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**A NEW APPROACH FOR MODIFICATION OF
ALUMINA AS A STATIONARY PHASE 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
Minggong Su**

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ABSTRACT

A NEW APPROACH FOR MODIFICATION OF ALUMINA AS A STATIONARY PHASE FOR HPLC

By Minggong Su

A new approach for modification of alumina as a stationary phase for HPLC has been investigated. Alumina was silanized with triethoxysilane (TES) reagent to form a hydride monolayer on the surface of alumina (TES alumina). The modified intermediate was reacted with a terminal olefin (1-octene or 1-octadecene) in the presence of a catalyst (dicyclopentadienyl platinum II) to form an alkyl-bonded TES-alumina as final product for the stationary phase for HPLC. Both intermediate TES-alumina and alkyl-bonded TES-alumina were evaluated by FTIR, silicon plasma emission, carbon analysis, DSC analysis and ^{29}Si , ^{13}C and ^{27}Al CP-MAS-NMR. All of the evaluation methods confirmed the success of chemically bonded silanized alumina as intermediate and alkyl-bonded TES-alumina as the final product. Hydrolytic stability tests showed no significant deterioration of bonded phase in pH 10 phosphate buffer and 1% TFA over an extended period. Three types of test mixtures were used to evaluate reverse-phase chromatographic performance (by peak asymmetry, resolution, and efficiency) for C_{18} -bonded TES alumina.

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1. INTRODUCTION

1.1 Background

High performance liquid chromatography (HPLC) has been widely used for analysis of a broad range of organic compounds, pharmaceuticals, food and biochemicals for several decades. As the heart of the HPLC system, stationary phases in columns have been developed for a variety of applications over a wide range. Silica-based modifications have dominated most HPLC applications for decades because this material exists on a variety of particle sizes, pore sizes and high mechanical strength. But a major drawback of silica and derivatized silica is limited pH stability. In general, silica dissolves if the pH is greater than 8. This has led to the development of novel modifications for silica and other alternatives such as alumina. In this work, an exploration of a new approach for the modification of alumina has been conducted because it possesses inherently higher pH stability.

Generally, HPLC can be divided into four different application modes, depending on several different mechanisms by which the chromatographic separation may be achieved [1]. Liquid-solid chromatography (LSC) is the original form of liquid chromatography. It also called adsorption or normal phase chromatography. M. S. Tswett (a Russian botanist) first introduced the method at the beginning of this century [1, 2]. The separation is carried out with a liquid mobile phase and a solid stationary phase that reversibly adsorbs the solute molecules. The stationary phase may be either polar such as silica gel or alumina, or non-polar such as polymer beads.

Exclusion chromatography, also referred to as gel chromatography, separates substances according to their molecular size and shape. Small molecules are eluted slower than bigger molecules because they can enter freely into the pores of stationary phase, and large molecules are completely excluded from all pores. Molecules are therefore eluted in order of decreasing in molecular size. The stationary phases could be aerogels, xerogels and xerogel-aerogels such as porous glass or silica, cross-linked dextran, polyacrylamide, polystyrene etc. Since the sample solvent molecules are usually much smaller than the molecules being separated by this method, they are eluted last. Hence, contrary to other forms of chromatography, the sample is eluted before the solvent.

Ion-exchange chromatography, a kind of adsorption chromatography, is based on exchange of ions from the mobile phase to the stationary phase. The stationary phase consists of a rigid matrix, the surface of which carries either a net positive or negative charge to give an exchange site. The separation is based on the strength of the interactions between the sample ions and the exchange site.

Partition chromatography has become the most widely used HPLC mode in recent decades. It is used in environmental, pharmaceutical and other industrial laboratories for most organic and biochemical separations. The separation in partition chromatography occurs when the sample is dispersed in the mobile phase and its components are partitioned between the stationary and mobile phases according to their partition coefficients. This partitioning leads to a different rate of migration. Compounds therefore are separated by their different migration rate.

Earlier partition chromatography could be described as liquid-liquid chromatography (LLC) in which the stationary phase is physically coated onto a finely divided inert support material. Compared with the later developed chemically bonded stationary phases, LLC has a major disadvantage of stability: its usage was limited due to loss of stationary phase with a soluble mobile phase.

The use of chemically bonded stationary phases for partition chromatography has become the dominant practice in the recent decades. Because covalent bonds between the stationary phase and the support material are involved, it results in enhanced stability. In addition, a large variety of polarities and superior selectivity are provided.

Chemically bonded stationary phases are prepared by bonding an organic moiety to a support material such as silica. The original chemically bonded phases were prepared by the reaction of the surface hydroxyl groups of silica with an alcohol to give ether-like linkage [2].

Due to the easy hydrolysis of these stationary phases they could only be used in non-aqueous systems.

Currently, the majority of commercially available bonded phases are prepared from silica by the reaction of the surface silanol groups with a triorgano-chlorosilane or triorgano-alkoxysilane to form an organosiloxane linkage [4].

The R' group is usually a methyl group and the R group may be a longer hydrocarbon (most commonly C₈ or C₁₈) or a hydrocarbon with a polar terminal group (such as –CN or –NH₂ etc.). This kind of linkage is more hydrolytically stable than the $\equiv\text{Si-O-R}$ linkage.

Despite considerable improvement in stability over the previous generation, organosiloxane bonded phases still lack the hydrolytic strength required in modern bio-separations of molecules such as polypeptides and proteins due to the $\equiv\text{Si-O-Si}$ bond [5, 6].

To respond to this demand, bonded phases comprising a direct Si-C linkage to silica have been developed recently. Based on steric considerations and relative bond strengths in silica chemistry, this kind of stationary phase provides not only a denser coverage of organic functionality onto silica, which reduce the non-reacted silanol groups on the surface, but it is also more hydrolytically stable than the others.

Numerous synthetic procedures have been developed to attach organic moieties directly onto the silica surface [5, 6]. Earlier synthetic procedures involved the chlorination of fully hydroxylated silica with a chlorinating reagent such as thionyl chloride, followed by reaction with organometallic compounds to give the Si-C linkage. The organometallic compounds are either Grignard reagents or organolithium compounds. The synthetic schemes are below:

After the reaction with the organometallic compound, the residual salts must be removed by a wash step. Compared with the organosiloxane type product, this kind of stationary phase possesses a definite advantage of hydrolytic stability, but the above synthetic procedure has remained unsatisfactory on practice, mainly because of the difficulties associated with moisture-sensitive reagents (SOCl_2 , XMgR and LiR) and intermediate (chlorinated silica).

A new alternate synthetic procedure for producing a direct Si-C linkage was reported recently in which surface silicon hydride (Si-H) was produced as an intermediate that is further reacted with a terminal olefin in the presence of a catalyst [7]. The hydride intermediate is much less sensitive to moisture than chlorinated silica, and can be isolated and stored under regular atmospheric conditions. The reaction, known as hydrosilylation can be described as following:

The terminal olefin can contain a variety of functional groups so that various Si-C linkage stationary phases are possible. This is in contrast to the limited number of functionalities that can be attached by regular organometallic procedures.

Two main methods are available for producing a silicon hydride intermediate: One, chemisorption of hydrogen from previously methylated silica, which is activated pyrolytically [8, 9]. The main concern with this process was that the high temperature required (about 800°C) might result in extensive deterioration of the pore structure, therefore precluding the possibility for making hydride intermediates for chromatographic purposes. Second, the reduction of a chlorinated silica surface with an inorganic hydride is extremely sensitive to moisture [7].

An alternative method to form a hydride surface as the intermediate has been investigated in our research group and the new approach produces a hydride-derivatized silica support by controlled chemisorption of triethoxsilane (TES) [7, 10, 11]. The TES become covalently attached to the silica surface by means of a condensation reaction:

The successful new approach limited or reduced the synthetic difficulties described above.

Even though chemically bonded silica has been widely available as stationary phases for HPLC applications, stability over a wide range of pH still persists after chemical modification. The most serious disadvantage of porous silica as a support material is that at $\text{pH} \geq 8$, it begins to dissolve [13]. This problem has limited certain applications in which strongly basic mobile phases are required for separation. To avoid this problem, the use of other support materials such as alumina, zirconia, etc had been explored for bonded stationary phases to be used with basic or acidic mobile phases [12-18]. Alumina is considered a suitable support material for chemically bonded stationary phases due to its excellent insolubility in both polar and non-polar organic solvents and great stability in basic and acidic solvents [16]. It offers an excellent alternative to the use of silica if appropriate bonding reactions can be developed.

Early modification for reverse phase alumina was tried by Knox and Pryde [16]. This modified alumina was synthesized using the same organosilane method used in making reverse phase (RP) silica as a standard synthetic procedure. The reaction is described as:

However, the product was not stable and no further work followed these initial reports.

Another approach for monomeric bonding can be accomplished by reacting organic phosphonic acids with alumina. This method leads to an $=\text{Al}-\text{O}-\text{P}-\text{R}$ linkage at the

surface. An octadecyl phase prepared by this method was used in pH > 10 solvent.

However, this modification is unstable in phosphate buffer solution [18].

In another study, a variety of modification schemes involving Grignard reagents as well as organolithium and organoaluminum compounds were evaluated [19]. These results were not totally satisfactory but they certainly indicated that other methods of modification should be explored.

1.2 Aims of This Work

Reaction schemes for modification of chromatographic alumina have not been as extensively documented as they have been for silica. In view of the need for more information on alumina, the aims in this research work for exploring modified alumina are outlined below.

The essential purpose of this research work is to develop an appropriate bonding reaction for alumina that can be used to prepare stationary phases with high stability over a wide pH range. It could be expected that since alumina has hydroxyl groups on the surface like silica, the same general chemical reaction schemes should be applicable although the reaction conditions could vary significantly.

First, the octyl group as the hydrocarbon chain for bonding directly onto the alumina surface was tried because preliminary studies on this type of material have led to a possible synthetic approach [19]. The synthesis includes three steps: (i) chlorinating of alumina, (ii) preparation of octyl-lithium reagent, (iii) reaction of chlorinated alumina with octyl-lithium to give a final octyl-alumina product. This type of modified alumina

might be expected to have high hydrolytic stability. However, the final product showed carbon-hydrogen stretching bands in the IR spectrum between 3000-2850 cm^{-1} that disappeared after washing by water, indicating that the organic groups were unstable.

Therefore, it was determined from the above experiment that the synthetic procedure is feasible, because the IR spectrum and the carbon content of the final product were measurable. The results also showed that the chlorinated alumina and octyl-lithium were very sensitive to moisture. It requires absolutely moisture free conditions when the reaction is carried out which makes the process relatively difficult. However, the aluminum carbon bond is easily hydrolyzed in water, so that it cannot be utilized as a stationary phase in HPLC with aqueous mobile phases. Finally, this synthetic method does not give satisfactory coverage of hydrocarbon which is an important parameter for chromatographic behavior.

From these results, it is clear that the direct Al-C type modification does not seem feasible because of the hydrolytic stability problems. Apparently, a suitable method for the modification of alumina must possess a stable layer on the alumina surface for improved hydrolytic stability as well as be relatively easy to produce.

The second method that was tried for the modification of alumina almost achieved the aforementioned requirements. This method was first developed by our research group as an alternate silane chemistry that combines the superior coverage and hydrolytic stability of the direct Si-C linkages with the preparative simplicity of silane derivatization [20]. The new synthetic procedure was applied successfully to the modification of alumina. From spectroscopic and elemental analysis, the new synthetic procedure not only

produced a very stable, covalent bonded silicon hydride monolayer on the alumina surface but also simplified the synthetic procedure.

This synthetic procedure involves two steps. The first step is formation of covalent-bonded silicon hydride monolayer on the alumina surface by controlled chemisorption of triethoxysilane (TES, $\text{HSi}(\text{OEt})_3$). TES is the hydrolysis product of hydrosilanes of the general formula HSiX_3 , where X is a hydrolytically labile group. The silanetriol becomes covalently attached to the alumina by means of a condensation reaction (8). The second step is attaching a terminal olefin to the silicon hydride alumina (TES alumina) to give a hydrocarbon directly bonded on the monolayer that is identical to attaching a hydrocarbon group to a hydride group. The reaction schemes are following:

Another objective of this work was to characterize and evaluate the new chemically bonded alumina as stationary phase of HPLC. Several analytical methods were employed for the evaluation. BET (Brunauer-Emmett-Teller) analysis was used to determine the pore size and surface area of alumina by nitrogen physisorption data. TGA (Thermogravimetric Analysis) provided a quantitative measurement of the amount of active hydroxyl groups on the surface of the alumina based on loss of water (weight loss) by heating the alumina from 120°C to 900°C. FTIR (Fourier Transform Infrared) spectra

provided a qualitative method for molecular identification of the bare alumina, TES alumina and the final hydrocarbon-bonded alumina. The 2260 cm^{-1} band is used to characterize the TES alumina by the typical Si-H stretching absorbance and the bands near 2950 cm^{-1} are used to characterize the final hydrocarbon-bonded alumina by the strong C-H stretching. In addition, plasma atomic emission spectroscopy and elemental analysis were employed for quantitatively determining the silicon content of TES alumina and the carbon content of the final hydrocarbon-bonded alumina, from which the coverage of hydrocarbon bonded on the surface of the alumina could be estimated. Furthermore, ^{29}Si , ^{13}C and ^{27}Al CP-MAS-NMR (Cross-Polarization Magic-Angle Spinning Nuclear Magnetic Resonance) spectra identified the chemical shifts on the hydride surface and bonded hydrocarbon surface vs. reference data [21-24]. DSC (Differential scanning Calorimetric) analysis determined the thermooxidative process of the hydride surface of TES alumina. Both methods provided additional evidence for the success of the modification.

A third objective was to determine the hydrolytic stability of the final hydrocarbon-bonded alumina over a wide pH range. The stability tests were conducted in two ways. Long-term static hydrolytic tests were performed on well-mixed suspensions of the C_{18} -bonded TES alumina in different pH solutions for extended periods. Samples were taken at certain time periods and monitored by FTIR and carbon analysis. Another hydrolytic stability test was performed with the final product packed in a HPLC column and washed with pH 10 phosphate buffer and 1%TFA (trifluoroacetic acid) solutions for 2,000 column volumes under chromatographic conditions (about 1000 psi). Then the column

was evaluated by the capacity factor (k') using the chromatographic performance with three organic test mixtures. The capacity factor (k') was determined by the solute migration time which reflected the change of partition coefficient if the stationary phase was degraded by hydrolysis.

Since this type of chemically bonded alumina has not been documented and used as a stationary phase in HPLC, the reverse phase chromatographic performance had to be tested as the fourth objective. A standard C_{18} -bonded silica column and a commercially modified C_{18} -bonded phosphate alumina column were used for comparison.

Three parameters, peak resolution (R_s), separation efficiency (N) and peak asymmetry (A/B), were used as evaluation parameters to determine the chromatographic performance of the stationary phases. Peak resolution (R_s) measures the degree of separation between two close peaks. Resolution (R_s) is defined as the ratio of the retention time difference of two close peak ($\Delta t = \text{peak}_{t1} - \text{peak}_{t2}$) over the average base width $[(W_{b1} + W_{b2})/2]$ of the two peaks (Figure 1). The equation is expressed below:

$$R_s = 2\Delta t / (W_{b1} + W_{b2})$$

Generally, resolution (R_s) greater than 1.5 is considered an optimum separation in HPLC [2]. Separation efficiency (N) which is also expressed as theoretical plate number is another important parameter for stationary phase evaluation. It measures the extent of zone broadening as a solute is eluted in a column. The distribution about the zone center

becomes greater when its migration distance is increased. The separation efficiency (N) is defined in terms of retention time and peak width according to the equation below:

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

Where t_R is the peak retention time and W represents the peak width at 50% peak height when $a = 5.54$ (Figure2). Peak asymmetry (A/B) is a useful parameter for characterizing the surface uniformity of stationary phase. The peak asymmetry could be affected by various chromatographic sources, which include slow kinetic processes, the formation of column voids and chemical reactions. But in this particular test slow kinetic processes were considered major sources because neither chemical reaction occurred nor columns void formed during the separation of the test mixture. Slow kinetic processes cause peak tailing when two types of sites are contained in the stationary phase. The chemically modified site allows for fast exchange of the solute between the mobile and stationary phases while slow adsorption and desorption proceeding on the residual silanol group causes peak tailing. The peak asymmetry is measured as a ratio of one half peak width (A) over another half peak width (B) at 10% the peak height. The expression is shown in Figure 2 and the equation below [2]:

$$\text{Peak asymmetry} = A/B$$

Three groups of organic compound mixtures were prepared for the chromatographic performance test. Group one contained acetophenone, propiophenone, n-butyrophenone, valerophenone, and hexanophenone. The compounds in this group have the same functional group and slightly different polarity that can be used for reverse phase behavior and the column efficiency test. Group two contained theophylline, p-nitroaniline, methylbenzoate, phenetole, and o-xylene. This mixture has a variety of functional groups with different polarities that can be used as an additional reverse phase behavior test. Group three contained N-methylaniline, N,N-dimethylaniline, and aniline. This mixture has strongly basic compounds which can be used to test the residual hydroxyl group on the modified surface because if there are residual hydroxyl groups peak tailing will occur [22, 25].

2. EXPERIMENTAL METHODS

2.1 Materials

Unisphere alumina (Biotage, Charlottesville, VA) with a mean particle size of 10 μm in diameter, mean pore size of 334 Å and a BET mean surface area of 49 m^2/g was used in the experiments as the support material. p-Dioxane (J.T. Baker Chemical Co., Phillipsburg, N.J.) was dried by contact with calcium hydride for several days and distilled prior to use. Triethoxysilane (Petrarch Systems Silanes & Silicons, Bristol, PA) was used as the hydrosilane reagent. IR-quality potassium bromide (Harshaw/Filtrol Partnership, Solon, OH) powder was used for the FT-IR spectra. 1-Octene and 1-octadecene 98% (Aldrich Chemical Company, Inc. Houston, TX) were employed as bonding materials. Deionized water was purified by a Milli-Q water purification system (Millipore Corp. Bedford, MA).

2.2. Synthetic Procedures

2.2.1. Synthetic Procedure for TES-Alumina

A 0.2 M solution of triethoxysilane (TES) was prepared by adding 37 mL of TES to 963 mL dried p-dioxane in a volumetric flask. Because TES is hydrolyzed and polymerized immediately with water, the preparation has to be performed in a glove bag with nitrogen gas flowing. All of the glassware must be clean and pre-dried. The TES solution was prepared freshly prior to use.

TES alumina was synthesized by weighing 5.0 g of pre-dried bare alumina in a 250-mL, 3-neck round bottom flask that was equipped with a west condenser, a magnetic

stirring bar and a self-equalizing funnel. One hundred ten mL of p-dioxane was transferred to the flask and mixed with the alumina. The mixture was stirred in the flask placed in an oil bath controlled at 80°C. Five mL of 3.1 M HCl solution was added as a catalyst giving a concentration of 0.1 M in the final mixture. Thirty five mL of 0.2 M TES solution was transferred to the self-equalizing funnel using a pre-dried syringe and added drop-wise into the stirred mixture. Once the addition of TES solution was finished, the mixture was gently refluxed for 5 hours at 80±5°C. TES alumina was collected when the reaction was finished and washed by centrifuge with the following steps: two 100-mL aliquots of 80:20 v/v p-dioxane/water; two 100-mL aliquots of p-dioxane; and two 100-mL aliquots of ethyl ether. The TES alumina was dried in air for several hours in a hood in order to remove any residual ethyl ether and then dried at 110°C in a vacuum oven at 25 torr for two hours.

2.2.2. Synthesis of C₁₈ Bonded TES Alumina

1-Octadecene bonded TES alumina was synthesized by weighing 5.0 g of TES alumina and loading it into a self-equalizing funnel. The funnel with the TES alumina was dried in a vacuum oven overnight at 110°C and 25 torr. One hundred mL of 1-octadecene and 0.5 mL of 50 mM dicyclopentadienyl platinum (II) chloride in dry chloroform as catalyst were transferred to a 250-mL 3-neck round bottom flask, equipped with a west condenser and a magnetic stirring bar. The pre-dried self-equalizing funnel containing TES alumina was inserted into one side of the flask neck. The mixture was stirred at 80°C for about one hour until it became clear. Then the TES alumina was

added slowly through the self-equalizing funnel by controlling the rate of addition. After adding the TES alumina, the slurry was stirred for 96 hours at $80 \pm 5^\circ\text{C}$. Once the reaction was complete, the product was collected as a solid phase by centrifugation. The washing steps consisted of four 50-mL aliquots of toluene, two 50-mL aliquots of dichloromethane, and two 50-mL aliquots of ethyl ether. Each washing step took about one minute with vortex stirring and was followed by centrifugation. The final product, C_{18} bonded TES alumina, was dried without heating in a hood for several hours and then moved to a vacuum oven and dried for 2 hours at 110°C and 25 torr.

2.3. Instrumentation and Methods

2.3.1. BET Analysis

Unisphere alumina and TES bonded alumina were evaluated for surface characteristics by BET (Brunauer-Emmett-Teller) on a Micromeritics model ASAP 2400 by the nitrogen adsorption method at Chevron Research and Technology in Richmond, California. It provided the particle pore size in Angstroms and the BET surface area in m^2/g .

2.3.2. TGA Analysis

A Perkin-Elmer model TGS-2 analyzer was employed for thermogravimetric analysis in order to determine the quantity of active hydroxyl groups on the surface of the bare alumina. The analysis was performed by loading 10 mg of sample into a platinum pan which was suspended in the analyzer, heated to 120°C at a rate of $40^\circ\text{C}/\text{min}$. and held at

this temperature until there was no deflection of the weight signal. Then the temperature was raised at the rate of 20°C/min. to 900°C and held until no further weight loss could be detected. Based on the weight loss from 120°C to 900°C, the hydroxyl group density on the alumina surface was calculated from the following equation:

$$\alpha(\mu\text{mol/g}) = [(W_{g120^\circ\text{C}} - W_{g900^\circ\text{C}})/18.01\text{g/mol}] \times 2 \times 10^6 / W_{g120^\circ\text{C}}$$

where $W_{g120^\circ\text{C}} - W_{g900^\circ\text{C}}$ is the weight difference in grams between 120°C and 900°C; 18.01 is the gram molecular weight of water; because one water molecule is formed from two-hydroxyl groups, a factor of 2 was used; 10^6 is the conversion factor for $\alpha(\mu\text{mol/g})$ and $W_{g120^\circ\text{C}}$ is the sample weight at 120°C. In order to remove adsorbed water on the surface of alumina, the sample was held at 120°C for about an hour until the baseline stabilized.

2.3.3. DSC Analysis

Differential Scanning Calorimetric (DSC) analysis was performed on a Perkin-Elmer Model DSC-7 system for evaluation of hydride alumina. About 10 mg of pre-dried TES alumina was loaded into a platinum sample pan and placed in a sample holder. The temperature was raised to 300°C until no heat exchange was observed, and then a temperature gradient from 300°C to 700°C at 20°C/min. with air flow was started. The heat flow was recorded with the temperature gradient and the thermooxidative curve was obtained.

2.3.4. FTIR Analysis

All bare alumina, TES alumina and final C₁₈ bonded alumina products were analyzed by diffuse reflectance Fourier transform infrared (FTIR) spectroscopy. A Perkin-Elmer Model 1800 FT-IR spectrometer equipped with a deuterated triglycine sulfate (DTGS) detector was employed for IR determination. DRIFT spectra of bare alumina and bonded alumina were taken in the region of 4000-450 cm⁻¹ in order to identify the modification layer on the surface of alumina qualitatively. A Spectra-Tech DRIFT accessory with a 2-mm diameter and 1-mm depth sample cup was used. KBr was ground in an agate mortar and pre-dried in vacuum oven at 110°C overnight. The alumina sample was mixed with an equal amount of KBr and filled into the sample cup. A very smooth sample surface was obtained by tapping and pressing the sample using a microscope slide. The height of the sample cup was adjusted by using the alignment routine provided with the standard Perkin-Elmer software in order to get a maximum signal throughput. A nominal resolution of 2 cm⁻¹ and fifty sample scans were programmed in the determination. Spectra shown were normalized to 100% transmittance.

2.3.5. Plasma Emission Analysis

TES alumina was evaluated by quantitatively determining amount of silicon as TES bonded on the surface of alumina. The silicon was analyzed by atomic spectroscopy on a Beckman SMI Model III DC Plasma Spectrometer. The procedure used 0.1 g of TES alumina in a 100-mL plastic beaker mixed and stirred with 10 mL of 0.1 N NaOH solution for one hour at 25°C in order to dissolve all of the silicon bonded on the surface

of alumina. The slurry was filtered. The filtrate was quantitatively transferred into a 50-mL plastic volumetric flask, and filled to volume with 0.1 N NaOH. A series of silicon standard solutions was prepared as 0, 10, 20, 30, 40, 50, and 100 ppm in 0.1N NaOH.

A blank sample was prepared by the same procedure but with 0.1 g of bare alumina. The filtrate was split to two aliquots. One aliquot was used as a blank and another aliquot for control sample preparation.

In order to determine the interference of aluminum ions that possibly dissolved while preparing TES alumina, a control sample was prepared by spiking a 40 ppm standard silicon in the blank solution. The control sample was determined while analyzing the TES alumina samples.

2.3.6. NMR Analysis

The solid state NMR spectra for ^{29}Si , ^{13}C , and ^{27}Al were obtained on a Bruker MSL 300 Spectrometer for identification of the intermediate and the final product. Cross-polarization magic-angle spinning (CP-MAS) NMR was used at a spinning rate of 4KHz. The typical spectrum of ^{27}Al was obtained by 2000 scans with a contact time of 5 ms and recycle time of 5 s. The parameters used for ^{29}Si were a pulse length of 5 μs with a pulse repetition time of 2 s, and a contact time of 5 ms. The acquisition of ^{13}C NMR spectra used a contact time of 12 ms, but otherwise were the same as the parameters used for ^{29}Si . Tetramethylsilane was used for ^{29}Si and ^{13}C NMR spectra as an external reference and the chemical shifts are

given in parts per million (ppm). Spectral simulation was done using the LINESIM program.

2.3.7. Carbon Analysis

The carbon content of the hydrocarbon bonded TES alumina was determined with a Perkin-Elmer Model 240C elemental analyzer equipped with a Perkin-Elmer Model 56 recorder and a CAHN Instrument Company Model G Electro-balance. About 5-10 mg of pre-dried alkyl bonded TES alumina was weighed in a platinum sample boat by the micro-balance with a precision of ± 0.0002 mg. The sample boat was then placed into a holder in the combustion tube and pushed to the combustion area of the furnace. The hydrocarbon bonded on the surface was oxidized to become carbon dioxide and recorded as a CO₂ peak. A previously characterized C₁₈ bonded silica was used as standard and the bare alumina was used as a blank.

2.4. Hydrolytic Stability Test

A static hydrolytic stability test for C₁₈ bonded TES alumina and commercial C₁₈ bonded phosphate alumina was performed with pH 2 HCl, pH 10 NaOH, and 1% TFA solutions. The procedure is described as follows; three 0.5 g portions of bonded alumina were placed in 50-mL centrifuge tubes with a magnetic stirring bar. The pH 2 HCl, pH 10 NaOH, and 1% of TFA solution were prepared in 10 % p-dioxane as the hydrolysis solution. Before adding the hydrolysis solution, 1 mL of p-dioxane was added to each test tube to wet the bonded material. Next 30 mL of each hydrolysis solution was added

to each test tube. The bonded material and solution were then agitated thoroughly until a uniform suspension was obtained. A three mL of aliquot of the well-agitated suspensions was taken every 12 hours and transferred to 12-mL centrifuge tubes. The suspensions were centrifuged for 3 minutes and then the solid was washed with two aliquots of 1:1 water/THF, THF and finally ethyl ether. The samples were dried in a hood at room temperature and then in vacuum oven at 110°C and 25 torr for 4 hours. The mother suspensions were centrifuged and replaced with fresh hydrolysis solution so that the ratio of bonded material and solution was kept constant.

2.5. Column Packing

For the chromatographic performance test, C₁₈ bonded TES-alumina was packed into a stainless steel HPLC column, 150mm x 4.6mm. Three grams of C₁₈ TES-alumina was mixed with 150 mL 100% HPLC-grade methanol and sonicated for 2 minutes. HPLC-grade methanol was used as the driving solvent. The column was packed under 6000 psi using a pneumatic amplification pump for 10 minutes. The column was then allow to sit for 30 minutes before removing the packing apparatus and sealed.

2.6. Chromatographic Performance

A Hewlett-Packard HP 1050 series HPLC system equipped with a quaternary pump and a multiple-wavelength UV-VIS detector was employed for chromatographic performance evaluation. The stability of packed C₁₈ bonded hydride alumina columns

were tested under extreme pH conditions and compared with commercial C₁₈ bonded phosphate alumina.

Three types of organic compound mixtures were prepared for chromatographic evaluation. Group one contained acetophenone, propiophenone, n-butyrophenone, valerophenone, and hexanophenone. The mixture was prepared by weighing and dissolving each compound in methanol as a stock solution and diluting to 100 ppm in the mobile phase. The mobile phase for the group one mixture was started at 40% MeOH/60% water and after 5 minutes a gradient was initiated to 100% methanol in 15 minutes. The mixture was detected at 250 nm with the UV-Vis detector. Group two contained theophylline, p-nitroaniline, methylbenzoate, phenetole, and o-xylene. The mobile phase for this mixture was 45% acetonitrile and 55% water. The mixture was detected at 254 nm with the UV-Vis detector. Group three contained N-methylaniline, N,N-dimethylaniline, and aniline. The mobile phase for this mixture was 35% acetonitrile and 65% water. The mixture was detected at 354 nm with the UV-Vis detector.

Chromatographic performance was evaluated by resolution (Rs), separation efficiency (N), and peak asymmetry (A/B). Resolution is defined as the degree of separation between two peaks as the ratio between the two peak maxima (Δt) and the average base width of the two peaks (Figure 1). The equation is defined as follows:

$$R_s = 2\Delta t / (W_1 + W_2)$$

Where R_s is the resolution ratio, Δt is the retention time difference between the neighboring peaks and W_1 and W_2 are the peak widths of the two neighboring peaks.

Separation efficiency (N), expressed as theoretical plate number, was calculated by the following equation:

$$N_{50\%} = 5.54(t_R/W_{50\%})^2$$

Where $N_{50\%}$ is the theoretical plate number by the 50% peak height method, 5.54 is the constant for calculating peak width at 50%, t_R is retention time for the peak measured and, $W_{50\%}$ is the width measured at half height in units of minutes.

Peak asymmetry (A/B) was calculated by the ratio of A/B at 10% peak height. Figure 2 shows an example of a peak symmetry measurement.

2.7. Column Ruggedness Test

Another hydrolytic stability determination referred to as the column ruggedness test was performed with a packed column under 1000 psi HPLC system pressure. The column was exposed to a total of 2,000 column volumes with two test buffers which are a pH 10 phosphate buffer and a 1% (15mM) TFA solution respectively. Columns were evaluated with the group two mixture after eluting 200-column volumes test solution. The capacity factor (k') was calculated for evaluation of degradation of column performance and plotted vs. the eluting column volume. The equation is expressed as follows:

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

Where t_R is the retention time of a measured peak and t_m is the column dead time, which is measured by the retention time of KNO_3 .

3. RESULTS AND DISCUSSION

3.1. Surface Characterization

The surface characteristics of bare alumina and TES-alumina were determined by the BET method of nitrogen adsorption. The analytical results for pore size and surface area are summarized in Table 1. Comparing the geometric pore diameter of TES-alumina (140Å) to that of bare alumina (198 Å), bonding TES to alumina reduced the pore diameter by 30%. However, the surface area of TES-alumina increased by 30% from 50m²/g to 65 m²/g which caused by more surface on the support beads created by the bonded phases. Both pore size and surface area changes indicate the successful bonding of TES to the bare alumina.

Hydroxyl groups on bare alumina surface are determined by the TGA method. It is important to know reasonably well the number of hydroxyl groups on the alumina surface, because the amount of TES reagent needed in the silanization reaction was based on this determination. Proper stoichiometric calculations can prevent polymerization of excess TES during the silanization reaction. The number of hydroxyls also provided a baseline for the calculation of silicon hydride coverage on the surface of alumina after the silanization process. The measurement was based on the weight loss of the bare alumina heated under nitrogen flow from 120°C to 900°C. The weight change represents loss of water on the alumina surface from combining two hydroxyl groups. The following scheme describes the process:

The mean value of 26 $\mu\text{mol}/\text{m}^2$ was obtained from the measurements shown in Table 2. This value is certainly higher than the one for most silica used in commercial stationary phase support (about 10 $\mu\text{mol}/\text{m}^2$). The higher value could be a tribute to either a large number of micropores and/or a higher TGA measurement in which some water held on the surface was not removed during the pre-heating period.

To avoid multilayer formation on the surface of alumina due to an excess of TES reagent, 1:1:1 molar ratio of TES was used in the silanization reaction with respect to the molar stoichiometry of hydroxyl based on the measurement of TGA. This was considered as the proper amount to get a monolayer with maximum hydride coverage on the alumina surface.

3.2. Reaction Time Optimization and Batch to Batch Reproducibility

The reflux time for the silanization reaction of TES with alumina was optimized. Five hours of refluxing gave the highest surface coverage by TES compared to one hour, three hours and seven hours. Table 3 shows the analysis of silicon content for the optimization experiments. The surface coverage increased to about 55% with refluxing for 5 hours compared to 35% in 3 hours, possibly because the more shielded hydroxyl sites react much slower.

The reproducibility of the synthetic procedure was obtained from three batches of silanization products and final C₁₈ bonded products. Data for TES alumina is shown in Table 4 and the final C₁₈ bonded products are shown in Table 5. In addition, chromatographic tests were conducted with different batches of products as well. The reproducibility was fairly good with $\pm 10\%$ in both silicon and carbon content from batch to batch for the synthetic procedure. The good reproducibility is a result of carefully following the experimental protocols for both silanization and hydrosilation which are free of any operating difficulties.

3.3. FTIR Spectra

The DRIFT method provided a qualitative determination of the success of the modifications of TES-alumina and 1-octadecene-bonded final product. Figure 3 shows the overlap spectra of bare alumina, TES-alumina, and 1-octadecene-bonded hydride alumina. Spectrum 3A is the bare alumina. Spectrum 3B is the TES-alumina which has a strong Si-H stretching band near 2260 cm^{-1} indicating the presence of a hydride siloxane layer on alumina surface. Spectrum 3C is the final product, 1-octadecene bonded hydride siloxane layer on alumina, that shows strong C-H stretching bands near 2950 cm^{-1} . The strong C-H stretching bands are typical of hydrocarbons and indicate that the C₁₈ moiety is present on the hydride siloxane alumina [7, 10, 11]. It is observed that a decrease in peak intensity of the Si-H band at 2260 cm^{-1} that coincides with the appearance of the C-H peak at 2950 cm^{-1} . However, not all of the hydride groups were bonded by olefin as shown by the presence of a small band near 2260 cm^{-1} in spectrum

3C. This might be due to steric hindrance in deeper pore sites and is apparently supported by the carbon analysis (Table 5). The C₈ bonded phase had higher coverage than the C₁₈ phase, which also demonstrates the steric hindrance effect. If necessary, the remainder of the hydrides could be diminished further by end capping with a smaller olefin, such as 1-butene or propylene.

3.4. TES Bonded Alumina Evaluation by Atomic Plasma Emission

Because the polysiloxane bonded monolayer dissolves in 0.1N NaOH but alumina does not, the ionic silicon in the solution was determined easily by the plasma emission method. Therefore, the coverage of TES on the alumina surface was obtained by calculation, based on the silicon concentration in the solution using the equation shown below:

$$\% \text{Coverage} = [(PPM_{Si} \times 50)/29/Wg/\alpha OH \mu mol/g] \times 100$$

Where *PPM_{Si}* is the measured silicon concentration in solution in ppm, 50 is the final volume in mL used for the silicon solution, 29 is the atomic weight of silicon, *Wg* is the sample weight of TES alumina, and *αOH μmol/g* is the hydroxyl group concentration on the alumina surface.

For control purposes, the remaining solid after the 0.1N NaOH wash was collected and evaluated by FTIR. Figure 4A shows that the strong Si-H stretching band near 2260 cm⁻¹ disappeared after washing. This result indicates the washing process is sufficient to

completely remove the polysiloxane layer on the TES-alumina. The original TES hydride alumina with a strong Si-H band is shown in Figure 4B for comparison.

In order to check any interference in the quantitative silicon measurement by the plasma emission method, a control sample was prepared by spiking a 40 ppm silicon standard to the blank sample that was obtained from bare alumina using the same washing procedure. The result shown in Table 3 demonstrates no interference for accurate quantitation of silicon.

The percent coverage of TES on the surface of alumina was about 50% ~ 65%. One of the reasons for the incomplete coverage is due to steric hindrance in deep pores on the alumina surface that were unable to react with the TES reagent. Another reason for low coverage might be inaccurate TGA measurements due to non-removable water resulting in a higher hydroxide calculation. However, the hydroxyl groups remaining in deeper pores might not affect chromatographic behavior because they are covered with a layer of siloxane. This was demonstrated by the good peak separation and symmetry with the three test mixtures in the chromatographic performance on the C₁₈ bonded alumina packed column that will be discussed later.

3.5 NMR Characterization of Modified Alumina

Additional evidence showing successful silanization on alumina and alkyl-bonded TES alumina were provided by ²⁹Si, ¹³C, and ²⁷Al CP-MAS-NMR analysis. Figure 5 shows typical ²⁹Si NMR spectra of TES-alumina (5A) and C₁₈-bonded TES-alumina (5B). The major Si-H peak observed at -85 ppm in (5A) can be used to confirm the

presence of a hydride siloxane monolayer. The two small peaks at -100 and -110 ppm can be assigned to a silicon with three siloxane and one hydroxide bond and four siloxane bonds respectively [21, 22]. This was an unexpected result for a monolayer silanization by TES and is likely due to polymerization on the surface. Figure 5B confirms the formation of the alkyl-bonded phase on silica with the additional Si-C peak at -65 ppm and a decrease in the Si-H peak compared to Fig. 5A. The presence of the unreacted Si-H at -85 ppm after the terminal olefin reaction is expected. This result agrees with the FTIR spectrum for the same sample. However, a relative increase in the -100 ppm peak, which is assigned to a silicon with three siloxane and one hydroxide probably due to the unbonded hydride be hydrolyzed to hydroxide. And decrease in the -110 ppm peak intensity, which is assigned to a silicon with four siloxane bonds is unclear. One possibility is that TES polymerization on the surface is rearranged during the alkylation reaction which either with alkyl group or hydrolyzed to hydroxide.

The ^{13}C CP-MAS-NMR spectrum, shown in Figure 6, also proves the alkyl bonding to the hydride monolayer. The chemical shift in the spectrum is almost identical to the spectrum obtained from alkyl bonding on silica surface [21]. The major peak at 30 ppm represents the resonance of carbon 4 to carbon 15 in the C_{18} hydrocarbon chain. The peak at 22 ppm is assigned as carbon 2 and carbon 17 in the C_{18} hydrocarbon chain and a peak between 10 to 20 ppm is the carbon 1 and 18. We also noticed that methyl group on the silica from alkyl bonding on silica surface does not appear in the C_{18} -bonded TES-alumina ^{13}C CP-MAS-NMR spectrum because it is absent of such methyl group.

In addition, the ^{27}Al CP-MAS-NMR spectrum, shown in Figure 7A, can also be used to characterize the modified surface of alumina. The smaller peak at about 60 ppm represents tetrahedrally (T_d) coordinated aluminum ions and the bigger peak at 1 ppm represents octahedrally (O_h) coordinated aluminum ions. Compared to most hydrated aluminas, about 18% of the total peak area for the T_d in the modified surface of the alumina (Figure 7A) is within the range from 15% to 20% reported in the literature on aluminas (Table 6) [24, 25]. Figure 7B is the simulated spectrum and Figure 7C is the individual components in the simulated spectrum. It is unclear why the individual components in simulated octahedrally coordinated aluminum ions show the three peaks in the spectrum. One of the possibilities might be due to the different conditions for the individual components. Another possibility might be due to the higher distortion of octahedral symmetry by modification process which generates higher quadrupolar effects caused by a non-symmetric electric field gradient. However, without the effective cross-polarization (CP), the distortion in the O_h peaks is lower. This gives the evidence that the hydrogens are close to the alumina surface. Table 6 shows the comparison of the bare alumina, hydride TES-alumina, C_{18} bonded TES-alumina, and commercial C_{18} bonded alumina in peak area. The areas for peak 1 (T_d) and peak 2 (O_h) are between 0.16 and 0.20 which is in good agreement for those four different aluminas. However, the area of the C_{18} bonded TES-alumina decreases slightly for peak 2, but increases for peak 4 which indicates the formation of hydride or alkyl bonded moiety, or the increase distortion of the octahedral symmetry during the modification process.

3.6. DSC study

The DSC/air method was also employed to evaluate the hydride-modified alumina. The thermooxidative process curve of the hydride-modified alumina is shown in Figure 8 in which a thermooxidation peak occurred at 525°C. It has been shown that polyhydrosiloxane (polymerization product of TES in the absence of alumina) has a maximum at 365°C while hydride silica occurs at 440°C [20]. Comparing the maximum thermooxidation temperature of TES-alumina to hydride silica and the polymerization product of TES, two strong evidences can be obtained; the higher thermooxidative temperature indicates chemically bonded TES on the surface of alumina, and in addition, TES-alumina has greater thermal stability than hydride silica. This result might also suggest greater chemical stability under aggressive solvent conditions than silica based modifications.

3.7. Bonded Phase Evaluation by Carbon Coverage

The quantitative determination for the final olefin bonded phase was obtained by carbon analysis. Coverage of the olefin bonded on the hydride TES-alumina can then be calculated. The results are shown in Table 5. The C₈ bonded phase has higher percent of coverage than the C₁₈ phase, 21.9 % vs. 14.3 % on bare alumina. This result can be explained by the steric hindrance effect, where the smaller C₈ can react with TES in the deeper pores on the surface of alumina, but the larger C₁₈ molecule cannot. Carbon analysis is also used for the hydrolytic stability test. The calculation of carbon coverage is following:

$$\% \text{Carbon Coverage} = [(\%C / 100 / 12 / N_c) / \alpha OH \mu\text{mol/g}] \times 100$$

Where %C/100 is the measured carbon content per gram of sample, 12 is the molecular weight of carbon, and N_c represents the number of bonded carbon atoms on alumina (C_8 or C_{18}).

3.8. Stability under Extreme pH Conditions

Hydrolytic stability tests that were conducted after the preliminary analysis demonstrated the potential usefulness of the olefin (C_8 or C_{18}) bonded on the TES alumina phase. The static hydrolysis tests were conducted at three critical conditions; pH 10, pH 2, and 1% trifluoroacetic acid (TFA) solutions. They were well-agitated for 108 hours at room temperature.

The C_{18} bonded TES-alumina showed good stability under the three conditions. Figure 9 shows the plots of carbon content vs. the hydrolytic time for the C_{18} bonded TES-alumina. It has been observed that the carbon content of the first sample point from the initial sample dropped significantly and thereafter, the carbon content is constant. This drop is believed that is not due to hydrolysis but most possibly caused by washing out of unbonded 1-octadecene residues by these strong buffers. Figure 10 is the same hydrolytic test for the commercial C_{18} bonded phosphate alumina, which shows good stability under these conditions as well. Figure 11 and 12 shows the FTIR spectrum for the C_{18} bonded TES-alumina and the commercial C_{18} bonded phosphate alumina after the stability test respectively, in which the strong absorbance between 3000 cm^{-1} and 2800

cm⁻¹ indicate the existence of a hydrocarbon phase on the surface of TES-alumina after 108 hours agitation. Table 7 and 8 show the carbon analysis data corresponding to Figure 9 and Figure 10.

3.9. Chromatographic Performance

A C₁₈-bonded hydride alumina column was evaluated for chromatographic performance and compared with a commercial C₁₈ bonded phosphate alumina and a C₁₈ bonded silica. Three groups of organic compounds were chosen for this evaluation for different chromatographic behavior. Group 1 is a mixture of alkyl phenyl ketones that have the same functional group (C₆H₅CO) and an increasing hydrocarbon chain that slightly decreased molecular polarity. This group can be tested for stationary phase uniformity and C₁₈ bonded coverage with reverse phase behavior. The expectation is that the more polar compounds should elute first from the reverse phase column. Figure 13A shows the chromatogram in which the alkyl phenyl ketones eluted in order of decreased polarity: acetophenone, propiophenone, n-butyrophenone, valerophenone, and hexanophenone from the C₁₈-bonded hydride alumina column. The chromatogram also shows baseline separation for these compounds. The commercial C₁₈ bonded phosphate alumina and C₁₈ bonded silica are compared in Figures 13B and 13C for the same alkyl phenyl ketone mixture. It should be noted that the C₁₈ bonded silica column was a previously used column with an unknown history. Therefore, the separation might not be optimum for this column. Group 2 is a mixture of aromatic compounds that have different functional groups, structures and molecular polarities. Figure 14A shows the

chromatogram of the group 2 mixture eluted from the C₁₈ bonded hydride alumina column. The compounds eluted in the order: theophylline, p-nitroaniline, methylbenzoate, phenetole, and o-xylene, from higher to lower polarity.

In order to determine the effect of unbonded hydroxyl groups on the surface of alumina, which might result in strong interaction between the stationary phase and the solute, a mixture of anilines was chosen [8, 9]. Figures 15A, 15B and 15C represent the chromatograms for the anilines on C₁₈ TES alumina column, commercial C₁₈-bonded phosphate alumina, and C₁₈-bonded silica respectively.

Tables 8 through Table 16 list calculations of the resolution (Rs), separation efficiency (N), and peak asymmetry (A/B at 10% peak height). The three parameters were used to evaluate chromatographic performance for reverse phase modification. Generally in HPLC, Rs $\geq$ 1.5 is considered baseline separation. The C₁₈ TES-alumina column shows resolution parameters between 2-5 for all of the three test mixtures. The peak symmetry parameter measures the adsorption effect between the solute and the stationary phase. Usually a ratio of A/B between 1 and 2 is considered a good symmetric chromatographic peak. The C₁₈ bonded TES-alumina column shows better symmetry (1.5 to 2.2) than the commercial C₁₈ bonded phosphate alumina and the C₁₈ bonded silica columns (2.5 to 3.5). Especially for aniline mixtures, The C₁₈ TES alumina column has peak asymmetry A/B between 1.85 to 2.76 vs. 2.93 to 3.15 for the C₁₈ bonded phosphate alumina, which apparently indicates fewer residual hydroxyl groups on the surface. The separation efficiency (N) was calculated as a relative number because the three columns used for the comparison have the same length (150cm). The C₁₈ bonded TES-alumina

shows lower separation efficiency than the other two columns for all the three types of mixture separation. It is believed that the lower efficiency is due to the lower carbon coverage (3.5%) than the commercial C₁₈ phosphate alumina (7.5%) and C₁₈ silica. The low carbon coverage could be improved by optimization of TES silanization hydrolylation or selection of another type of alumina. Based on the resolution, efficiency, and asymmetry for each of the three mixtures, the C₁₈ bonded alumina column packing shows promising performance of reverse phase chromatography.

3.10 Column Ruggedness

Two-packed C₁₈ bonded TES alumina columns were evaluated for bonded phase ruggedness with pH 10-phosphate buffer and 1% TFA solution respectively. The test was performed on the HPLC system using a column pressure of about 1000 psi. The commercial C₁₈ bonded phosphate alumina columns were compared under the same testing conditions. Because the C₁₈ silica column is known to be unstable at these pH conditions, it was not involved in this test.

Tables 17 to 21 as well as Figures 16 to 19 show the columns stability with the two buffers vs. the eluting column volume evaluated by k' factors. The C₁₈ TES alumina column with pH 10 buffer displayed same degradation during the total 2000 column volume test (k' from 2.28 to 1.47) except the first measurement at 200 column volumes. The k' drops significantly (k' from 3.94 to 2.28 for o-xylene) after the first 200 column volume wash that obviously agreed with the static hydrolytic stability test (Figure 9). This degradation is probably due to the same reason. However, the commercial C₁₈

bonded phosphate alumina shows continuous degradation (Figure 17) and the k' dropped from 2.76 to 1.50 for o-xylene.

With the 1% TFA mobile phase, the C₁₈ TES-alumina shows significant stability (Figure 18) over the commercial C₁₈ bonded phosphate alumina column. The k' of o-xylene drops 22% (from 5.86 to 4.60) for C₁₈ TES-alumina compared to 48% (from 2.63 to 1.40) for C₁₈ bonded phosphate alumina column (Figure 19) for the total 2000 column volume test. Figures 20 and 21 are FTIR spectra for the packing material of the commercial C₁₈ bonded alumina column and the C₁₈ TES-alumina after the 1% TFA and pH 10 phosphate buffer tests.

4. CONCLUSIONS

From the overall evaluation of the C₁₈ bonded TES-alumina, it appears that the TES silanization procedure and various alkyl-bonded syntheses on alumina are very promising, and alkyl modified alumina has potential value as an alternative stationary phase. The new modified alumina shows good separation for various organic mixtures and good peak shape in the reverse phase mode. The more important advantage compared to commercial silica and alumina columns is that the new modified alumina possesses better hydrolytic stability under pH10 phosphate buffer and 1% TFA solution.

Several analytical methods have been established for monitoring the intermediate and the final product of alumina modification. FTIR, NMR, and DSC provided an efficient qualitative analysis and silicon plasma emission and carbon% analysis provided quantitative measurement. With the established analytical methods, the new approach for preparing alternative HPLC stationary phases becomes feasible and easy to control.

This research is a preliminary exploration for alumina modification. Further work needs to be done for procedure optimization and variations. Different type of alumina (manufacture, particle size, surface area, pore size, and surface hydroxyl group etc.) might result different surface coverage by TES silanization. The surface coverage of hydride TES could be optimized by variation of TES amount and refluxing temperature. More efficient washing solvent for the intermediate and final product should be tried in order to wash completely. Different stationary phase such as terminal -CN and -NH₂ can be explored on alumina and chromatographic behavior with high pH mobile phase would be interesting.

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Fig. 1. Measurement of peak symmetry (A/B ratio) at 10% of peak height.

Fig. 2. Measurement of resolution for two closely spaced peaks.

Fig. 3. FTIR spectra of (A) bare alumina, (B) TES-alumina and (C) C₁₈-bonded TES-alumina.

Fig. 4. FTIR spectra of (A) 0.1N NaOH washed TES-alumina and (B) TES-alumina.

Fig. 5. ^{29}Si CP-MAS-NMR spectra of (A) TES-alumina and (B) C_{18} -bonded TES-alumina.

Fig. 6. ^{13}C CP-MAS-NMR spectra of C_8 bonded TES-alumina.

Fig. 7. ^{27}Al CP-MAS-NMR spectra: (A) experimental spectrum of C_{18} -bonded TES-alumina, (B) simulated spectrum of C_{18} -bonded TES-alumina and (C) simulated spectrum with individual components.

Fig. 8. DSC curve for the thermooxidation of TES-alumina.

Fig. 9. Hydrolytic stability of C₁₈-bonded TES-alumina with different buffers

Fig. 10. Hydrolytic stability of commercial C_{18} -bonded phosphate alumina with different buffers

Fig. 11. FTIR spectra of C₁₈-bonded TES-alumina after the 108 hour hydrolytic test with (A) HCl, pH 2, (B) NaOH, pH 10 and (C) 1% TFA

Fig. 12. FTIR spectra of commercial C₁₈-bonded phosphate alumina after the 108 hour hydrolytic test with (A) HCl, pH 2, (B) NaOH, pH 10 and (C) 1% TFA

Fig. 13. Chromatogram of test mixture for reversed-phase behavior: (A) C₁₈-bonded TES-alumina, (B) commercial C₁₈-bonded phosphate alumina and (C) commercial C₁₈-bonded silica. Mobile phase: 40% MeOH and 60% water for 5 minutes and gradient to 100% MeOH in 15 minutes. Peaks: 1= acetophenone, 2= propiophenone, 3= n-butyrophenone, 4= valerophenone and 5= hexanophenone.

Fig. 14. Chromatogram of test mixture for reversed-phase behavior: (A) C₁₈-bonded TES-alumina, (B) commercial C₁₈-bonded phosphate alumina and (C) commercial C₁₈-bonded silica. Mobile phase: 45% acetonitrile and 55% water. Peaks: 1= theophylline, 2= p-nitroaniline, 3= methylbenzoate, 4= phenetole and 5= o-xylene

Fig. 15. Chromatogram of test mixture for residual hydroxyl groups on the stationary phase: (A) C_{18} -bonded TES-alumina, (B) commercial C_{18} -bonded phosphate alumina and (C) commercial C_{18} -bonded silica. Mobile phase: 35% acetonitrile and 65% water. Peaks: 1= aniline, 2= N-methylaniline, 3= N,N-dimethylaniline.

Fig. 16. Column ruggedness evaluation of C_{18} -bonded TES-alumina with pH 10 phosphate buffer

Fig. 17. Column ruggedness evaluation of commercial C_{18} -bonded phosphate alumina with pH10 phosphate buffer

Fig. 18. Column ruggedness evaluation of C_{18} -bonded TES-alumina with 1% TFA

Fig. 19. Column ruggedness evaluation of commercial C_{18} -bonded phosphate alumina with 1% TFA

Fig. 20. FTIR spectra of C₁₈-bonded TES-alumina after the column ruggedness test with (A) pH 10 phosphate buffer and (B) 1% TFA

Fig. 21. FTIR spectra of commercial C₁₈-bonded phosphate alumina after the column ruggedness test with (A) pH 10 phosphate buffer and (B) 1% TFA

Table 1. Surface Characterization of Bare Alumina and TES-Alumina by BET

Sample Description	Particle Diameter μm	Surface Area m^2/g	Pore Diameter $\text{\AA}$
Alumina	10	50	198
TES-Alumina	-	65	140

Table 2. Hydroxyl Group Measurement on the Bare Alumina Surface by TGA

Analysis #	Weight at 120°C mg	Weight at 900°C mg	Weight Loss %	α Al-OH $\mu\text{mol/g}$	α Al-OH $\mu\text{mol/m}^2$
1	9.534	9.422	1.176	1305	26.11
2	8.626	8.523	1.195	1327	26.50

Table 3. Plasma Emission Analysis for Reaction Time Optimization of TES-Alumina Synthesis

Reaction Time hrs	Sample Weight g	P.E. Intensity unit	Conc. Si ppm	Coverage on Surface $\mu\text{mol}/\text{m}^2$	Coverage on Al-OH %Mean
1 hour	0.0772	2553	15.7	7.26	27.6
3 hours	0.0824	3300	21.1	9.14	34.7
5 hours	0.1011	2796	42.6	15.04	54.9
7 hours	0.1167	7167	44.3	13.56	51.3

Calibration equation for 1 hour: Conc. (ppm) = $-2.71 + 0.0072 X$

Calibration equation for 3 hours: Conc. (ppm) = $-2.71 + 0.0072 X$

Calibration equation for 5 hours: Conc. (ppm) = $-4.34 + 0.0168 X$

Calibration equation for 7 hours: Conc. (ppm) = $-3.04 + 0.0066 X$

Table 4. Plasma Emission Analysis for Evaluation of TES-Alumina

Sample #	Sample Weight g	P.E. Intensity unit	Conc. Si ppm	Coverage on Surface $\mu\text{mol}/\text{m}^2$	Coverage on Al-OH %
Blank 1 ^a	0.100	5	0.0		
Control 1 ^b	0.100	2669	40.5		
Batch 1-1	0.101	2796	42.6	15.0	56.5
Batch 1-2	0.107	2747	41.8	14.0	53.3
Blank 2 ^a	0.100	23	0.0		
Control 2 ^b	0.100	6523	40.0		
Batch 2-1	0.117	7167	44.3	13.6	51.6
Batch 2-2	0.089	5480	33.2	13.4	50.9
Blank 3 ^a	0.100	14	0.0		
Control 3 ^b	0.100	3230	39.9		
Batch 3-1	0.104	3979	49.4	16.9	64.5
Batch 3-2	0.099	3868	47.9	17.2	65.4

^aBlank sample was prepared with bare alumina

^bControl sample was spiked 40 ppm Si solution in blank.

Calibration equation for Batch 1: $\text{Conc.}(\text{ppm}) = -4.34 + 0.0168 X$

Calibration equation for Batch 2: $\text{Conc.}(\text{ppm}) = -3.04 + 0.0066 X$

Calibration equation for Batch 3: $\text{Conc.}(\text{ppm}) = -0.79 + 0.0126 X$

Table 5. Elemental Analysis (%C) for Evaluation of C₈ and C₁₈-Bonded TES-Alumina

Batch #	Bonded Phase Type	%C	Coverage on Alumina $\mu\text{mol/g}$	Coverage on Alumina $\mu\text{mol/m}^2$	Coverage on TES-Al %
1	C ₈	2.77	288	5.77	39.9
1	C ₁₈	4.07	188	3.77	26.1
2	C ₁₈	4.72	218	4.37	32.4
3	C ₁₈	4.68	217	4.33	25.4

Table 6. Relative Peak Area for Simulated Components in ²⁷Al CP-MAS-NMR Spectra

Type of Alumina	Peak 1 (T _d)	Peak 2 (O _h)	Peak 3 (O _h)	Peak 4 (O _h)
Bare alumina	0.18	0.48	0.33	-
Hydride alumina	0.16	0.52	0.28	0.05
C ₁₈ bonded TES alumina	0.20	0.43	0.24	0.14
Biotage C ₁₈ phosphonate	0.17	0.51	0.24	0.08

Table 7. Elemental Analysis (%C) for Evaluation of Hydrolytic Stability of C₁₈-Bonded TES-Alumina with Different Buffers

Sampling	pH 10 NaOH	pH 2 HCl	1% TFA
hours	%C	%C	%C
0	4.67	4.67	4.67
12	3.22	3.35	3.07
24	3.25	3.31	3.16
36	3.01	3.29	3.12
48	2.99	3.34	3.06
60	2.94	3.28	3.04
72	2.84	3.31	2.95
84	2.97	3.21	3.16
96	3.00	3.15	3.03
108	2.92	3.18	2.97

Table 8. Elemental Analysis (%C) for Evaluation of Hydrolytic Stability of Commercial C₁₈-Bonded Phosphate Alumina with Different Buffers

Sampling	pH 10 NaOH	pH 2 HCl	1% TFA
hours	%C	%C	%C
0	7.50	7.50	7.50
12	7.28	7.19	7.14
24	7.28	7.165	7.20
36	7.36	7.34	7.17
48	7.36	7.27	7.13
60	7.33	7.52	7.25
72	7.35	7.48	7.29
84	7.37	7.47	7.41
96	7.30	7.52	7.31
108	7.37	7.66	7.33

Table 9. Chromatographic Performance Evaluation of C₁₈-Bonded TES-Alumina with an Alkylphenone Mixture

Compound	t _R	W _{10%}	W _{50%}	A _{10%}	B _{10%}	Rs	N _p	Symmetry
Name	min.	min.	min.	min.	min.			A/B
Aceto-phenone	4.07	0.470	0.175	0.324	0.144		2,997	2.25
propio-phenone	5.90	0.675	0.270	0.437	0.239	3.20	2,645	1.83
n-Butyro-phenone	9.40	1.020	0.443	0.640	0.381	4.13	2,494	1.68
Valero-phenone	14.8	0.900	0.407	0.546	0.353	5.63	7,326	1.55
Hexano-phenone	19.6	0.825	0.352	.518	0.305	5.62	17,264	1.70

Table 10. Chromatographic Performance Evaluation of Commercial C₁₈-Bonded Phosphate Alumina with an Alkylphenone Mixture

Compound	t _R	W _{10%}	W _{50%}	A _{10%}	B _{10%}	Rs	N _p	Symmetry
Name	min.	min.	min.	min.	min.			A/B
Aceto-phenone	4.41	0.420	0.145	0.306	0.114		5,124	2.68
propio-phenone	7.37	0.720	0.243	0.520	0.203	5.19	5,096	2.56
n-Butyro-phenone	11.1	0.580	0.180	0.420	0.158	5.77	21,143	2.66
Valero-phenone	13.8	0.470	0.144	0.349	0.121	5.07	50,732	2.88
Hexano-phenone	15.7	0.430	0.134	0.319	0.111	4.27	76,050	2.87

Table 11. Chromatographic Performance Evaluation of Commercial C₁₈-Bonded Silica with an Alkylphenone Mixture

Compound	t _R	W _{10%}	W _{50%}	A _{10%}	B _{10%}	Rs	N _p	Symmetry
Name	min.	min.	min.	min.	min.			A/B
Aceto-phenone	8.2	1.000	0.303	0.755	0.245		4,057	3.08
propio-phenone	11.7	0.670	0.224	0.494	0.177	4.17	15,063	2.79
n-Butyro-phenone	13.9	0.600	0.199	0.444	0.156	3.57	27,224	2.85
Valero-phenone	15.8	0.515	0.176	0.362	0.153	3.35	44,761	2.37
Hexano-phenone	17.3	0.480	0.163	0.355	0.125	2.93	62,262	2.84

Table 12. Chromatographic Performance Evaluation of C₁₈-Bonded TES-Alumina with an Aromatic Mixture

Compound	t _R	W _{10%}	W _{50%}	A _{10%}	B _{10%}	Rs	N _p	Symmetry
Name	min.	min.	min.	min.	min.			A/B
Theophylline	2.30	0.272	0.110	0.185	0.087		2,420	2.13
p-Nitro-aniline	3.11	0.222	0.106	0.130	0.092	3.28	4,770	1.41
Methyl-benzoate	4.14	0.410	0.180	0.252	0.158	3.26	2,930	1.59
Phenetole	5.57	0.510	0.240	0.291	0.219	3.11	2,980	1.33
o-Xylene	8.21	0.748	0.370	0.418	0.330	4.20	2,730	1.27

Table 13. Chromatographic Performance Evaluation of Commercial C₁₈-Bonded Phosphate Alumina with an Aromatic Mixture

Compound	t _R	W _{10%}	W _{50%}	A _{10%}	B _{10%}	Rs	N _p	Symmetry
Name	min.	min.	min.	min.	min.			A/B
Theophylline	2.14	0.175	0.082	0.118	0.057		3,770	2.07
p-Nitro-aniline	2.74	0.210	0.090	0.150	0.060	3.12	5,140	2.50
Methyl-benzoate	3.54	0.340	0.145	0.248	0.092	2.91	3,300	2.70
Phenetole	4.62	0.460	0.180	0.340	0.120	2.70	3,650	2.83
o-Xylene	6.55	0.658	0.270	0.498	0.160	3.45	3,260	3.11

Table 14. Chromatographic Performance Evaluation of Commercial C₁₈-Bonded Silica with an Aromatic Mixture

Compound	t _R	W _{10%}	W _{50%}	A _{10%}	B _{10%}	Rs	N _p	Symmetry
Name	min.	min.	min.	min.	min.			A/B
Theophylline	1.44	0.270	0.110	0.197	0.073		949	2.69
p-Nitro-aniline	2.65	0.260	0.102	0.179	0.081	4.57	3,739	2.21
Methyl-benzoate	5.12	0.700	0.216	0.540	0.162	5.15	3,113	3.33
Phenetole	8.52	1.140	0.340	0.891	0.252	3.70	3,479	3.54
o-Xylene	14.4	1.960	0.574	1.520	0.436	3.79	3,487	3.49

Table 15. Chromatographic Performance Evaluation of C₁₈-Bonded TES-Alumina with an Aniline Mixture

Compound	t_R	W_{10%}	W_{50%}	A_{10%}	B_{10%}	Rs	N_p	Symmetry
Name	min.	min.	min.	min.	min.			A/B
Aniline	3.20	0.780	0.330	0.580	0.210		521	2.76
N-methyl-aniline	4.68	0.740	0.320	0.480	0.260	1.95	1,185	1.85
N,N-dimethyl-aniline	8.51	1.23	0.547	0.855	0.376	3.89	1,341	2.27

Table 16. Chromatographic Performance Evaluation of Commercial C₁₈-Bonded Phosphate Alumina with an Aniline Mixture

Compound	t _R	W _{10%}	W _{50%}	A _{10%}	B _{10%}	Rs	N _p	Symmetry
Name	min.	min.	min.	min.	min.			A/B
Aniline	3.23	0.330	0.110	0.246	0.084		4,777	2.93
N-methyl-aniline	4.60	0.410	0.127	0.308	0.103	3.70	7,268	2.99
N.N-dimethyl-aniline	7.77	0.670	0.207	0.510	0.162	5.87	7,806	3.15

Table 17. Chromatographic Performance Evaluation of Commercial C₁₈-Bonded Silica with an Aniline Mixture

Compound	t _R	W _{10%}	W _{50%}	A _{10%}	B _{10%}	Rs	N _p	Symmetry
Name	min.	min.	min.	min.	min.			A/B
Aniline	3.28	0.470	0.157	0.360	0.110		2,418	3.27
N-methyl-aniline	6.78	0.980	0.284	0.767	0.213	4.83	3,157	3.60
N,N-dimethyl-aniline	15.9	2.50	0.700	2.030	0.475	5.24	2,858	4.27

Table 18. Column Ruggedness Evaluation of C₁₈-Bonded TES-Alumina with pH 10 Phosphate Buffer

Eluted column volume	Theophylline		p-Nitro-aniline		Methyl-benzoate		Phenetole		o-Xylene	
	Rt	k'	Rt	k'	Rt	k'	Rt	k'	Rt	k'
0	2.30	0.380	3.11	0.871	4.14	1.49	5.57	2.35	8.21	3.94
200	1.76	0.0565	2.68	0.610	3.22	0.939	3.94	1.37	5.46	2.28
400	1.84	0.104	2.71	0.628	3.19	0.916	3.83	1.31	5.24	2.15
600	1.85	0.109	2.68	0.611	3.11	0.870	3.70	1.22	4.91	1.95
800	1.73	0.0403	2.59	0.559	2.99	0.797	3.52	1.12	4.69	1.82
1,000	1.74	0.0475	2.62	0.573	3.01	0.811	3.53	1.12	4.67	1.81
1,200	1.82	0.0956	2.57	0.545	2.92	0.757	3.43	1.06	4.49	1.71
1,400	1.66	0.000	2.56	0.541	2.90	0.741	3.36	1.02	4.36	1.62
1,600	1.66	0.000	2.57	0.546	2.87	0.724	3.29	0.975	4.18	1.52
1,800	1.73	0.0378	2.58	0.552	2.86	0.722	3.25	0.957	4.13	1.48
2,000	1.62	0.000	2.54	0.525	2.82	0.696	3.23	0.941	4.11	1.47

Table 19. Column Ruggedness Evaluation of Commercial C₁₈-Bonded Phosphate Alumina with pH 10 Phosphate Buffer

Eluted column volume	Theophylline		p-Nitro- aniline		Methyl- benzoate		Phenetole		o-Xylene	
	Rt	k'	Rt	k'	Rt	k'	Rt	k'	Rt	k'
0	2.14	0.231	2.74	0.576	3.54	1.032	4.62	1.66	6.55	2.76
100	1.91	0.0954	2.66	0.528	3.32	0.909	4.19	1.41	5.83	2.35
200	1.87	0.0724	2.65	0.525	3.31	0.902	4.15	1.39	5.73	2.29
400	1.85	0.0615	2.59	0.490	3.17	0.819	3.89	1.23	5.26	2.02
600	1.80	0.0356	2.60	0.495	3.15	0.811	3.85	1.21	5.14	1.95
800	1.79	0.0305	2.57	0.475	3.08	0.770	3.73	1.14	4.94	1.84
1,000	1.78	0.0052	2.52	0.446	2.97	0.707	3.54	1.03	4.61	1.65
1,200	1.86	0.0672	2.56	0.471	3.04	0.747	3.65	1.10	4.79	1.75
1,400	1.88	0.0816	2.56	0.471	3.04	0.749	3.66	1.10	4.81	1.76
1,600	1.68	0.000	2.48	0.424	2.89	0.661	3.41	0.958	4.39	1.53
1,800	1.71	0.000	2.46	0.414	2.87	0.648	3.37	0.934	4.31	1.48
2,000	1.70	0.000	2.48	0.425	2.88	0.658	3.39	0.947	4.34	1.50

Table 20. Column Ruggedness Evaluation of C₁₈-Bonded TES-Alumina with 1% TFA

Eluted column volume	Theophylline		p-Nitro- aniline		Methyl- benzoate		Phenetole		o-Xylene	
	Rt	k'	Rt	k'	Rt	k'	Rt	k'	Rt	k'
0	2.08	0.253	3.04	0.831	4.65	1.80	6.99	3.21	11.39	5.86
200	2.10	0.265	2.96	0.783	4.44	1.67	6.47	2.89	10.49	5.32
400	2.15	0.295	3.08	0.855	4.52	1.72	6.63	2.99	10.77	5.48
600	2.14	0.289	2.97	0.789	4.43	1.67	6.43	2.87	10.36	5.24
800	2.16	0.301	2.99	0.801	4.42	1.66	6.38	2.84	10.33	5.22
1,000	2.12	0.277	2.93	0.765	4.13	1.48	6.24	2.75	10.11	5.09
1,200	2.14	0.289	2.94	0.771	4.32	1.60	6.16	2.71	9.95	4.99
1,400	2.13	0.283	2.93	0.765	4.3	1.59	6.15	2.70	9.84	4.92
1,600	2.14	0.289	2.89	0.741	4.22	1.54	5.99	2.60	9.58	4.77
1,800	2.13	0.283	2.87	0.729	4.19	1.52	5.91	2.56	9.44	4.68
2,000	2.13	0.283	2.84	0.711	4.15	1.50	5.85	2.52	9.29	4.59

Table 21. Column Ruggedness Evaluation of Commercial C₁₈-Bonded Phosphate Alumina with 1%TFA

Eluted column volume	Theophylline		p-Nitro-aniline		Methyl-benzoate		Phenetole		o-Xylene	
	Rt	k'	Rt	k'	Rt	k'	Rt	k'	Rt	k'
0	2.14	0.0486	2.89	0.418	3.82	0.874	5.13	1.52	7.39	2.63
200	2.11	0.0373	2.73	0.339	3.47	0.705	4.52	1.22	6.40	2.14
400	2.09	0.0250	2.61	0.278	3.26	0.600	4.14	1.03	5.79	1.84
600	2.08	0.0187	2.58	0.268	3.23	0.585	4.10	1.01	5.73	1.81
800	2.09	0.0260	2.57	0.261	3.16	0.550	3.95	0.940	5.48	1.69
1,000	2.09	0.0255	2.59	0.273	3.21	0.576	4.05	0.989	5.65	1.77
1,200	2.08	0.0230	2.55	0.250	3.13	0.538	3.92	0.925	5.43	1.66
1,400	2.08	0.0230	2.55	0.251	3.14	0.540	3.93	0.930	5.45	1.67
1,600	2.08	0.0230	2.45	0.203	2.92	0.433	3.55	0.743	4.80	1.36
1,800	2.09	0.0230	2.46	0.208	2.94	0.442	3.56	0.761	4.87	1.39
2,000	2.09	0.0230	2.45	0.202	2.94	0.443	3.59	0.763	4.89	1.40