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Studies of physicochemical and chromatographic properties of mixed amino-alkylamide bonded phases[☆]

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Abstract

Mixed amino-alkylamide chemically bonded phases have been prepared via two-step reaction which involved the initial modification of silica with aminopropyltrimethylsilyl groups under environmentally sealed conditions followed by a secondary derivatization with different acid chlorides (*i.e.*, acetyl, hexanoyl and stearoyl chlorides). In addition, for comparative purposes two conventional C₁₈ phases with different coverage densities have been prepared. Subsequently, the physical properties of the mixed phases have been studied by liquid chromatography as well as by elemental analysis and solid-state nuclear magnetic resonance spectrometry. The resulting data have provided information about how the hydrophobic chains and the specific interaction sites in the bonded phases influence solute retention under reversed-phase conditions. A potentially important application of the mixed amino-alkylamide materials is for separating basic compounds such as amines and amides.

1. Introduction

Chemically bonded phases (CBPs), especially materials with alkyl ligands, are important in various areas of analytical chemistry including high-performance liquid chromatography (HPLC), filtration, solid-phase extraction, etc. [1–4]. Their general use and popularity are due to their hydrophobic character as well as good chemical, mechanical and thermal stabilities [3,5–8]. In addition, many analytical separations

require more selective phases which contain simple polar functionalities such as amino and nitrile groups [1–4,8–12] to more complex chiral selective materials such as immobilized cyclodextrins, proteins and Pirkle-type phases [1,2,8,13–15].

Recently, the preparation of CBPs with a specific interaction site localized in the interior region of hydrophobic ligands (*i.e.*, bonded alkylamide groups) has been described [3,16,17]. In preliminary studies, the packings were found to be useful for separating polar compounds, particularly, those which contain basic functionalities [3,16,17]. Under such conditions enhanced peak symmetry was obtained compared to more conventional alkyl-bonded phases. A possible explanation for this enhanced behavior is via an internal masking mechanism [18].

In the current work mixed amino-alkylamide

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phases have been synthesized and their chromatographic properties evaluated. Emphasis has been placed on developing a better understanding of how changes to and the interplay between the terminal alkyl groups, the specific interaction site in the bonded alkylamide ligands, and the underlying amino groups used for attachment, influence the retention and separation of various solutes. In doing this, alkylbenzene and alkylniline homologues, as well as phenol and nitrobenzene have been chromatographed as a function of temperature under reversed-phase conditions using mixed hydro-organic eluents. For reference purposes comparative measurements have been carried out on two conventional C₁₈ phases with low and high coverage densities. In addition to the chromatographic studies, the surface properties of the mixed amino-alkylamide phases have been characterized by solid-state nuclear magnetic resonance spectrometry (NMR).

2. Experimental

2.1. Materials

The two types of 5- μm spherical silica used to prepare the bonded phases were Separon SGX (Tessek, Prague, Czech Republic) and LiChrospher Select B (SB; E. Merck, Darmstadt, Germany). These materials had nitrogen BET surface areas of 347 and 570 m²/g, respectively.

The surface modification reagents, octadecyldimethylchlorosilane and γ -aminopropyltrimethylmethoxysilane, were from Huls (Bristol, PA, USA) and the acetyl, hexanoyl and stearoyl chlorides as well as morpholine were from Aldrich (Milwaukee, WI, USA). The remaining chemicals used in the synthetic procedure, methanol, toluene and *n*-hexane (reagent grade) were also purchased from Aldrich.

The chromatographic solvents, methanol and acetonitrile (HPLC grade) were from Fisher Scientific (Fairlawn, NJ, USA) and the deionized water was purified in the laboratory using a Millipore (El Paso, TX, USA) Milli-Q reagent water system.

2.2. Synthesis

The preparation of the two C₁₈ phases were as follows. A 3-g amount of silica was placed in an environmentally isolated glass reactor [3] and then heated to 185°C under vacuum (10⁻² Pa) for 10 h. In addition, thermogravimetric studies of the silica sample showed that its heating up to 185°C primarily eliminates adsorbed water and does not affect significantly the surface silanols. Materials with high (^hC₁₈) and low (^lC₁₈) density coverages were prepared by varying the molar ratio of the octadecyldimethylchlorosilane and morpholine [3,20].

Similarly the mixed amino-alkylamide phases were synthesized in a sealed glass reactor by a two-step process [17], in which an initial aminopropyltrimethylsilyl phase was prepared under environmentally isolated conditions [3,11] and subsequently reacted with either acetyl, hexanoyl or stearoyl chlorides [3,17,18].

2.3. Spectroscopic and elemental analysis

The ²⁹Si and ¹³C cross-polarization magic-angle-spinning (CP-MAS) NMR spectra were obtained by using a General Electric (Fremont, CA, USA) Model GN-300 spectrometer and acquisition parameters similar to those reported previously [17]. The elemental analysis was performed on a Perkin-Elmer (Norwalk, CT, USA) Model 240B CHN analyzer and these results used to calculate the coverage density of the groups bonded to the surface during the silanization reactions [*i.e.*, for the octadecyl (α_{RP}) and the amino (α_{NH_2}) phases] using Eq. 1 and the subsequent degree of amidization in the case of the mixed amino-alkylamide surfaces (α_{AA}) using Eq. 2.

$$\alpha_{\text{RP}} \text{ or } \alpha_{\text{NH}_2} \text{ (mol/m}^2\text{)} = \left[\frac{P_c}{1200n_{c(1)} - P_c(M_1 - 1)} \right] / S_{\text{BET}} \quad (1)$$

$$\alpha_{\text{AA}} \text{ (mol/m}^2\text{)} = \left[\frac{P_{c(\text{AA})} + P_{c(\text{AA})}N_1(M_1 - 1) - 1200N_1n_{c(1)}}{1200n_{c(2)} - P_{c(\text{AA})}(M_2 - 1)} \right] / S_{\text{BET}} \quad (2)$$

where P_c = carbon percentage for the bonded phase prepared via one-step reaction (e.g., phases with octadecyl or amino ligands); $P_{c(AA)}$ = carbon percentage for the mixed amino-alkylamide phase; N_1 = concentration of the ligands bonded in the initial silanization; in the current work N_1 denotes the concentration of amino ligands in mol/g, which can be calculated from the carbon or nitrogen percentages for the NH_2 phase according to Eq. 1 (cf. Table 1); $n_{c(1)}$ = number of carbon atoms in the bonded ligand from the initial silanization; $n_{c(2)}$ = number of carbon atoms added in the second-step amidization reaction; M_1 = molecular mass of the bonded ligand from the initial silanization; M_2 = molecular mass of the added group in the second-step amidization reaction; and S_{BET} = BET specific surface area in m^2/g .

The coverage density of unreacted aminopropyl groups following the second-step amidization was calculated by subtracting the concentration of alkylamide ligands from the initial concentration of aminopropyl dimethylsilyl groups calculated for the NH_2 phase.

2.4. Chromatographic studies

Chromatographic measurements were carried out on an IBM (Danbury, CT, USA) Model 9560 ternary gradient liquid chromatograph equipped with an LDC (Riviera Beach, FL, USA) Model III UV detector set at 254 nm, and a Rheodyne (Berkeley, CA, USA) Model 7100

sampling valve with 10- μ l injection loop. The eluent was pre-equilibrated to the correct temperature before it reached the column. The column temperature was controlled to ± 0.1 K in a Hotpack (Philadelphia, PA, USA) refrigerated water bath. The flow-rate of the mobile phase was monitored with a Phase Separations (Queensferry, UK) Model FLOSOA1 flow meter connected to the detector outlet.

2.5. Column packing

About 1.2 or 2.2 g of the modified silicas were packed, respectively, into 60 mm \times 4.6 mm I.D. or 125 mm \times 4.6 mm I.D. stainless-steel column blanks which were purchased from Supelco (Bellefonte, PA, USA). Prior to packing a slurry was prepared using 35 ml of 2-propanol and sonicating the mixture for 5 min. Subsequently, the columns were packed using a Haskel (Burbank, CA, USA) Model DST-52 pump at a pressure of 50 MPa and methanol as the carrier solvent.

3. Results and discussion

The non-specific and specific interactions which occur in the stationary layer of chemically bonded phases are a complex function of the residual silanols, the bonded ligands, and intercalated solvent molecules. Various approaches have been used to characterize these including

Table 1
Surface coverages of the octadecyl and mixed amino-alkylamide monomeric phases studied

| Silica support | Phase | n_c | P_c | P_N | α_{RP} | α_{NH_2} |
|----------------|------------|-------|-------|-------|---------------|-----------------|
| Separon SGX | $^hC_{18}$ | 20 | 23.4 | — | 4.03 | — |
| LiChrospher SB | $^lC_{18}$ | 20 | 23.2 | — | 2.42 | — |
| | NH_2 | 5 | 6.9 | 1.6 | — | 2.33 |
| | AA_1 | 7 | 8.3 | 1.7 | 1.38 | 0.96 |
| | AA_2 | 12 | 9.9 | 1.6 | 0.98 | 1.35 |
| | AA_3 | 24 | 19.7 | 1.6 | 1.59 | 0.74 |

n_c = Total number of carbon atoms in the bonded ligands; α_{RP} and α_{NH_2} denote the surface concentrations (in $\mu\text{mol}/m^2$) of the alkyl-ended and amino ligands, respectively. P_N = nitrogen percentage for a chemically bonded phase.

the measurement of $\ln k'$ vs. $1/T$ dependencies as well as variations in these relationships as a function of solvent composition [21–26]. Such procedures have been used in the current work in order to examine how differences in mixed amino-alkylamide phases effect both the specific and non-specific interactions of the solute molecule with the surface layer, as well as, the interaction of the immobilized amine and amide groups with the underlying residual silanols.

Listed in Table 1 are the coverage densities for three mixed amino-alkylamide phases with differing terminal chain lengths, the initial amino phase used to prepare these materials, and for comparative purposes two conventional C_{18} phases. As has been reported elsewhere [3,17,18], the amino-alkylamide phases were prepared via two-step synthesis which involved an initial silanization reaction using 3-aminopropyltrimethoxysilane (NH_2) followed by a secondary amidization using either acetyl chloride (AA_1), hexanoyl chloride (AA_2) or stearyl chloride (AA_3). During the initial silanization more than 70% of the accessible silanols were converted to aminopropyltrimethylsilyl groups. In the subsequent step the extent of conversion of these groups to the alkylamide moiety ranged from approximately 40% for the AA_2 phase to about 60–70% for the AA_1 and AA_3 phases. Based on a statistical analysis of the nitrogen data listed in Table 1 the overall variations in the estimated coverages are approximately 10%. Thus, the small differences in alkylamide coverage (α_{AA}) for the AA_1 and AA_3 phases are within expected statistical variations. However, in contrast to these phases, the AA_2 material contained more unreacted aminopropyl groups (*i.e.*, see the α_{NH_2} values in Table 1). Based on reactivity and steric considerations these latter results are unexpected and presumably reflect experimental differences in the reaction conditions used [18,19].

For both C_{18} phases the total amount of bound carbon was greater than 23%. However, the estimated surface densities (*i.e.*, the α_{RP} values in Table 1) which are based on the specific surface area of the unmodified silicas, were significantly different (*i.e.*, $4.03 \mu\text{mol}/\text{m}^2$ for the

Separon SGX material compared to $2.42 \mu\text{mol}/\text{m}^2$ for the LiChrospher SB material). The density of the Separon material was about 1.7 times that of the LiChrospher material. It should be noted that although the coverage density depends on the synthetic procedure, it also is controlled by the nature of the native silica in terms of its porosity, specific surface area, type and distribution of surface silanols, etc. [1,3]. The surface area of the Separon SGX silica is smaller than that of the LiChrospher SB silica; however, it has been reported to contain a greater density of free silanols accessible for modification [3]. Thus, the lower coverage densities of both the C_{18} and NH_2 which are statistically similar (*i.e.*, within 4% of each other) are consistent with reported reactivity differences of the native silicas [3].

For illustrative purposes a ^{13}C CP-MAS NMR spectrum for the AA_1 phase is shown in Fig. 1. The spectrum is consistent with previously published work [3,17,18] and contains six resonances at $\delta = -2.5, 15.9, 21.5, 43.4, 50.9$ and 171.9 ppm which are due to the dimethylsilyl, C1, C2, C5, C3 and C4 carbons. Spectra for the AA_2 and AA_3 phases contained additional resonances for the interior chain carbons and they were similar to those published elsewhere [3,17–19].

Variations in solute retention as a function of temperature were measured for benzene, toluene, aniline, nitrobenzene and phenol using binary mixtures of methanol–water (40:60, v/v) as mobile phases over a range from 263 to 313 K. These measurements were made in order to evaluate the physicochemical properties of the

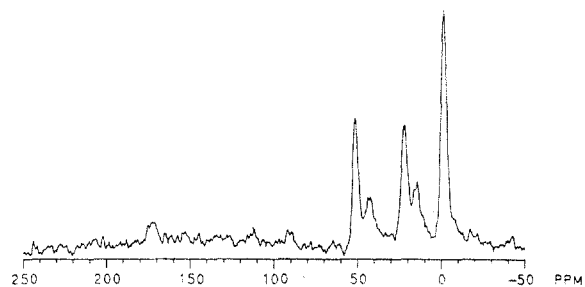


Fig. 1. ^{13}C CP-MAS NMR spectrum for the monomeric AA_1 phase.

three mixed AA materials vs. those of conventional C₁₈ phases. Shown in Fig. 2 are the experimental dependencies of $\ln k'$ vs. $1/T$ in K for the solutes chromatographed on the AA₂, AA₃ and ¹C₁₈ phases. Since almost all of the

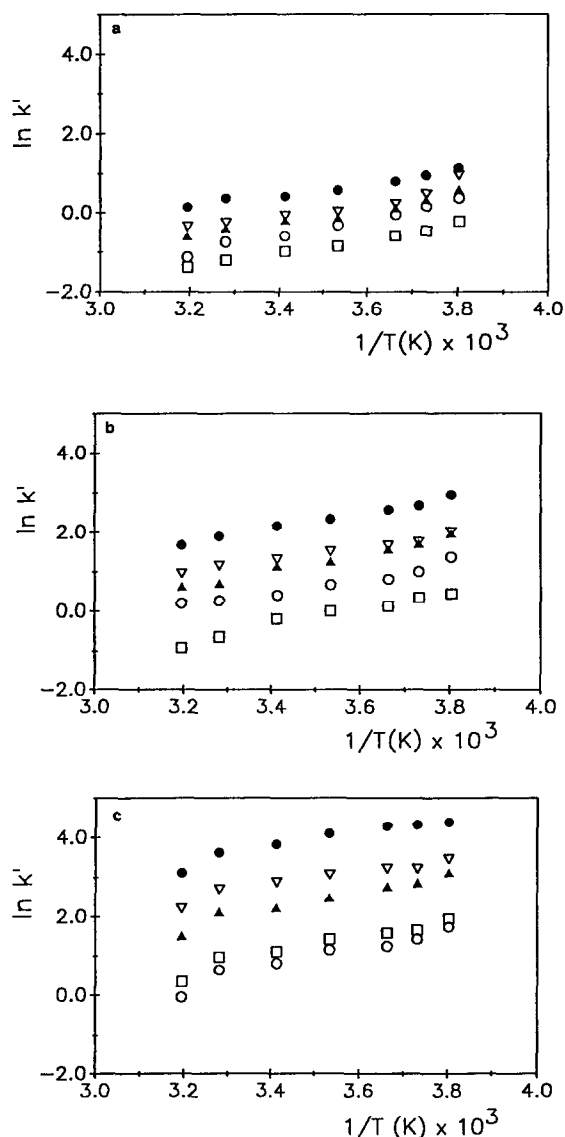


Fig. 2. Linear dependencies of the $\ln k'$ on the inverse temperature ($1/T$) measured for phenol (\square), aniline (\circ), nitrobenzene (\blacktriangle), benzene (\triangle) and toluene (\bullet) on the columns with the AA₂ (a), AA₃ (b) and ¹C₁₈ (c) packings using the methanol–water (40:60, v/v) mobile phase. Chromatographic conditions: flow-rate 1 ml/min and UV detection at 254 nm.

capacity factors for the solutes on the AA₁ phase were smaller than unity, they were not considered to be accurate enough to be used in further calculations.

A comparison of the dependencies presented in Fig. 2 shows that the values of k' obtained on the ¹C₁₈ phase are higher than those on the amino-alkylamide phases and that they decrease with decreasing length of terminal alkyl portion for the amino-alkylamide phases. Further, the data in Fig. 2 demonstrate that while the elution order for toluene, benzene and nitrobenzene was the same for the ¹C₁₈ and amino-alkylamide packings, this was not the case for phenol and aniline. Phenol was retained longer on the ¹C₁₈ column than aniline, however, on the amino-alkylamide material the elution order was reversed (cf. Fig. 2b and c).

Another feature of the dependencies shown in Fig. 2 are their significant deviations from linearity at lower temperatures which appears to be associated with degrading peak shapes. For all phases, the peak asymmetry increased with decreasing temperature. These same trends were also observed when acetonitrile–water was used as the eluent as is illustrated later. This was especially apparent for both of the conventional octadecyl materials. At temperatures below 269 K the peaks merged into highly convoluted irregular shaped profiles. However, in the case of the mixed amino-alkylamide phases, even at the lowest temperature studied, 263 K, the elution profiles although asymmetrical were less convoluted.

Enthalpy values (ΔH) were estimated on the basis of the linear regions of the $\ln k'$ vs. $1/T$ dependencies (i.e., from 273 to 305 K) as illustrated in Fig. 3. This was done for the test solutes as a function of eluent composition using Eq. 3 [23,24]:

$$\ln k' = \frac{\Delta S}{R} + \ln \phi - \frac{\Delta H}{RT} \quad (3)$$

where ϕ is the phase ratio. For the octadecyl phase, the values of ΔH were between -14 and -15 kJ/mol for aniline, phenol and nitrobenzene compared to approximately -13 kJ/mol for toluene and -10 kJ/mol for benzene. The slight-

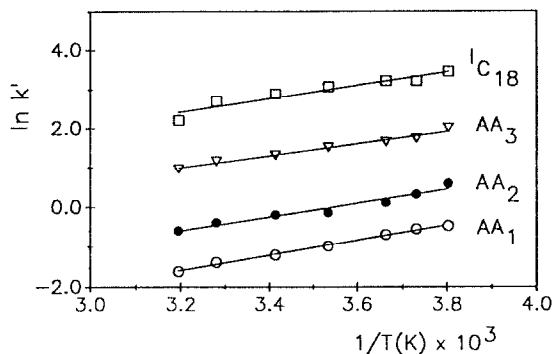


Fig. 3. Comparison of the linear dependencies of $\ln k'$ on the inverse temperature ($1/T$) for benzene on the amino-alkylamide and ${}^1C_{18}$ phases listed in Table 1. Chromatographic conditions as in Fig. 2.

ly larger heats of adsorption for the aromatic solutes containing polar substituents are consistent with increased specific interactions of the polar substituents with the residual silanols. Assuming an initial silanol concentration of $5.2 \mu\text{mol}/\text{m}^2$ for unmodified LiChrospher SB [3,20], the concentration of residual surface silanols on the ${}^1C_{18}$ material after modification was about $2.8 \mu\text{mol}/\text{m}^2$. Similarly, for the amino surface (*i.e.*, the NH_2 phase), as well as for the mixed amino-alkylamide materials the residual silanols were $2.8 \mu\text{mol}/\text{m}^2$. These values were obtained by subtracting the concentration of bonded groups given in Table 1 from $5.2 \mu\text{mol}/\text{m}^2$. In the case of the mixed amino-alkylamide phases, only the aminopropyltrimethylsilyl ligands reacted with the acid chlorides and thus the number of remaining silanols was identical to the starting amino phase. In addition, the mixed amino-alkylamide phases contained unreacted aminopropyltrimethylsilyl groups, as well as alkylamide groups. The concentration of each on the three mixed amino-alkylamide materials is given in Table 1. Thus, the chromatographic properties of the mixed amino-alkylamide materials should reflect interactions of the solute with the residual silanols, the unmodified amino groups, and the alkylamide ligands. However, since the number of residual silanols was the same for all of the phases, it seems reasonable to assume that the observed differences in retention behavior should be attributable to differences in

the concentration of amino and alkylamide groups. Unfortunately, a direct comparison between the phases is complicated by the fact that the midrange AA_2 phase contained a different degree of amidization than the AA_1 and AA_3 materials. Although reproducibility of the amino-alkylamide phases under controlled conditions is good, further studies need to be done in order to specify experimental conditions for achieving the same degree of amidization for various alkylamide phases. Thus, the current data are limited in terms of developing a detailed description of the mechanisms controlling separation on the mixed amino-alkylamide surface. However, they are significant in that they demonstrated improved symmetry for polar solutes on the new mixed phases compared to more conventional alkyl type materials, *e.g.*, the values of the asymmetry factor for aniline on the ${}^1C_{18}$ and AA_3 phases were, respectively, 1.1 and 1.0. This improved performance is believed to be due to amino-silanol and amide-silanol interactions which act to mask or attenuate unwanted solute-silanol interactions.

Although large variations in solute retention were observed between the ${}^1C_{18}$ and AA_3 phases, differences in ΔH were not as significant. These trends suggest that the dominant interactions on both materials were hydrophobic and that the reduced retention volumes on the mixed amino-octadecylamide phase were due to the reduced density of the terminal alkyl groups. However, differences in the underlying polar groups between the AA_3 and conventional ${}^1C_{18}$ materials were manifested in the observed variations in solute selectivity for the polar solutes.

The chromatographic properties of the ${}^1C_{18}$ and AA_3 phases are compared further in Fig. 4 which contains plots of $\ln \alpha$ vs. $1/T$. In each case, the selectivity values were calculated using benzene as the reference solute. The line for toluene/benzene, which lies above zero because toluene was retained longer on both phases than benzene, provides information about the non-specific (methylene) selectivity of the phases. The relative magnitude of the data are consistent with the reduced alkyl density on the AA_3 phase compared to the ${}^1C_{18}$ phase. The remaining α -

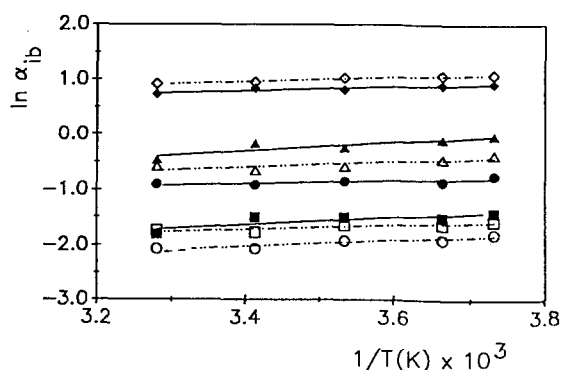


Fig. 4. Comparison of the temperature-dependent selectivities for the following pairs: aniline/benzene (\square , \blacksquare); phenol/benzene (\circ , \bullet); nitrobenzene/benzene (\triangle , \blacktriangle) and toluene/benzene (\diamond , \blacklozenge) on the ${}^1C_{18}$ (dashed line with open symbols) and AA_3 (solid line with filled symbols) phases; α_{ib} denotes selectivity for the i -th solute with respect to benzene.

plots in Fig. 4 lie below zero because benzene was retained longer on the ${}^1C_{18}$ and AA_3 phases than either phenol, aniline or nitrobenzene. These latter plots clearly demonstrate differences in the chromatographic behavior between the ${}^1C_{18}$ and AA_3 phases with respect to some of the polar compounds. This is illustrated by comparing the selectivity plots for aniline/benzene on the ${}^1C_{18}$ and AA_3 phases, which are similar to the lines for phenol/benzene, which are separated to a significant degree indicating that the OH group interacts differentially with the ${}^1C_{18}$ and AA_3 phases. Similarly the same effect was observed for the NO_2 group, but to a smaller degree.

In addition to the above studies, the hydrophobic interactions on the ${}^hC_{18}$, ${}^1C_{18}$ and AA_3 phases were examined further by measuring the methylene selectivity for alkylbenzene homologues using methanol–water (60:40) as the mobile phase. Shown in Fig. 5 is a plot of $\ln k'$ vs. the number of carbon atoms in the terminal alkyl portion of the mixed amino-alkylamide phases. The slopes of these plots (*i.e.*, methylene selectivity) for both the ${}^1C_{18}$ and AA_3 phases are similar and equal to about 0.57 which is also consistent with the idea that when the dominant mechanism controlling retention is based on hydrophobic interactions both materials behave similarly.

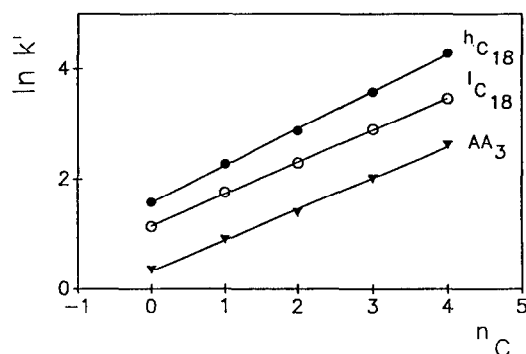


Fig. 5. Linear relationships between $\ln k'$ and the number of methylene groups (n_C) in alkylbenzenes measured on the ${}^1C_{18}$, ${}^hC_{18}$ and AA_3 packings (*cf.* Table 1) using the methanol–water (60:40, v/v) mobile phase. Other chromatographic parameters as in Fig. 2.

In addition to the above measurements, the ${}^hC_{18}$, ${}^1C_{18}$ and AA_3 phases were evaluated using acetonitrile–water (60:40) as the mobile phase. Shown in Fig. 6 are plots of $\ln k'$ vs. $1/T$ for benzene over a temperature range from 263 to 333 K. A cursory look at these data would seem to indicate that linearity was observed over a narrower range for the high-density octadecyl phase (293–323 K) compared to either the lower-density octadecyl or the AA_3 phases. As noted previously, a significant feature of the lower-temperature measurements were dramatic changes in peak symmetry and shape as illustrated in Fig. 7. Although these differences may

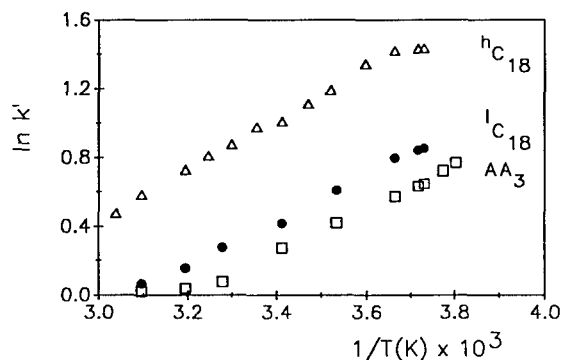


Fig. 6. The $\ln k'$ vs. $1/T$ plots for benzene measured on the AA_3 and C_{18} packings (*cf.* Table 1) using the acetonitrile–water (60:40, v/v) mobile phase. Chromatographic conditions as in Fig. 2.

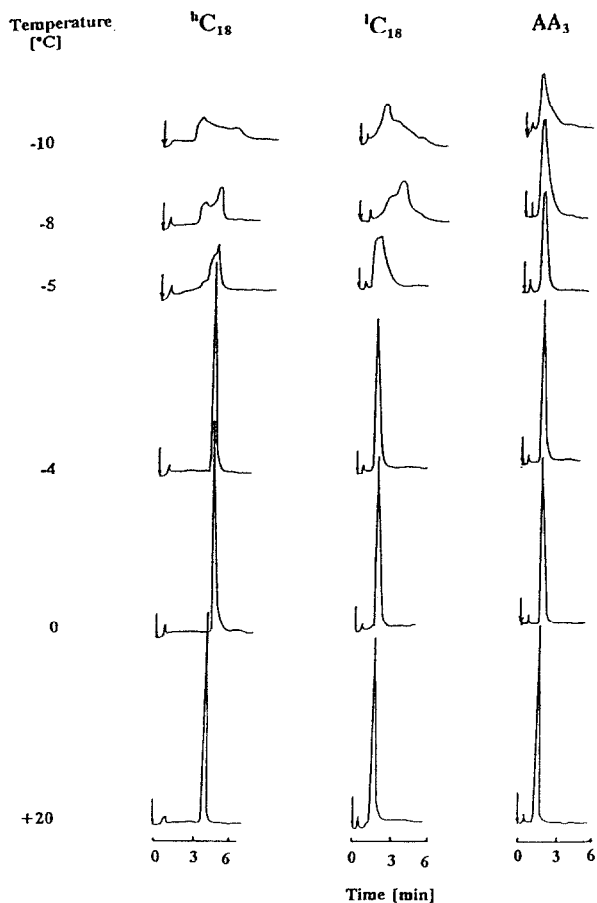


Fig. 7. Chromatograms for benzene recorded at different temperatures on the $^{13}\text{C}_{18}$, $^{12}\text{C}_{18}$ and AA_3 phases. Chromatographic conditions as in Fig. 6. The injected amount of benzene was equal to 40 ng.

be significant [27–29], based on the limited data obtained in the current study, it does not seem prudent to extract too much from them at the present time. However, additional work is now in progress to further elucidate the non-linear behavior and degradation of peak shape observed at the lower temperatures studied. The higher-temperature regions of the data shown in Fig. 6 were linear with slopes consistent with the methanol–water studies discussed above.

4. Conclusions

Comparative studies of mixed amino-alkylamide and conventional C_{18} bonded phases

have shown that the specific interaction sites in the amino-alkylamide phases have a significant influence on solute retention and selectivity under reversed-phase conditions. In addition, improved peak symmetry was observed for polar solutes such as amines which makes these phases attractive for carrying separation of base type solutes. Systematic studies are now in progress to examine how differences in amidization influence solute retention. In addition, spectrometric investigations are being carried out in order to examine possible ligand–silanol interactions and their influence on peak symmetry.

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