm downfield from TMS.[25] Such peaks are not seen in the spectrum. In addition, the spectrum doesn't show the presence of the alkyl groups of the pentenoic acid. Hence, it is clear that the esterification reaction didn't take place.

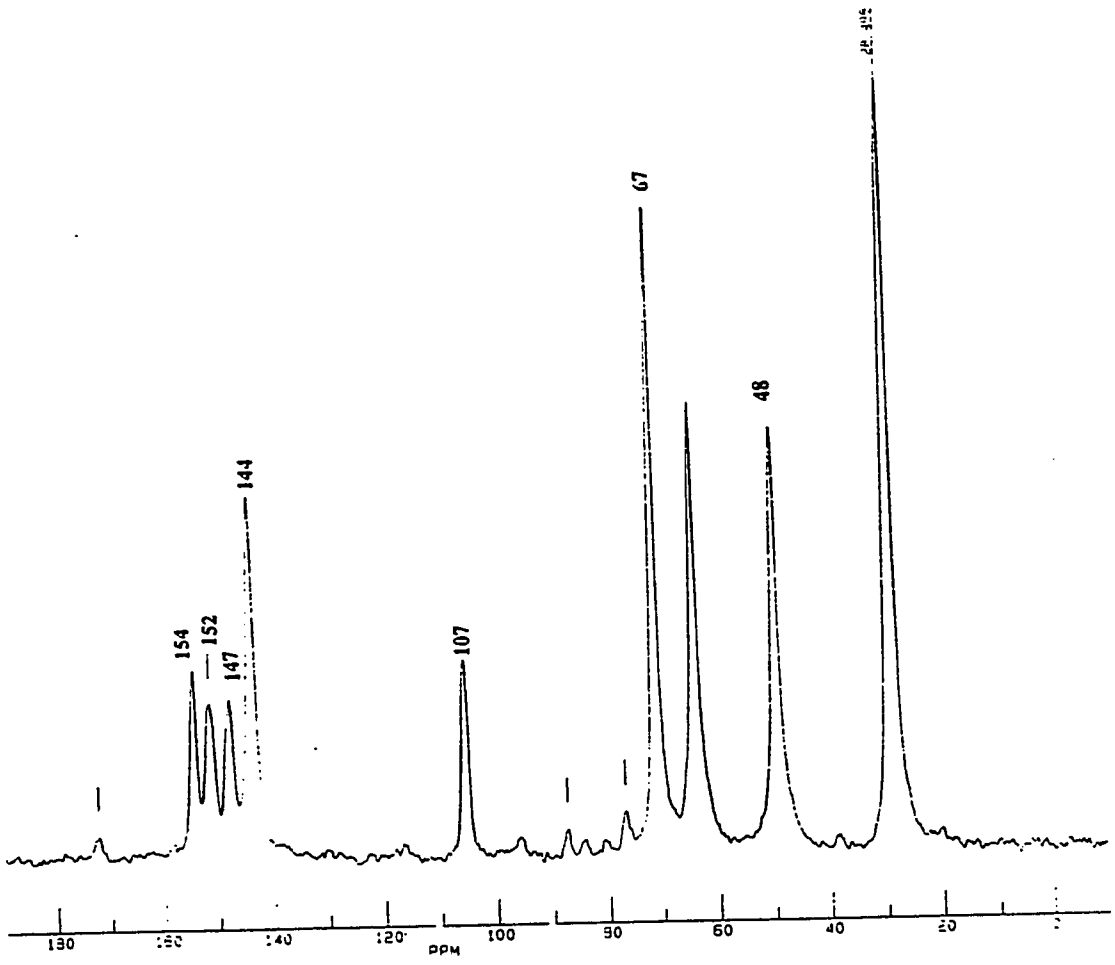


Figure 8. ^{13}C spectrum of 7-(2,3-dihydroxypropyl) theophylline.

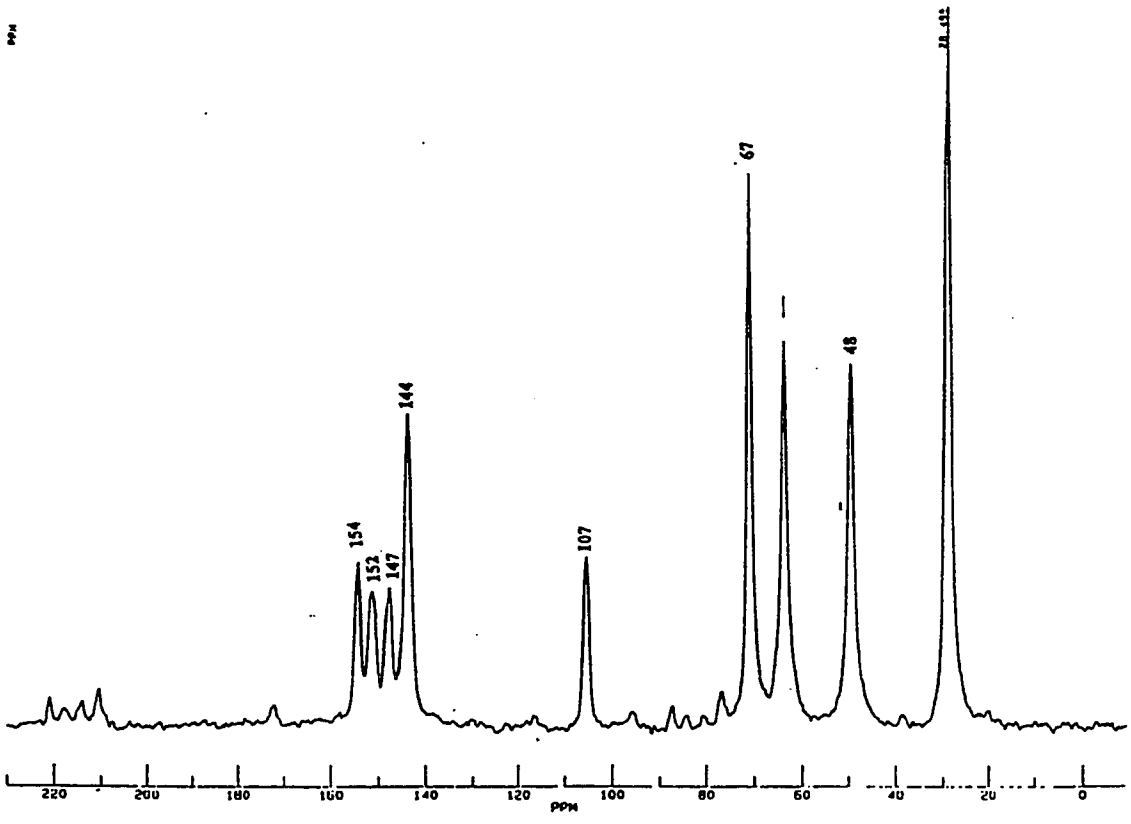


Figure 9. ^{13}C spectrum of 7-(2,3-dihydroxypropyl) theophylline material after esterification reaction.

In order to further assess the extent of the esterification reaction, the proton NMR of 7-(2,3-dihydroxypropyl) theophylline was taken before and after the esterification reaction. Figure 10 shows the proton NMR of the 7-(2,3-dihydroxypropyl) theophylline material. The spectrum of the 7-(2,3-dihydroxypropyl) theophylline (starting material) is not shown. Both the spectra have the same features. The two peaks at 3.45 ppm and 3.75 ppm represent the primary and the secondary alcohols, respectively. If the esterification reaction had occurred (if 100% of the product is formed), the primary alcohol peak should either disappear or diminish in intensity. Since the derivative spectrum shows the primary alcohol peak at 3.45 ppm, it is clear that the esterification reaction did not occur.

5. ^{13}C Spectrum of bonded Silica

Figure 11 shows the CP-MAS NMR spectrum of the theophylline material bonded to the Si-H intermediate. Two peaks around 16 and 60 ppm correspond to the methyl and methylene resonances of the ethoxy moiety. Two peaks for the primary and the secondary groups appear at 48 and 72 ppm. In addition, the two peaks at 143 and 106 ppm correspond to the carbons at C5 and C8 positions. This clearly indicates that the 7-(2,3-dihydroxypropyl) theophylline material is bonded to the silica hydride.

The DRIFT and the ^{13}C NMR spectra of the 7-(2,3-dihydroxypropyl) theophylline material does not contain a terminal vinyl group. Since the bonded silica spectra of both DRIFT and ^{13}C NMR show the distinctive features of the 7-(2,3-dihydroxypropyl) theophylline, it is obvious that the bonding has occurred. But, the exact location of the bonding of the silica hydride to the 7-(2,3-dihydroxypropyl) theophylline material can not be determined from the spectroscopic data. The addition of the 7-(2,3-dihydroxypropyl) theophylline material to the silica hydride can occur either at the primary alcohol, the

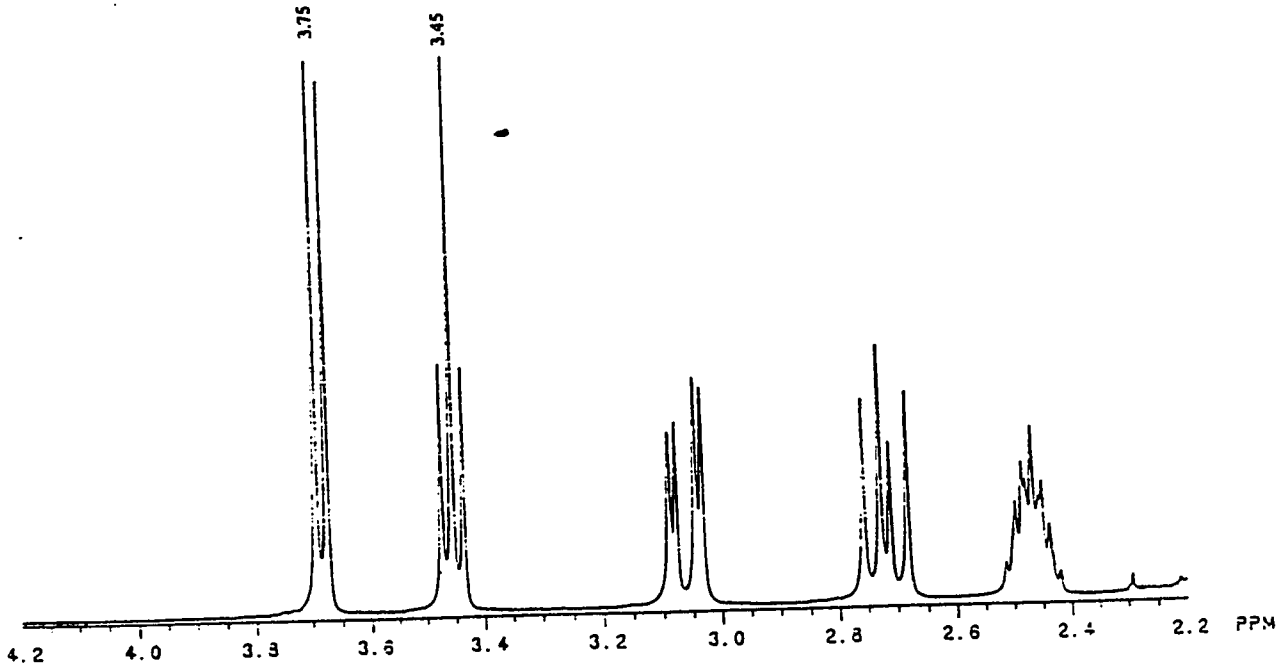


Figure 10. Proton NMR spectrum of 7-(2,3-dihydroxypropyl) theophylline material after esterification reaction.

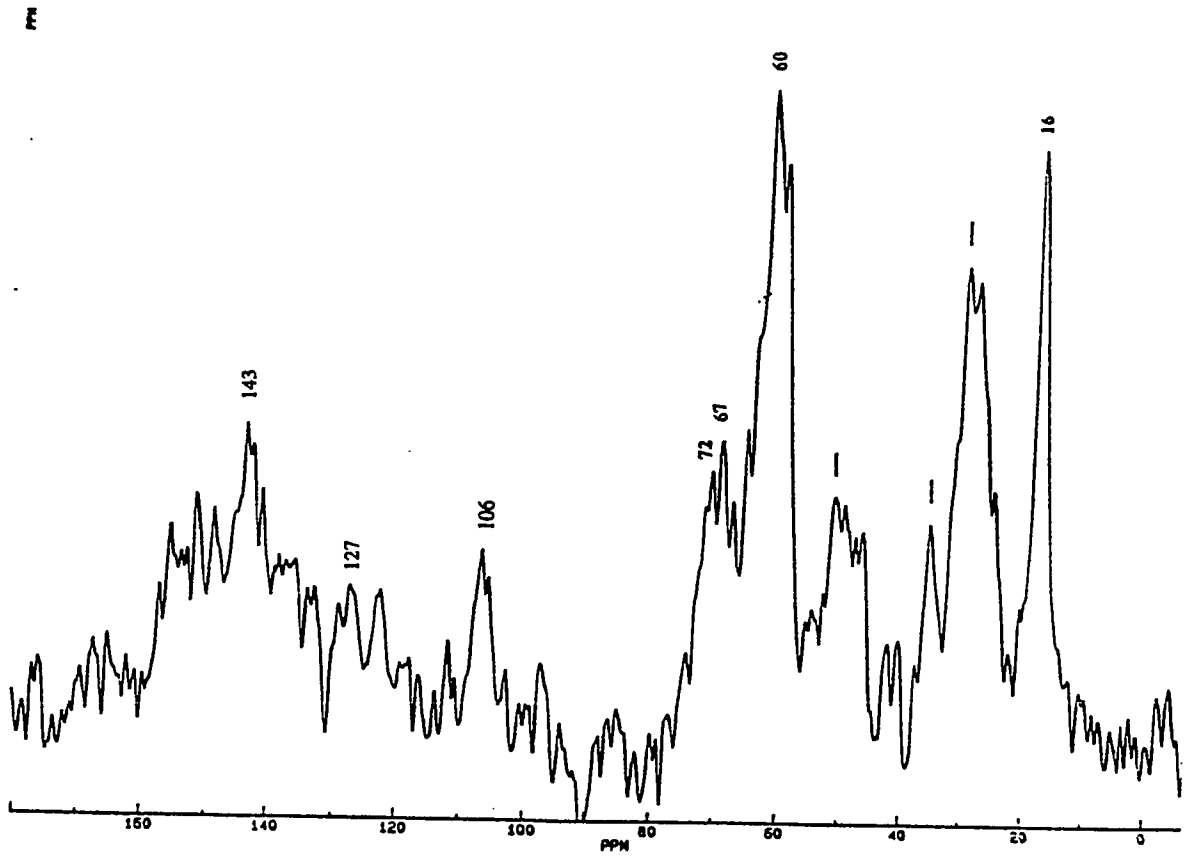


Figure 11. ^{13}C NMR spectrum of the 7-(2,3-dihydroxypropyl) theophylline material bonded to the silica hydride intermediate.

secondary alcohol, or the nitrogen atom (at the N9 position) that has lone pair of electrons. Of the two alcohol groups, the primary alcohol would be more likely to bond than the secondary alcohol because the secondary alcohol is more sterically hindered. If the bonding has occurred through the primary alcohol, the Si-C linkage peak should be seen around 12 ppm in ^{13}C NMR spectrum of the bonded silica. However, the Si-C peak is not visible in the ^{13}C spectrum. Although possible explanation is that this peak can not be distinguished from the noise. If the addition has occurred through the nitrogen (N9 position), then Si-N linkage can be obtained from ^{15}N NMR. Therefore, ^{15}N NMR might be one way to confirm the addition of the silica hydride at nitrogen (N9 position).

C. THERMAL CHARACTERIZATION

The DSC thermograms for 7-(2,3-dihydroxypropyl) theophylline and the 7-(2,3-dihydroxypropyl) theophylline material after the esterification reaction are shown in Figure 12 and Figure 13 respectively. An exothermic peak for 7-(2,3-dihydroxypropyl) theophylline is observed at 160 °C and while the 7-(2,3-dihydroxypropyl) theophylline esterification product varies between 147 °C - 138 °C. Although esterification process does not occur, there is a large shift in the melting point of the theophylline material which indicates that the material obtained after the reaction is not pure. At this point, there is no clear explanation for the varying melting point of the material. It is not likely that such a large shift in the melting point is just due to an impurity. Although, one possible reason might be that the molecule has undergone some minor structural change. However, the spectroscopic data does not show the change.

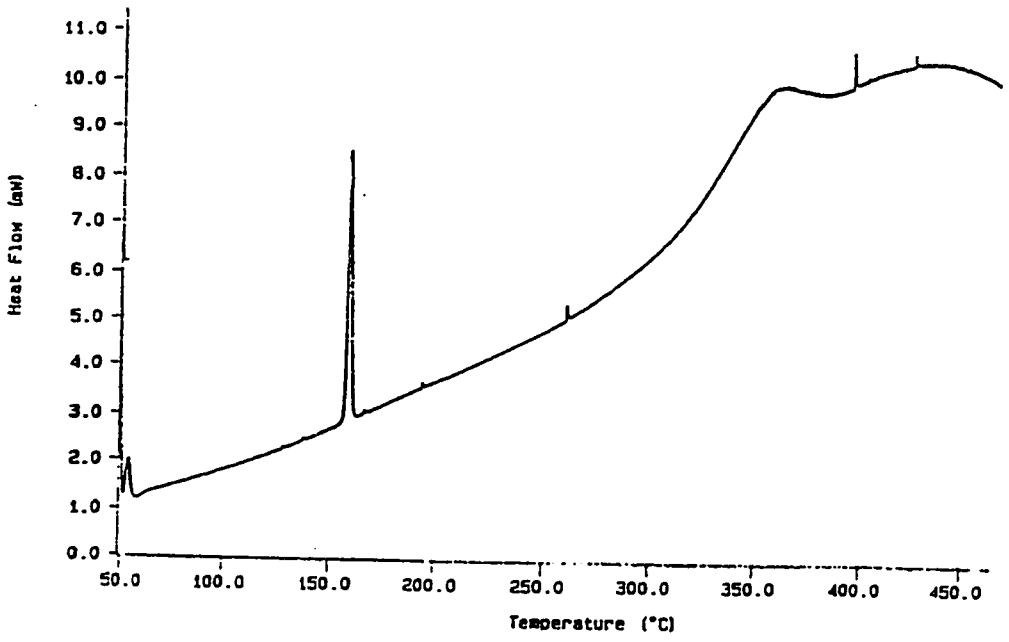


Figure 12. DSC thermogram of 7-(2,3-dihydroxypropyl) theophylline.

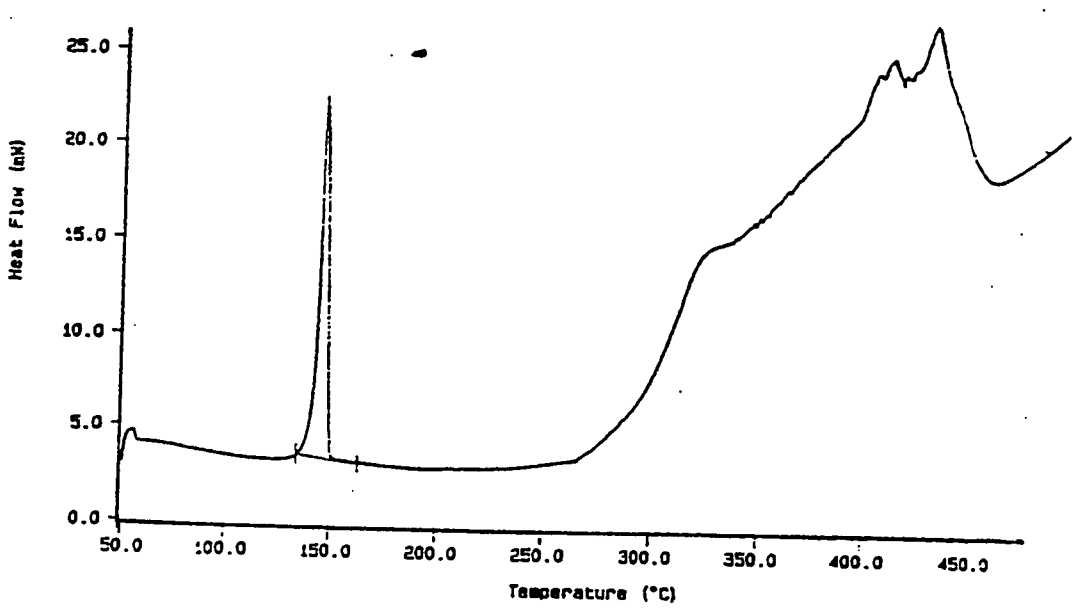


Figure 13. DSC thermogram of 7-(2,3-dihydroxypropyl) theophylline material after esterification reaction.

D. OPTIMIZATION

As mentioned earlier in Section IF, optimum surface coverage was obtained by changing one experimental parameter at a time while keeping the other constant. The variables such as time, catalyst/olefin ratio, different catalysts, temperature and the solvent were changed one at a time.

The experiment for a particular catalyst/olefin ratio was repeated multiple times under the same reaction conditions. The surface coverages reported in Table 4 show that the data is not very reproducible. The problem observed during the course of the reaction was that some of the 7-(2,3-dihydroxypropyl) theophylline material was condensed on the thermometer bulb. Hence, the amount of the theophylline material present in the solution that reacts with the silica hydride differs each time. This could significantly affect the surface coverage. The condensation of the 7-(2,3-dihydroxypropyl) theophylline material on the thermometer bulb is attributed to the low solubility of this compound in the chloroform (8.4×10^{-3} g/mL). Therefore, future investigations should involve selecting a solvent in which this material has high solubility.

1. Effect of time on Surface Coverage

Generally, most of the bonding occurs during the early stages of the reaction, while at longer reaction times, increases in surface coverage are less pronounced. Table 5 shows the surface coverage of bonded silica at different reaction times. The maximum surface coverage of 1.12 $\mu mol/m^2$ was obtained at 72 hours. The surface coverage decreased to 0.69 $\mu mol/m^2$ at 96 hours. A similar study performed by J. Sandoval et al., for 1-octadecene on a hydride modified silica shows an increase in surface coverage up to 100 hours reaction time.[6] The reason for the decrease in surface coverage of 7-(2,3-

Table 4. Surface coverage as a function of catalyst/olefin ratio.

Catalyst/Olefin Ratio*	% Corrected Carbon	Si-H Carbon %	Surface Coverage $\mu mol/m^2$
7.69×10^{-5}	1.39	0.80	1.12
7.69×10^{-5}	1.65	0.75	1.34
5×10^{-6}	0.51	0.90	0.40
5×10^{-6}	1.20	0.90	0.96
5×10^{-6}	0.49	0.79	0.39

** The reaction was repeated under similar conditions at different times. The reaction was carried out for 72 hours at 52 °C.*

Table 5. Surface coverage as a function of reaction time.

Elapsed time in hour*	Corrected % Carbon	Surface coverage ($\mu mol/m^2$)
24	0.79	0.63
48	1.16	0.93
72	1.39	1.12
96	0.87	0.69

** The catalyst/olefin ratio used was 7.69 x 10^{-5} and temperature 52 °C.*

dihydroxypropyl) theophylline at 96 hours reaction time is not known at this point. No further study was carried out to examine the decrease. With one data point it is difficult to explain the decrease in surface coverage. Therefore, multiple hydrosilation experiments at longer reaction times should be performed to determine whether the decreasing trend is real or not. One potential explanation is that at longer reaction times the organic material is removed from the silica surface. As a result, the surface coverage will drop. This is especially true since the nature of the linkage to the silica surface is unknown.

2. Effect of Catalyst/Olefin Ratio on Surface Coverage

Surface coverage is also strongly dependent on the catalyst/olefin ratio. The graphic presentation of these results is given in Figure 14. The data indicate that the maximum surface coverage was obtained with a 7.69×10^{-5} ratio. Surface coverage increases up to a ratio of 7.69×10^{-5} catalyst/olefin. However, at values larger than a 7.69×10^{-5} ratio, the surface coverage decreases. Hence, 7.69×10^{-5} catalyst/olefin ratio was chosen as an optimum.

During the induction period, the 7-(2,3-dihydroxypropyl) theophylline material forms a complex with the catalyst. After the addition of silica hydride, the complex diffuses into the pores of the silica and reacts with the silica hydride. At higher concentrations of the catalyst, it may be possible that the theophylline material forms a strong bond with the activation complex and may not react with the silica hydride. Hence, surface coverage would decrease at higher concentrations of the catalyst. Similarly, the bonded 4-methyoxyphenol-4-allyloxybenzoate (MPAB) shows an increase in surface coverage up to a specific catalyst/olefin ratio and then decreases.[26]

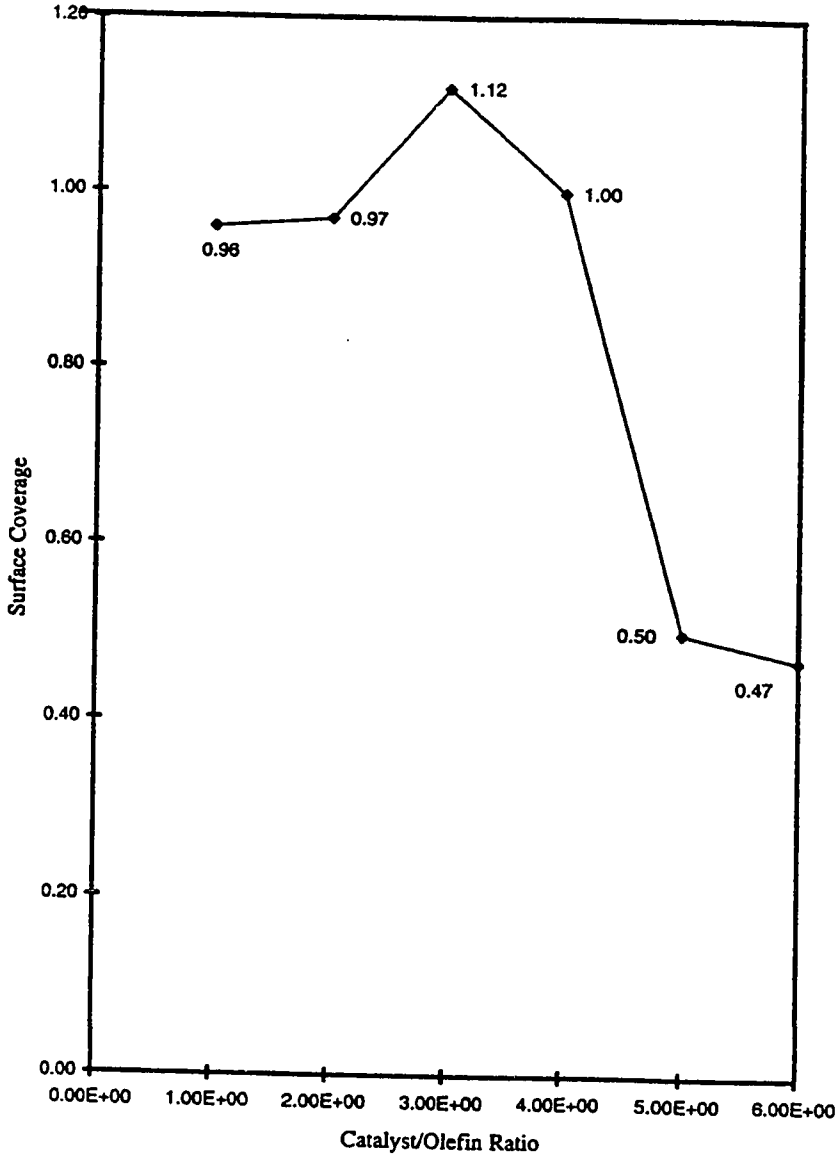


Figure 14. Effect of catalyst/olefin ratio on surface coverage.

3. Effect of Different Catalysts on Surface Coverage

Speier's Catalyst, Wilkinson's Catalyst and Ni(II) Chloride were used as catalysts. The surface coverages are listed in Table 6. All the experiments used the same 4.5×10^{-4} catalyst/olefin ratio, a reaction time of 72 hours and temperature of 52 °C. Higher surface coverage was obtained with Wilkinson's catalyst at a constant catalyst/olefin ratio. However, a higher surface coverage was obtained with Speier's catalyst at a different catalyst/olefin ratio (surface coverage of 1.50 μmol/m^2 at a 7.69×10^{-5} ratio). For this reason, most of the work was repeated using Speier's catalyst.

4. Effect of Temperature on Surface Coverage

Generally, a higher temperature leads to greater surface coverage. The highest allowed temperature, however, is determined by the boiling point of the solvent. Since the boiling point of the chloroform is 60 °C, the maximum temperature that could be used for the hydrosilation reaction was 58 °C. Holding other variables constant, the hydrosilation reaction was repeated at different temperatures for two different catalyst/olefin ratios. Carbon analysis data are given in Table 7 and Table 8. The general trend observed is that the surface coverage increased with increasing temperature. This observation is consistent with the work of J. Sandoval et al., reported for bonding of 1-octadecene to a hydride modified silica.[6]

5. Effect of Solvent Type and the Amount of the Solvent

Dimethylformamide and chloroform were used as solvents. The reaction was performed at 52 °C using Speier's catalyst in the presence of DMF and $CHCl_3$. The surface coverage obtained with DMF was 0.13 μmol/m^2. Higher surface coverage (0.96

Table 6. Hydrosilation of the 7-(2,3-dihydroxypropyl) theophylline material on hydride silica with three different catalysts.

Catalyst/Olefin Ratio*	Catalyst	Surface Coverage ($\mu mol/m^2$)
4.5×10^{-4}	Speier's	0.50
4.5×10^{-4}	Wilkinson's	1.13
4.5×10^{-4}	Ni(II) Chloride	0.56

** The other conditions were time 72 hours and temperature 52 °C.*

Table 7. Surface coverage as a function of temperature at 5×10^{-6} catalyst/olefin ratio.

Catalyst/Olefin Ratio*	Temperature (°C)	Corrected % Carbon	Surface Coverage ($\mu mol/m^2$)
5×10^{-6}	48	1.07	0.86
5×10^{-6}	52	1.20	0.96
5×10^{-6}	58	1.41	1.14

* *Speier's catalyst was used and the reaction time was 72 hours.*

Table 8. Surface coverage as a function of temperature at 7.69×10^{-5} catalyst/olefin ratio.

Catalyst/Olefin Ratio*	Temperature (°C)	Corrected % Carbon	Surface Coverage ($\mu mol/m^2$)
7.69×10^{-5}	48	1.14	0.91
7.69×10^{-5}	52	1.65	1.34
7.69×10^{-5}	58	1.85	1.50

* *Speier's catalyst was used and the reaction time was 72 hours.*

$\mu mol/m^2$) was obtained with $CHCl_3$ as the solvent. In the presence of DMF, the reaction was also repeated at 75 °C and 100 °C. Higher surface coverages were obtained (1.34 and 1.35 $\mu mol/m^2$). However, the ^{13}C CP-MAS NMR of bonded silica in the presence of DMF does not show strong peaks in the aromatic region of the spectrum that are seen with bonded silica formed in the presence of $CHCl_3$ and in the 7-(2,3-dihydroxypropyl) theophylline. Hence, chloroform was used as the solvent for the hydrosilation reaction.

The reaction was also repeated using 15 mL, 20 mL and 25 mL of solvent. The surface coverages were 1.05, 0.97 and 0.78 $\mu mol/m^2$ respectively. It is possible that at lower amount of solvent, there is more interaction between the reactants contributing to the formation of product and hence higher surface coverage. At higher amounts of solvent, the interaction between the reactants decreases considerably and, as a result, lower surface coverage will be obtained.

When 15 mL of solvent were used, the bonded phase turned gray in color once (out of three experiments) after the washing procedure. Normally the bonded phase is white in color. Similar observations have been reported by Sandoval and Pesek.[6] They suggested that the platinum catalyst was reduced from Pt (II) in the complex to Pt (0) which precipitated as fine particles and remain trapped in the porous silica. The trapped platinum particles might react with the analytes during the chromatographic separation. A similar observation was observed by my colleagues as well. Although the graying of the product at lower solvent volumes is not consistent, larger solvent volumes should be used to eliminate graying.

E. CHROMATOGRAPHIC STUDIES

In spite of the uncertainty in the reproducibility of the hydrosilation reaction, preliminary chromatographic studies were conducted to investigate the separation of a

steroid mixture using the 7-(2,3-dihydroxypropyl) theophylline column. The structures of the four steroids used in this study are shown in Figure 15.

The 7-(2,3-dihydroxypropyl) theophylline bonded silica with a surface coverage of 1.11 μmol/m^2 was used to pack the column. All organic solvents used in chromatographic separation were filtered through a 0.20-μm nylon membrane. In every case the detection at 254 nm was used. The injection volume for all samples was 5 μl. The determination of the retention of a non-retained component, the dead time (t_0) was carried out using n-pentane.

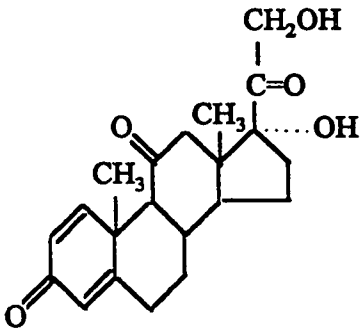
1. Separation of Steroid Mixture in 100% Hexane

The analysis was carried out in 100% hexane (20 minutes) under isocratic conditions (single solvent at constant composition), with a flow rate of 1 mL/min. No signals were observed. In a previous study, the same four compounds were separated using 7-octene-1,2-diol column and hexane with varying amounts of methylene chloride as the mobile phase. All the test compounds were eluted with the diol column.[27] The reason for not getting any separation with 7-(2,3-dihydroxypropyl) theophylline column might be that the steroid mixture is more polar than the hexane (mobile phase).

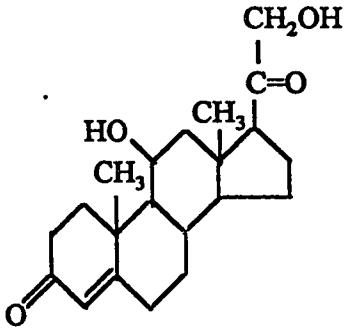
2. Separation of Steroid Mixture in 100% Methylene Chloride

The separation was carried out in 100% methylene chloride (more polar than hexane) under isocratic conditions. The solvent flow used was 1.5 mL/min. All four steroid compounds were resolved (Figure 16). The retention times of the individual compounds were determined by running each solute separately under the same condition as the steroid mixture. Two signals at 2.84 and 3.24 minutes were identified as 4-

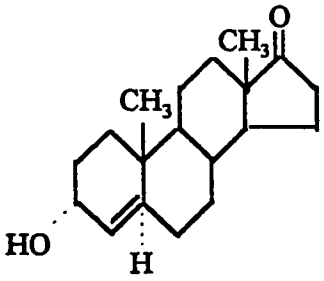
Prednisone



Corticosterone



4-Androstene-3,17-dione



Adrenosterone

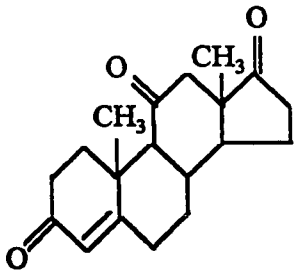


Figure 15. Structures of the four steroids.

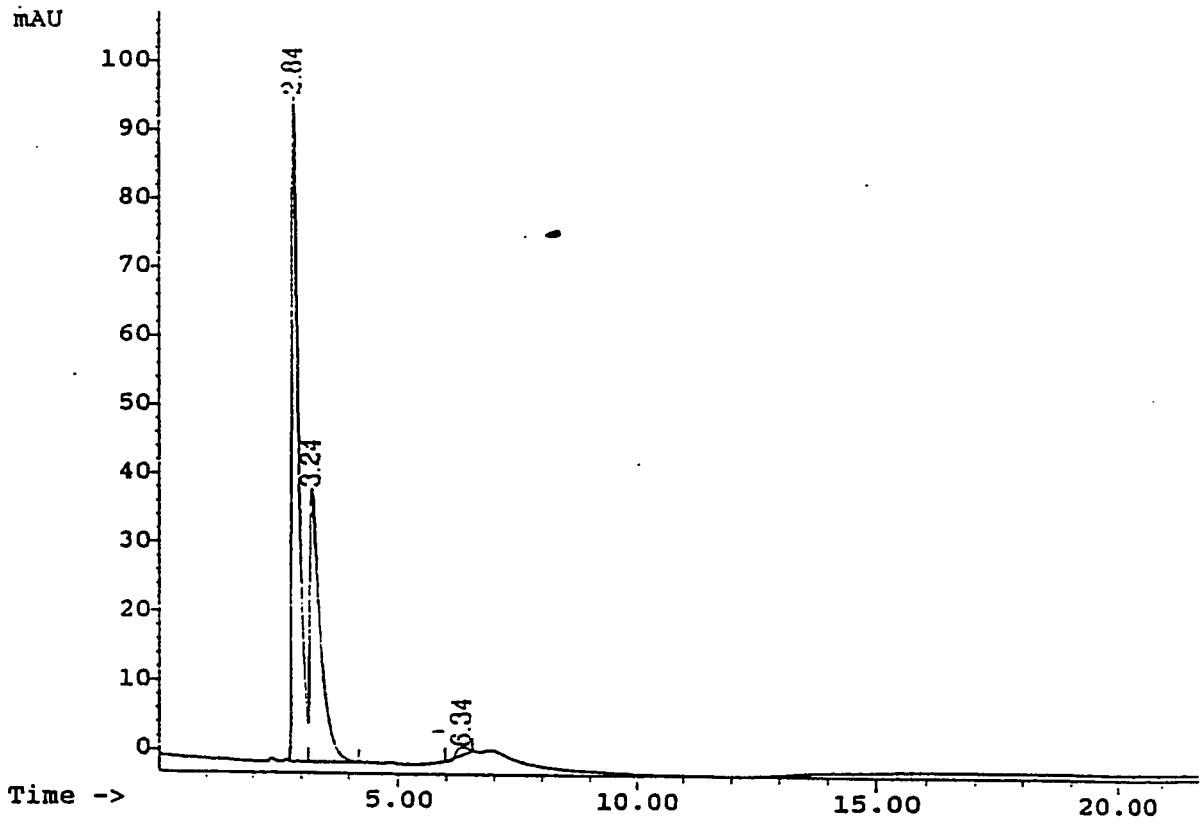


Figure 16. Separation of steroid mixture in 100% methylene chloride.

androstene-3,17-dione and adrenosterone while the other two signals at 6.34 and 6.90 minutes were corticosterone and prednisone, respectively.

3. Separation of Steroid Mixture in 90% Methylene Chloride and 10% Tetrahydrofuran

In order to test a more polar mobile phase, the separation was carried out in 90% methylene chloride and 10% tetrahydrofuran (more polar than methylene chloride). The flow rate used was 2.0 mL/min. The steroid mixture was resolved into three signals (Figure 17). Both prednisone and corticosterone were resolved (3.88 and 2.30 minutes). 4-androstene-3,17-dione and adrenosterone were eluted very close together (retention time 1.42 and 1.49). Since the polarity of these two compounds are similar, it is expected that they would elute together. Separation should be improved by using a stationary phase that has higher surface coverage. In addition, separation might also be improved by changing the mobile phase composition or by altogether trying new mobile phase components.

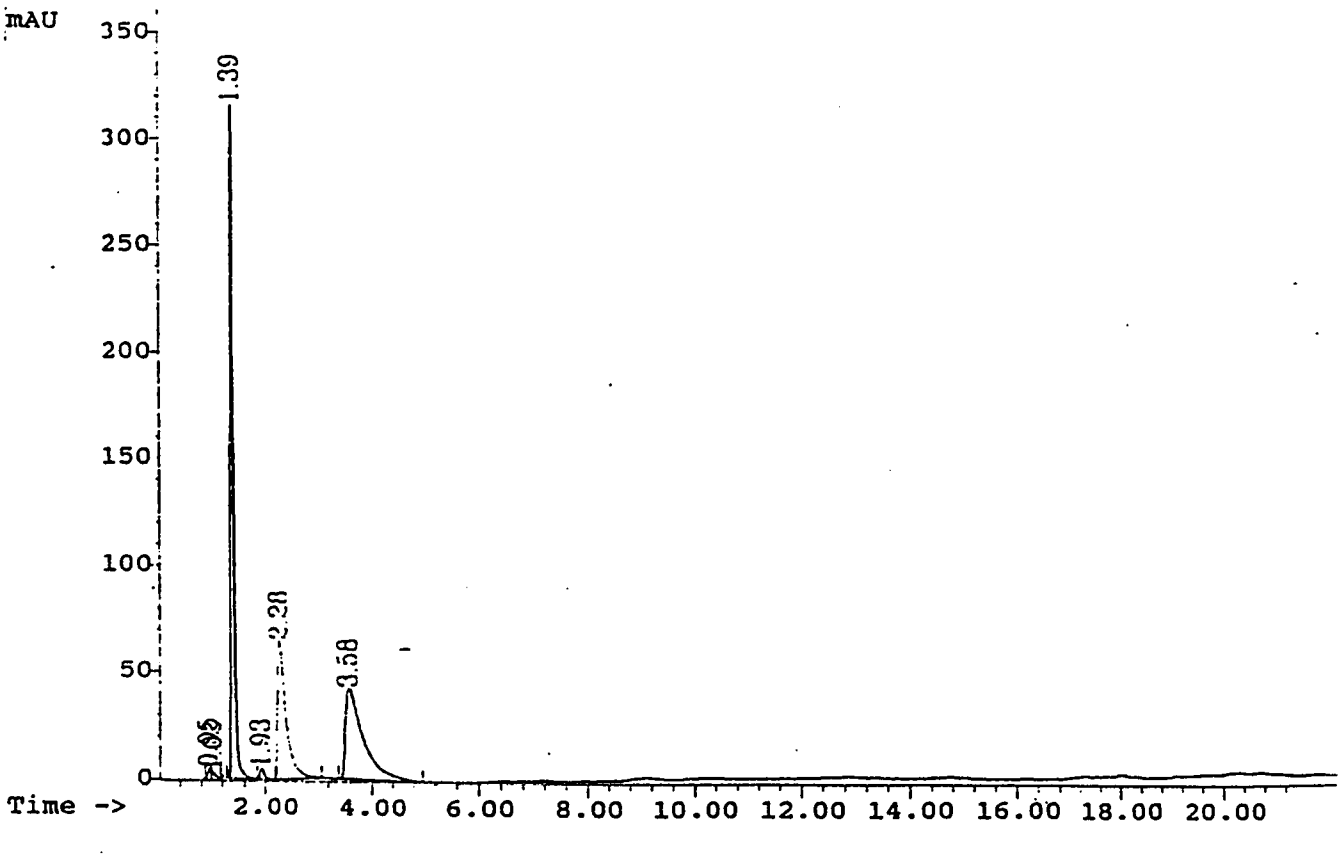


Figure 17. Separation of steroid mixture in 90% methylene chloride and 10% tetrahydrofuran.

IV. CONCLUSION

Hydrosilation provides a suitable method for the preparation of silica-based stationary phases for liquid chromatography. Hydrosilation is a reaction between an organic moiety containing a terminal double bond with a silica hydride intermediate. Esterification of 7-(2,3-dihydroxypropyl) theophylline with 4-pentenoic acid was attempted to produce a derivative that has a terminal double bond. However, the esterification reaction did not occur. The spectroscopic analysis of the 7-(2,3-dihydroxypropyl) theophylline esterification product confirms the absence of the double bond. In spite of the absence of the double bond, hydrosilation occurred between 7-(2,3-dihydroxypropyl) theophylline and the silica hydride. Carbon analysis showed a maximum surface coverage of 1.50 $\mu mol/m^2$. However, the spectroscopic data does not reveal the exact location of the bonding of 7-(2,3-dihydroxypropyl) theophylline to the silica hydride.

Future work should focus on improving the synthetic process in order to obtain consistent yields and higher surface coverage. Also, additional research should include determining the reaction mechanism between 7-(2,3-dihydroxypropyl) theophylline and the silica hydride.

The stationary phase with a surface coverage of 1.11 $\mu mol/m^2$ was used in the separation of four steroid compounds in the normal phase. The column successfully resolved the four steroid compounds using 100% methylene chloride. The chromatographic studies have shown that the stationary phase is promising for the separation of some steroids.

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