Reordering/resolvation studies of alkylamide phases

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ABSTRACT

A series of chemically bonded liquid chromatographic packings containing a specific interaction site localized in the interior region of the attached ligands has been prepared by a two-step synthetic route. In the first-step an amino phase is formed and then it is converted to an alkylamide group via reaction with an acid chloride. Using this procedure materials have been prepared with immobilized terminal alkyl groups with chain lengths ranging from C_5 to C_8. Subsequently, the conformation of these modified surfaces have been investigated as a function of temperature under totally aqueous mobile phase conditions. Plots of ln k' vs. $1/T$ curves have been found to be similar to those reported for alkyl phases. However, the onset of reordering/resolvation of the attached ligands is shifted to lower temperatures and the incremental change per methylene group is smaller.

INTRODUCTION

Separations in reversed-phase liquid chromatography are based on a combination of specific and non-specific interactions which arise between the solute, the eluent and the bound interfacial layer. The dominance of particular interactions over others is controlled by electronic and structural properties of the solute, composition and secondary additives in the mobile phase [1–3], and the structure and dynamics of the bonded layer [1,3–6] as well as experimental variables such as temperature. Although a large volume of experimental data have been collected using reversed-phase systems, many aspects of the mechanisms controlling separations still are not understood clearly, especially in terms of the microscopic properties of the surface and the complex interplay between the bonded ligands, intercalated solvent and underlying silanol groups.

The most often used reversed-phase systems are alkyl-modified silica (*i.e.*, which contain either C_8 or C_{18} ligands) in combination with aqueous binary eluents such as methanol–water and acetonitrile–water [1,2,7]. Thus, a considerable amount of effort has been directed towards characterizing the surface of these packings in terms of the degree of coverage, the influence of alkyl chain length, the effect of structure and heterogeneity of the underlying surface [1,4–6,8] as well as the importance of more complex features such as conformation, organization and dynamics of the interface [1–3,9,10]. Under certain conditions large differences in conformation and organization of the immobilized ligands may be observed [1–6,9–12].

Previously, systematic investigations have been carried out using high to totally aqueous eluents in order to examine the orientation of chemically immobilized alkyl chains [11,13–18]. In these studies various system parameters have been examined such as alkyl chain length [13–21], bonding density [13], attachment chemistry [19], heterogeneity of the silica [20], solute structure [21] as well as temperature and concentration of trace organic modifier present in hydro–organic mobile phases [13–17]. Under

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such conditions non-linear thermal behavior is observed which can be explained on the basis of a two-state model where the alkyl chains assume either a folded or extended configuration depending on the experimental conditions. The initial temperature at which there is a change in the conformation of the bound alkyl chain has been termed the reordering/resolving temperature (T_0). In the current work this same approach has been used to examine a new class of phases which contain an alkyl tail attached to the surface via an alkylamide group.

EXPERIMENTAL

Reagents and materials

The LiChrosorb Si-60 silica, which was used to prepare the chemically bonded phases, was from EM Science (Cherry Hill, NJ, USA) and the remaining reagents used for surface modification, γ-aminopropyltriethoxysilane, pentanoyl chloride, hexanoyl chloride, heptanoyl chloride, octanoyl chloride and morpholine were purchased from Aldrich (Milwaukee, WI, USA). Likewise, the solvents, methanol, acetonitrile, 2-propanol, toluene and n-hexane, were from Aldrich. The remaining chemicals which were used as test solutes, 4-hydroxybiphenol (HBP) and 4-ethylphenol (EP), were obtained from Sigma (St. Louis, MO, USA). The deionized water, was purified in-house using a Millipore (El Paso, TX, USA) Model Milli-Q reagent water system.

Column preparation

Chemically bonded phases were prepared via a two-step synthesis as previously described [4]. In the first step, the silica was placed in a glass reactor, dried under vacuum (10^{-2} Pa) for 12 h, and reacted with γ-aminopropyltriethoxysilane. In the second step, chain lengths ranging from C_5 to C_8 were attached to the initially modified aminopropyl surface by treating it with a given acid chloride [4,22,23]. Subsequently, about 1.0 g of the modified silica was made into a slurry with 30 ml of 2-propanol and placed in a dynamic packing apparatus [24]. The 60 mm × 2.1 mm I.D. stainless-steel columns were packed in upward fashion under a pressure of 50 MPa

using a Haskel (Burbank, CA, USA) Model DSTV-52C air-driven fluid pump and methanol as the delivery solvent.

Equipment

The chromatographic system consisted of a Spectra-Physics (San Jose, CA, USA) Model SP-8810 precision isocratic pump, a Model Spectra 100 variable-wavelength UV detector, set at 254 nm, and a Model Chromjet integrator. Samples were injected using a Rheodyne (Berkeley, CA, USA) Model 7125 valve equipped with 20-µl loop. In all chromatographic experiments the flow-rate was monitored using a Phase Separation (Queensferry, UK) Model F108OA flow meter and the columns were maintained to within ± 0.1 K using a Fisher Scientific (Pittsburgh, PA, USA) Model 9500 Isotemp refrigerated circulator bath.

Thermal studies

All thermal investigations were carried out according to methods described previously [13–20]. In doing this, columns were initially conditioned with 100 ml of acetonitrile followed by an equal volume of water. Subsequently, solute retention (HBP and EP) was measured every 5 K from 278 K to 333 K at a flow-rate of 1 ml/min using water as the eluent (initial evaluation). Following this initial run the column was cooled to the starting temperature and a second set of data collected in a similar fashion (reevaluation).

RESULTS AND DISCUSSION

In the current study, four different alkylamide phases were prepared by a two-step reaction which has been discussed elsewhere [4,22,23]. In the first step, the silica was treated with γ-aminopropyltriethoxysilane in a sealed glass reactor in order to form aminopropyl modified silica which had a coverage of 4.66% carbon and 1.09% nitrogen. Based on the elemental nitrogen results, the bonding density of the attached aminoalkylsilyl groups was 0.88 mmol per gram of reacted silica [25,26]. Further by assuming that the elemental carbon data also should give the same bonding density on an average one residual ethoxy group remained per bonded amino-

alkylsilyl molecule. The presence of residual alkoxy groups following surface modification of silica with trialkoxysilanes have been observed by others [26,27]. Subsequently, materials with C_5, C_6, C_7 and C_8 terminal alkyl groups were prepared by treating the aminoalkyl phase with the corresponding acid chloride. Each of the final phases were examined by solid-state NMR spectrometry [8,12,22,23] as well as elemental analysis. The corresponding coverages for the four different phases are summarized in Table I. The results from the elemental carbon analysis are summarized in the second column and the corresponding calculated bonding densities, assuming no loss of the remaining ethoxy groups in the first-step of the reaction, appear in the third column. Based on these numbers the degree of amidization was statistically equivalent and was approximately 75% complete for the four different phases. However, the overall degree of amidization becomes better if some of the ethoxy groups were lost during the secondary modification (i.e., the ethoxy groups were converted to hydroxyl groups). For example, a 50% loss in the ethoxy groups would result in a 93% reaction yield. Unfortunately, based only on the elemental analysis data it is not possible to determine the exact extent of the amidization reaction. As such, isotopic labelling work is in progress in order to further elucidate the chemical nature of the final alkylamide phases.

Shown in Fig. 1 is a representative plot of ln k' vs. $1/T$ for 4-ethylphenol on the octylamide phase. Similar plots were obtained for the other phases with C_5, C_6 and C_7 terminal alkyl chains.

TABLE I

SURFACE COVERAGE AND ON-SET TEMPERATURE FOR THE ALKYLAMIDE-MODIFIED MATERIALS

R = Length of the terminal alkyl chain; P_c = carbon loading (%); N_A = bonding density (mmol/g); T_0 = mean values determined from multiple injections of the two solutes (K).

R	P_c	N_A	T_0
$-C_5H_{11}$	8.38	0.66	284.0
$-C_6H_{13}$	9.01	0.66	293.7
$-C_7H_{15}$	9.47	0.64	300.2
$-C_8H_{17}$	10.29	0.68	306.8

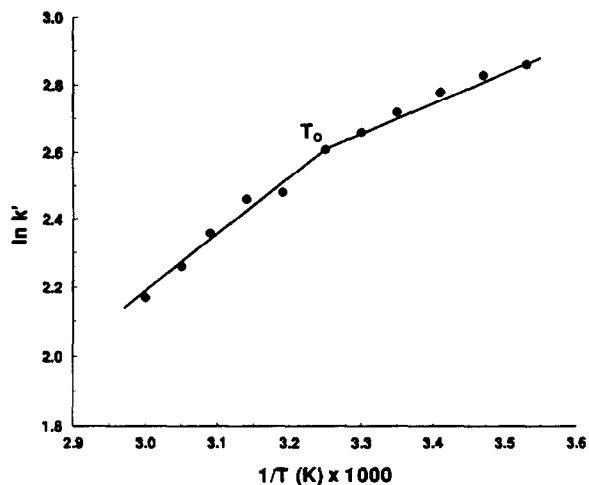Fig. 1. Plot of ln k' vs. $1/T$ for 4-ethylphenol chromatographed on the octylamide phase using neat water as the eluent. Flow-rate: 1 ml/min. The onset temperature for reordering/resolvation is 306.8 K.

In all cases biphasic behavior was observed which is consistent with trends reported in other studies of silica modified with linear alkyl groups [13–20] and phases containing alkyl chains with terminal polar groups [18]. In these earlier studies the biphasic behavior was explained in terms of a major conformational change in the immobilized ligands between a folded aggregated state and an extended solvated state.

Listed in Table I are the mean onset temperatures (T_0) obtained from plots of ln k' vs. $1/T$ for the four different phases prepared in the current study. These values were obtained by fitting the lower and higher temperatures region of the Van 't Hoff plots by linear regression analysis and using the slope and intercept values of the fits to determine the onset temperature. In most cases the regression fits were 0.99 or better. Shown in Fig. 2 is a plot of T_0 vs. carbon number in the terminal alkyl portion of the immobilized ligand. Also shown in the same figure are the error bars for the mean T_0 values which reflect the precision of the measurements made using two different solutes. As has been reported for normal alkyl phases [28], an incremental increase in T_0 was observed per addition of a methylene unit. However, for the current phases the change was smaller (i.e., 7.4 ± 0.7 K per methylene unit)
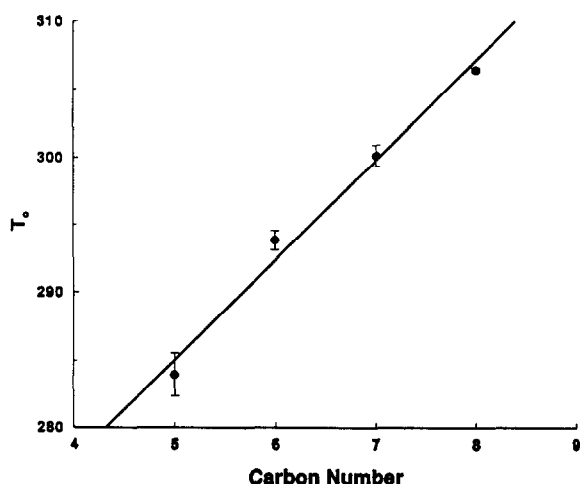
Fig. 2. Plot of T_0 (K) vs. number of carbon atoms in the terminal alkyl chain.

compared to normal hydrocarbon phases (i.e., 9.5 ± 1.1 K per methylene unit). Based on a statistical analysis of the two data sets (i.e., t-test) the values are significantly different at the 95% probability level and presumably arises from variations in structural features of the bonded phases and their influence on surface solvation.

Under totally aqueous conditions, the thermally induced reordering/resolution of bonded hydrophobic ligands has been attributed to a combination of cohesive (chain–chain), hydrophobic (chain–solvent) and specific (solvent–surface) interactions. The non-linear biphasic behavior presented in Fig. 1 and the incremental change in T_0 are consistent with these same ideas except the degree and type of interactions are influenced by differences in bonding as well as the underlying amino groups and the amide groups within the chain.

CONCLUSIONS

The current study further demonstrates that totally aqueous non-linear chromatographic experiments are useful for characterizing the conformational aspects of hydrophobic phases including the new type of alkyl-modified materials based on immobilization via an amide linkage. Currently spectroscopic studies are in progress in

order to characterize further the structural features of the surfaces in an effort to explain the differences in T_0.

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