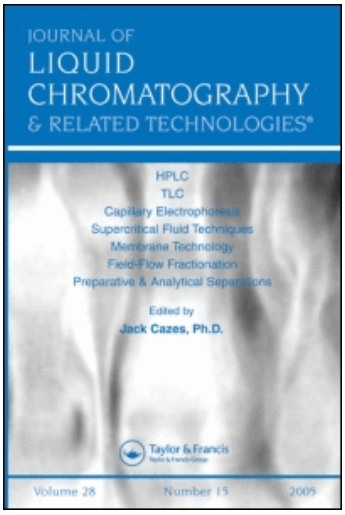

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CHROMATOGRAPHIC PROPERTIES OF MIXED CHEMICALLY BONDED PHASES WITH ALKYLAMIDE AND AMINOPROPYL LIGANDS

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ABSTRACT

A series of mixed chemically bonded phases, which contained alkylamide and aminopropyl ligands, were prepared by subsequent reaction of the silica-based aminopropyl phase with suitable alkanoyl chlorides. Physicochemical properties of these phases were studied by using various techniques such as elemental analysis, ^{29}Si and ^{13}C solid-state NMR and adsorption.

In addition, suitable chromatographic measurements were carried out in order to study the effect of the length of the terminal alkyl chain on the solute's retention under reversed phase conditions.

INTRODUCTION

In spite of the development of many novel packing materials for reversed-phase high performance liquid chromatography (RP HPLC), e.g., polymeric and carbonaceous packings,[1-4] chemically bonded phases (CBP) have been increasingly used.[5] This is due to their interesting physicochemical properties and relatively high stability under reversed-phase conditions.[6] At these conditions the retention and selectivity depend strongly on the specific and nonspecific interactions of the analyte with the components of the mobile and stationary phases. It should be noted, that molecular interactions in the stationary phase are more complex in comparison to those in the mobile phase because they are influenced by many factors such as: the type and structure of the bonded ligands, their coverage density as well as the surface properties and porosity of siliceous supports.[2,6-14] The situation becomes even more complex for chemically bonded ligands, which possess some specific functionalities, e.g., internal and/or external $-NO_2$, $-NH_2$, -OH, -CN, $-C_6H_5$, -NHCO- groups.[2,6,11,15-18]

In order to improve chromatographic separations of polar substances, new types of chemically bonded phases, which contain the specific internal groups were synthesized.[5] Among them, alkylamide phases attracted special attention because of their utility for separation of various basic compounds.[19-27] Amide groups are also present in the silica-based phases synthesized by immobilization of proteins, e.g., bovine serum albumin (BSA).[28,29] The BSA phases were used for normal-phase, as well as reversed-phase separations, including chiral separations. Retention mechanism on these phases is complex and not fully explained yet. Another example of the specific phase was reported by Feibush and Santasania,[30] who studied the shielded hydrophobic phase (SHP), which contained ion-exchange groups in the hydrophobic zone. This phase has been shown to be useful for direct injection analysis of drugs in biological fluids.

In the current work, a series of mixed chemically bonded phases, which in addition to residual surface silanols containing two different ligands, alkylamide and aminopropyl, was studied. The amide ligands were obtained by the secondary reaction of aminopropyl ligands, created in the first step of the silica modification, with various alkanoyl chlorides. Thus, the resulting phases contained terminal alkyl chains of different lengths, e.g., $-CH_3$, $-C_6H_{13}$, $-C_{12}H_{25}$, $-C_{16}H_{33}$, and $-C_{18}H_{37}$. Surface properties of these packing materials were characterized by various physicochemical methods such as elemental analysis, ^{29}Si, and ^{13}C solid-state NMR and adsorption. Their chromatographic properties were investigated using two different test mixtures: homologous series of alkylbenzenes and alkylanilines.

EXPERIMENTAL

Materials

Chemically bonded phases were prepared by using 5 μm LiChrospher Select B silica from E. Merck, Darmstadt, Germany. Its BET specific surface area and total pore volume were equal to 570 m^2/g and 0.96 cm^3/g, respectively. The average pore width was about 5.8 nm. The concentration of accessible surface silanols was estimated to be equal to 5.18 $\mu mol/m^2$.

The following surface modification reagents were used: γ-aminopropyldimethyl-metoxysilane from Huls (Bristol, PA, USA), acetylchloride from Fluka (Buchs, Switzerland), hexanoyl chloride, lauroyl chloride, palmitoyl chloride, and stearyol chloride from Aldrich (Milwaukee, WI, USA). Specially prepared dry morfoline[31] (Riedel de Haën, Seelze, Germany) was used as an activator.

The remaining chemicals: methanol, toluene, and n-hexane, all analytical grade purity, were purchased from Aldrich. The deionized water was purified in laboratory using a Millipore (El Paso, TX, USA) Milli-Q reagent water system.

Synthesis of Chemically Bonded Phases

Mixed alkylamide phases were synthesized in a sealed glass reactor by a two-step process.[19-26] Prior to the synthesis, the silica gel (SG, about 15 g) was dried at 180°C under vacuum (10^{-3} Pa) in a glass reactor without contact with the environment for 12 h.[32] Then, it was reacted with 20 mL of monofunctional aminopropylsilane under environmentally isolated conditions at 110°C ± 5°C for 5 hrs. The product, aminopropyldimethylsilyl phase (SG-NH_2), was filtered, washed with dry toluene, methanol, and n-hexane, and finally dried by flowing nitrogen. In each second modification step about 3 g of the SG-NH_2 phase was placed in the glass reactor and heated at 120°C under vacuum (10^{-3} Pa) for 12 h, and then allowed to react with suitable alkanoyl chloride in the presence of dry morpholine at 50, 60, 80 and 100°C ± 5°C, respectively. The resulting material was filtered, washed with dry toluene, methanol, and n-hexane, and then dried at room temperature.The following alkanoyl chlorides were used: acetylchloride (SG-AP_1 - code of the resulting phase), hexanoyl chloride (SG-AP_2), lauroyl chloride (SG-AP_3), palmitoyl chloride (SG-AP_4), and stearoyl chloride (SG-AP_5). Note that the synthesized

Table 1

Physicochemical Properties of Mixed Alkylamide Phases

Phase	Terminal Alkyl Chain	P_C (%)	P_N (%)	α_a(μmol/m^2)[a]
SG-NH_2	----	6.93	1.58	2.33
SG-AP_1	-CH_3	8.32	1.68	1.38
SG-AP_2	-C_5H_{11}	9.93	1.57	0.98
SG-AP_3	-$C_{11}H_{23}$	15.24	1.62	1.45
SG-AP_4	-$C_{15}H_{31}$	18.33	1.65	1.55
SG-AP_5	-$C_{17}H_{35}$	19.75	1.62	1.59

[a] Concentration of aminopropyl groups in the case of the SG-NH_2 phase or alkylamide groups in the case of the SG-AP phases; the concentration of residual aminopropyl groups in the SG-AP phases is equal to 2.33 - α_a.

alkylamide phases possessed different terminal alkyl chains: -CH_3, -C_5H_{11}, -$C_{11}H_{23}$, -$C_{15}H_{31}$, -$C_{17}H_{35}$, respectively. Physicochemical properties of the resulting phases are summarized in Table 1, whereas the scheme of the modification process is shown in Figure 1.

Column Packing

About 1.2 g of a given phase was added gradually to 35 mL of 2-propanol. The slurry was sonicated in an ultrasonic bath for 5 min and packed in stainless steel columns (60 x 4.6 mm I.D.) using a Haskel (Burbank, CA, USA) model DST-162-52 air driven fluid pump. Prior to packing, the system was pressurized to about 6500 psi. Methanol was used as the carrier solvent.

Physicochemical Measurements

The specific surface area, total pore volume, and average pore width of the LiChrospher Select B silica were determined form low temperature nitrogen adsorption data measured using a Model 1800 Sorptomatic instrument (Carlo Erba, Milan, Italy). The concentration of surface silanols was determined using the method proposed by Nondek and Vyskocil[33] based on the GC determination

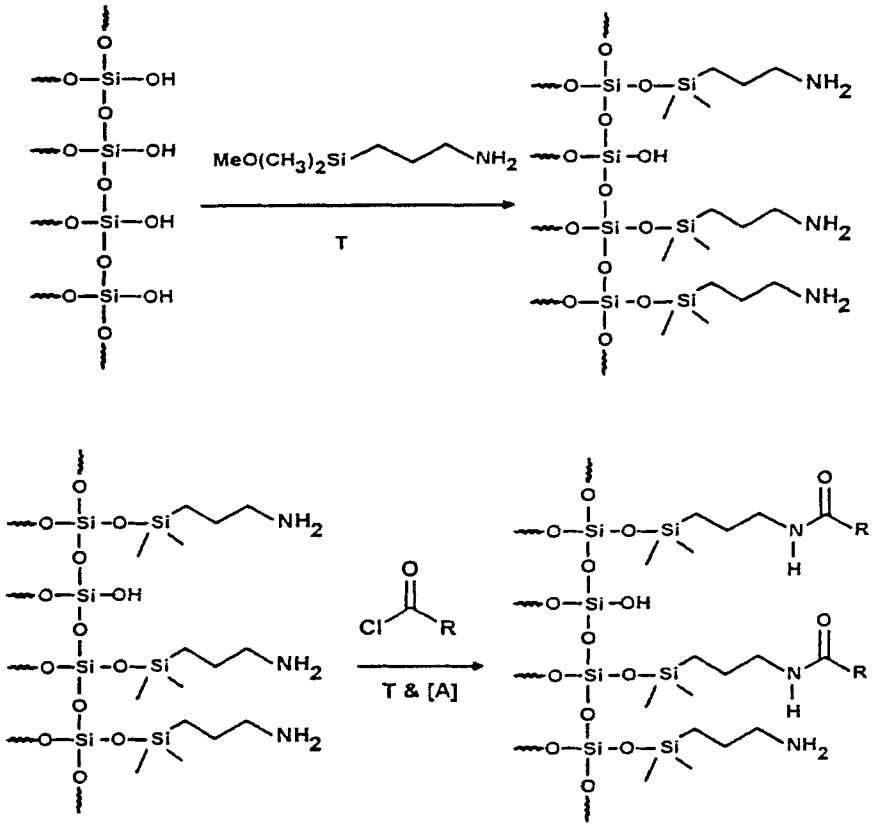

Figure 1. Two-step modification of the silica surface; T denotes heating, [A] denotes activator, and R = $-CH_3$, $-C_5H_{11}$, $-C_{11}H_{23}$, $-C_{15}H_{31}$, $-C_{17}H_{35}$, respectively.

of methane formed during the reaction between dimethylzinc- tetrahydrofurane complex and silanol groups. The surface coverage of bonded ligands was calculated from the carbon (P_C) and nitrogen (P_N) loadings[21] determined by using a Perkin Elmer (Norwalk, CT) Model 240 CHN elemental analyzer.

Solid state NMR measurements, before and after chemical modification, were performed using a Bruker (Rheinstatten, Germany) Model MSL 200 spectrometr. Magic-angle-spinning (MAS) was used at a spinning rate of 4 KHz. ^{29}Si cross-polarization (CP) MAS NMR spectra were recorded with a pulse repetition time of 2 s.

In the case of ^{13}C CP/MAS NMR spectra, a contact time of 12 ms was applied. All NMR spectra were externally referenced to liquid tetramethylsilane. The chemical shifts (L) are given in ppm.

Chromatographic Measurements

HPLC measurements were carried out using a Hewlett Packard (Waldbronn, Germany) Model HP-1050 liquid chromatograph system with a UV detector and a Rheodyne (Berkeley, CA) Model 7125 sampling valve fitted with 20 μL loop. The retention data were recorded and processed using a Hewlett Packard Vectra QS/16S computer with ChemStation. All chromatographic measurements were performed at room temperature using a flow rate of 0.5 cm^3/min. Methanol-water (35 - 65 % v/v) mixture was employed as the mobile phase. The capacity factor (k') values were calculated by utilizing the void volume determined by means of D_2O.

RESULTS AND DISCUSSION

The ratio of aminopropyl bonded ligands (see Table 1) to the surface silanols (about 5.18 μmol/m^2 for the silica studied) indicate that only about 45% of accessible silanols were reacted during the first step of chemical modification (see Figure 1). The second step of modification involved the reaction of suitable alkanoyl chlorides with aminopropyl bonded ligands (see Figure 1) and led to formation of alkylamide ligands. Note, that during the second modification step the concentration of residual surface silanols (about 55%) did not change. The values of the surface coverage, summarized in Table 1, for all alkylamide phases studied indicate that about 60% of aminopropyl ligands, i.e., only about 28% of the initial concentration of surface silanols, were converted to amide ligands. The SG-AP_2 phase was an exception because the conversion was about 40% only. Thus, on average, each alkylamide phase possessed about 55% of residual silanols, 17% of residual aminopropyl ligands, and 28% of alkylamide ligands. It appears that the length of the terminal alkyl chain did not influence the final composition of mixed alkylamide phases.

Shown in Figure 2 are the ^{29}Si CP/MAS NMR spectra for the bare (a) and modified (b) silicas. The spectrum b was recorded for the SG-AP_5 phase. A comparison of both spectra shows that the chemical modification of the silica caused a substantial reduction of the number of geminal (Q_2, δ = -91 ppm) and free (Q_3, δ = -100 ppm) surface silanols. Simultaneously, the number of siloxane groups (Q_4, δ = -108 ppm) increased.

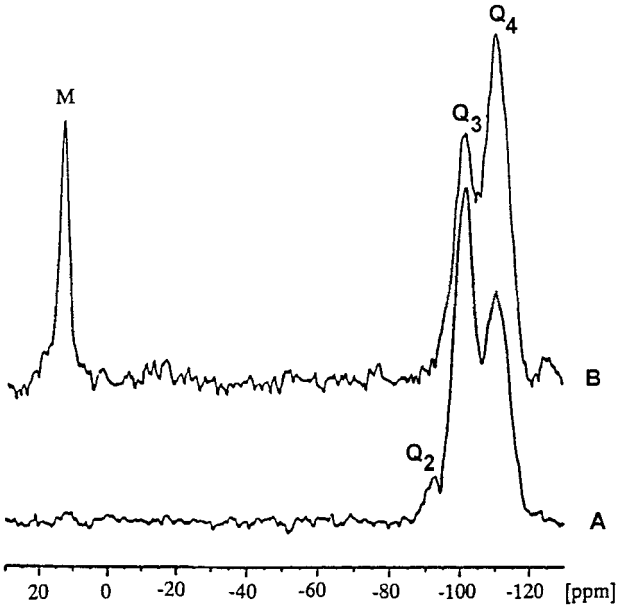

Figure 2. ^{29}Si CP/MAS NMR spectra for LiChrospher Select B silica before (A) and after (B) chemical modification.

In addition, the spectrum b contains the peak M (δ = +14.4 ppm) that corresponds to chemically bonded monofunctional silyl ligands.[12,18] That peak clearly demonstrates the formation of a covalent bonding during attachment of monosilane. An additional confirmation of this bonding was obtained by recording the ^{13}C CP/MAS NMR spectra for the SG-NH_2 and SG-AP_1 phases, which are shown in Figure 3.

As can be seen on both spectra the peak A (δ = -2.5 ppm) corresponds to the formation of the -$Si(CH_3)_2$- bond. The peaks localized in the range of chemical shifts between δ = +10 ppm to δ = +60 ppm correspond to the remaining -CH_2 groups. An additional peak 4 (δ = +174 ppm) is observed on the spectrum for the SG-AP_1 phase and can be related to the carbon atom present in amide group.

Chromatographic properties of the alkylamide phases listed in Table 1 were evaluated on the basis of the RP HPLC data measured for alkylbenzenes and alkylanilines. An exemplary chromatogram is shown in Figure 4 for alkylanilines separated under reversed phase conditions on the SG-AP_5 phase.

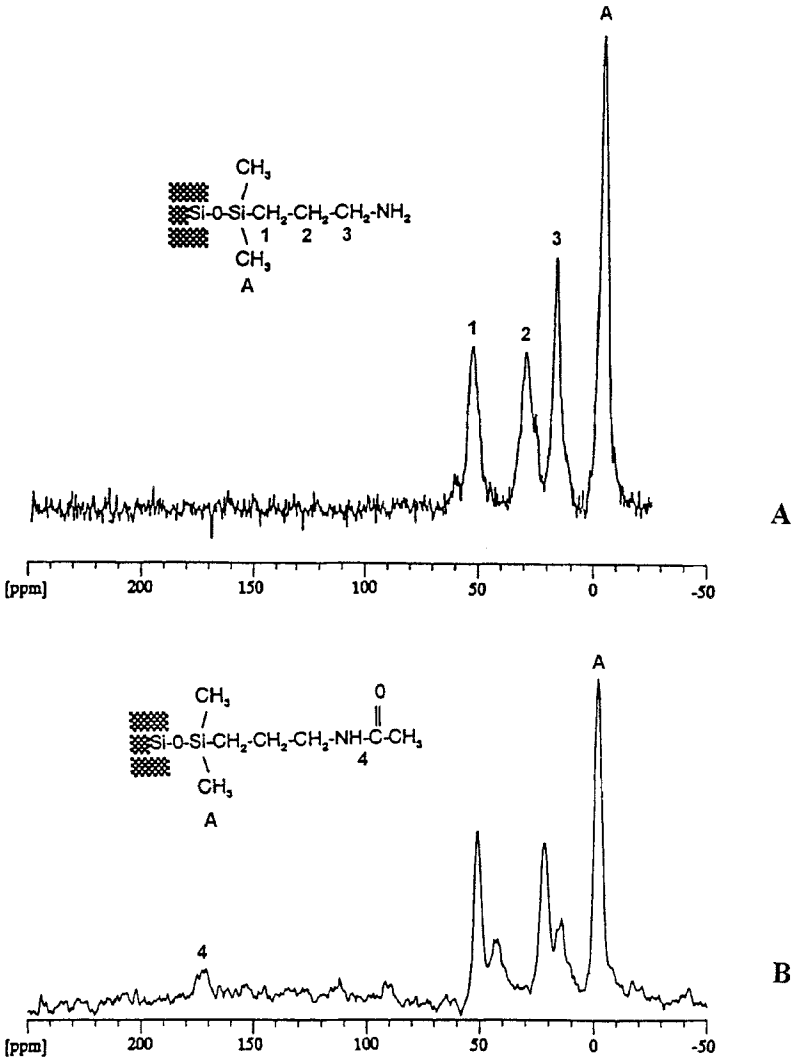

Figure 3. ^{13}C CP/MAS NMR spectra for monomeric SG-NH_2 (A) and SG-AP_1 (B) bonded phases.

Although retention times for alkylanilines are relatively long, the peak symmetry is very good. Also, as can be seen in Figure 5, the retention data measured under reversed phase conditions for homologous compounds on alkylamide phases show normal behavior, i.e., the plots of the logarithm of the capacity factor (log k') on the number of carbon atoms (n_C) in the alkyl chain of

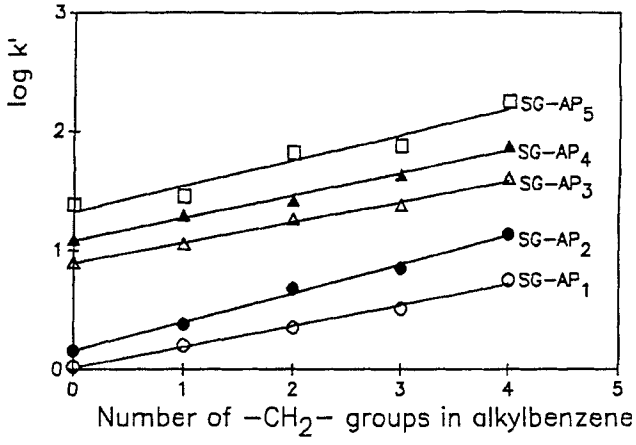

Figure 4. Exemplary chromatographic separation of alkylanilines (s - solvent peak, 1 - aniline, 2 - methylaniline, 3 - dimethylaniline, 4 - diethylaniline) on the SG-AP_i phase; conditions as in Figure 5.

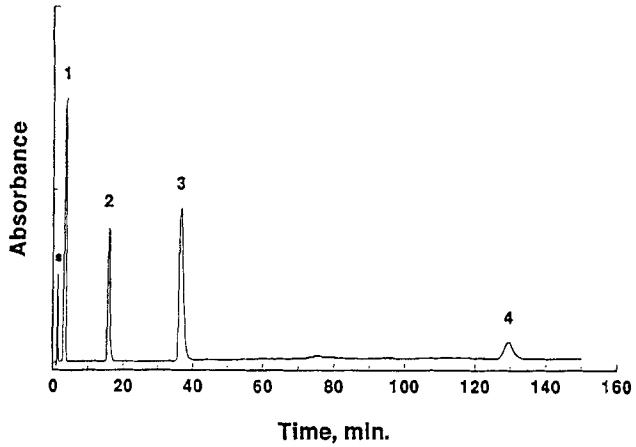

Figure 5. Dependence of log k' *vs.* the number of methylene groups in alkylbenzenes chromatographed using 35% v/v methanol in water and 1 mL/min flow rate.

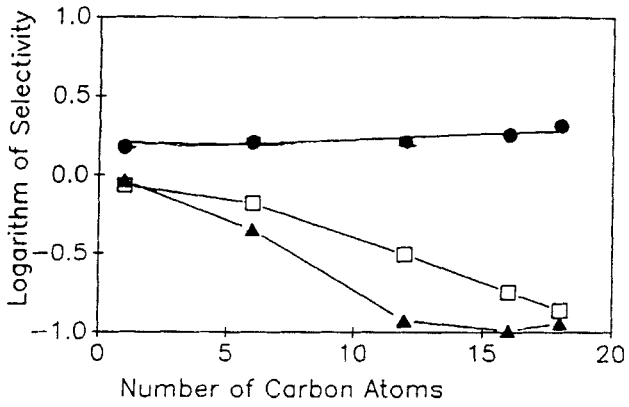

Figure 6. Logarithm of the selectivity *vs.* the number of carbon atoms in the terminal alkyl chain of the alkylamide phases studied; filled circles refer to the methylene selectivity, whereas open squares and filled triangles refer to the hydroxyl and amine selectivities, respectively.

the solute are linear. The slope of each linear plot shown in Figure 5 is equal to the logarithm of the methylene selectivity, which is proportional to the free energy of transfer of the methylene group between the mobile and stationary phases. This quantity is useful to estimate the hydrophobicity of chemically bonded phases because its value increases with increasing contribution of hydrophobic interactions in the stationary phase. Hence, the logarithm of the methylene selectivity increases with increasing number of carbon atoms in the terminal alkyl chain of the bonded ligands.

As can be seen from Figure 6 (see the solid line with filled circles), the methylene selectivity data for the alkylamide phases studied satisfy this condition. Simultaneously, the contribution arising from specific interactions should decrease when the length of the terminal alkyl chain increases.

Shown in Figure 6, plots for the hydroxyl and amine selectivities fulfill this condition too; their values decrease when the length of the terminal alkyl chain increases. The nonspecific and specific selectivity data presented in Figure 6 demonstrate that the length of the terminal alkyl chain in the bonded alkylamide phases plays an important role in controlling the hydrophobicity of these phases as well as their chromatographic performance.

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