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Comparative studies of chromatographic properties of silica-based amide-bonded phases under hydro–organic conditions

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Abstract

The basic chromatographic properties of silica-based amide-bonded phases possessing unsubstituted or substituted aromatic rings were evaluated using retention measurements carried out for simple probe molecules such as alkylbenzenes and aromatic hydrocarbons. An octylamide phase, the chromatographic properties of which have been investigated previously, was used as a reference material in the current work. © 1998 Elsevier Science B.V.

Keywords: Stationary phases, LC; Alkylbenzenes; Hydrocarbons, aromatic

1. Introduction

Surface modified porous inorganic oxides are used as separation media in high-performance liquid chromatography (HPLC) under hydro–organic conditions, i.e. reversed-phase HPLC. In particular, silica is very popular because its surface properties can be tailored with a variety of ligands resulting in bonded phases of good mechanical, thermal and chemical stability [1–3]. Silicas modified with alkyl groups (e.g., octyl and octadecyl) are probably the most widely used chromatographic packings. However, alkyl-bonded phases can also have some drawbacks. For example, these materials are generally not compatible with mobile phases having a high water content.

Recently, bonded phases containing amide functional groups have become popular column packings in reversed-phase HPLC [4–7]. These phases can be employed even under fully aqueous conditions [7]. Moreover, the presence of the specific interaction site, i.e. amide functional group, can be advantageous for certain types of separations. For example, amide bonded phases have proven to be very useful for separations of polar compounds containing basic functionalities [6,8–10]. Much physicochemical characterization of amide-bonded phases to-date has been performed using alkylamide phases prepared by a two-step process [4,6,7]. The amide bonded phases prepared in such fashion are actually mixed amine–amide phases [6] because a quantitative conversion of amine groups to amides is usually difficult to achieve [11]. However, if desired, a fraction of the residual amines can be ‘‘removed’’ by reaction with acetyl chloride [10]. It is possible to avoid residual amine groups entirely by performing the surface modification in a single step [11–13], where this approach involves the preparation of the appropriate silane [11]. For example, an alkylcarbamate phase was synthesized by this method [13], which contained bonded ligands nearly identical to alkylamide ligands with carbamate functional groups replacing amide groups.

In the current work, amide-bonded phases con-

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taining unsubstituted or substituted aromatic rings (i.e. phenylamide, cyanophenylamide and phenylacetylamide phases), prepared by the two-step procedure [14], were studied under hydro–organic conditions using simple probe molecules such as alkylbenzenes and aromatic hydrocarbons. The octylamide phase [15], also synthesized via the two-step process, was used for comparative purposes. The main objective of the current work is the physicochemical characterization of amide-bonded phases containing aromatic rings as potential chromatographic packings, and not the optimization of some specific chromatographic separations. It is shown that the solute retention is influenced by the presence of methylene and nitrile groups in the bonded ligands. In addition, the stability of the bonded phases under hydro–organic conditions was evaluated.

2. Experimental

2.1. Materials

LiChrospher Si-100 silica, which had a 10 μm average particle diameter and a BET surface area of about 350 m^2/g was purchased from EM Separations (Gibbstown, NJ, USA). The γ-aminopropyltriethoxysilane and the acid chlorides (i.e. benzoyl, 4-cyanobenzoyl, phenylacetyl, octanoyl) were from Aldrich (Milwaukee, WI, USA). Toluene was purchased from Fisher Scientific (Pittsburgh, PA, USA) and isopropyl ether was from Aldrich. All materials were used as received.

Chromatographic solvent methanol (HPLC grade) was from Fisher and the deionized water was purified in the laboratory using an Ionpure Plus 150 (Lowell, MA, USA) reagent water system.

2.2. Bonded phase synthesis

The synthesis of the aminopropyl-bonded phase was carried out according to a procedure similar to that described by Engelhardt and Orth [16]. Prior to the reaction approximately 5 g of silica was washed with deionized water and dried at 393 K for 20 h. Subsequently, the silica was dispersed in water-saturated toluene and refluxed for 17 h in the presence of 20 ml of γ-aminopropyltriethoxysilane. The aminopropyl-bonded phase was washed with dry toluene and deionized water and dried at 393 K for 24 h. Two separate preparations of aminopropyl silica were made and verified to be nearly identical on the basis of elemental analysis and thermogravimetric measurements. Fig. 1 compares the thermogravimetric mass-loss curves for the two preparations of aminopropyl silica (denoted as 1 and 2), washed with toluene followed by water. It is evident that the water treatment, which serves to remove physically adsorbed silane and/or its hydrolysis products is necessary for obtaining the reproducible, well-defined aminopropyl silica [14,16,17].

The amide-bonded phases were prepared by reacting aminopropyl silica with benzoyl, 4-cyanobenzoyl, phenylacetyl or octanoyl chloride according to the procedures similar to that reported previously [4], where the same sample of aminopropyl silica was used to prepare all amide phases under study. The aminopropyl-bonded phase was refluxed for 4 h with an isopropyl ether solution containing an excess of the appropriate acid chloride. The resulting amide bonded phases were washed with isopropyl ether, methanol–water (1:1, v/v) mixture and pure methanol and subsequently dried under vacuum at ambient

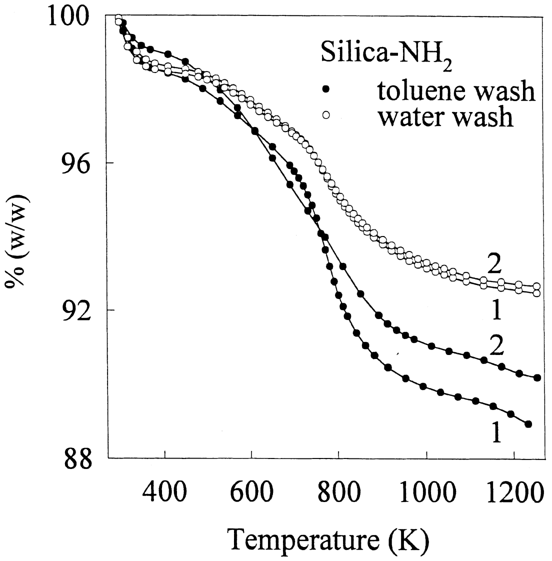


Fig. 1. Comparison of thermogravimetric mass-loss curves for two replicate preparations of aminopropyl silica (denoted as 1 and 2) washed with toluene followed by water.

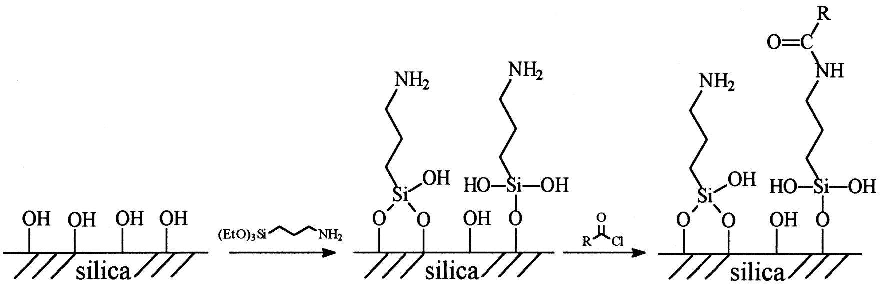


Fig. 2. Schematic representation of the modification of the silica surface.

temperature for 40 h. The materials were placed in brown bottles and stored in the desiccator prior to chromatographic measurements. Shown in Fig. 2 is the schematic representation of the step-wise modification of the silica surface.

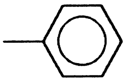
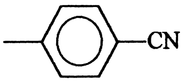
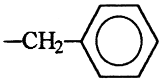
The surface and structural, i.e. porous, properties of all materials under study were previously evaluated by nitrogen adsorption at 77 K, high resolution thermogravimetry and elemental analysis [14]. Shown in Table 1 are the surface properties of the modified silicas. The surface coverages (per m^2 of unmodified silica) of aminopropyl ligands (α_{NH_2}) bonded to the surface and amide groups (α_{amide}) attached in the second synthesis step, were estimated from the carbon contents of the materials using Eqs. (1) and (2), respectively [6]:

$$\alpha_{\mathrm{NH}_2}\,(\mathrm{mol/m^2}) = \left[\frac{P_{\mathrm{C}(1)}}{1200n_{\mathrm{C}(1)} - P_{\mathrm{C}(1)}(M_1 - 1)}\right]/S_{\mathrm{BET}} \qquad (1)$$

$$\alpha_{\mathrm{amide}}(\mathrm{mol/m^2}) = \left[\frac{P_{\mathrm{C}(2)} + P_{\mathrm{C}(2)}N_1(M_1 - 1) - 1200N_1n_{\mathrm{C}(1)}}{1200n_{\mathrm{C}(2)} - P_{\mathrm{C}(2)}(M_2 - 1)}\right]/S_{\mathrm{BET}} \qquad (2)$$

Table 1

Surface properties of modified silicas under study

Modified silica	Notation	R[a]	Percentage carbon	Percentage nitrogen	α_{amide} [b]	α_{NH_2} [c]
Aminopropyl	Silica-NH_2	—	3.17	1.22	—	2.90
Phenylamide	Silica-Ph		7.59	1.13	1.94	0.96
Cyanophenylamide	Silica-CNPh		8.45	1.81	2.07	0.83
Phenylacetylamide	Silica-PhAc		8.17	1.08	1.93	0.97
Octylamide	Silica-AA_8	—C_7H_{15}	7.36	1.11	1.61	1.29

[a] R-Group attached to the amide group.

[b] Surface concentration of amide ligands (in μmol/m^2).

[c] Surface concentration of unreacted (residual) aminopropyl groups (in μmol/m^2).

where $P_{C(1)}$ and $P_{C(2)}$ is the percent carbon in the aminopropyl and amide bonded phases, respectively, N_1 is the surface coverage of aminopropyl ligands in mol/g obtained in the first synthesis step, which can be calculated from the carbon content according to Eq. (1), M_1 and M_2 are the molecular masses of ligands attached in the first and second synthesis steps, respectively, $n_{C(1)}$ and $n_{C(2)}$ are the numbers of carbon atoms per ligand attached in the first and second synthesis steps, respectively, and S_{BET} is the BET specific surface area in m^2/g of unmodified silica. The carbon percentage was used to calculate the bonding densities because three of the ligands attached in the second synthesis step did not contain nitrogen. However, the nitrogen percentage gave very similar bonding densities to those shown in Table 1. The conversion of amine groups to amides was about 65–70% for the aromatic amide phases and approximately 55% for the octylamide bonded phase. These values are in good agreement with results previously reported for different alkylamide phases [6,15]. Thermogravimetric studies have shown that all amide phases exhibit very similar mass-loss profiles and are thermally stable up to about 600 K. Moreover, nitrogen adsorption measurements indicate that the chemical modification induced no appreciable changes in the surface area and pore size distribution of the starting silica. However, the bonding of ligands to the silica surface had a notable effect on the interaction energies of the materials with nitrogen, where all aromatic amide phases exhibited nearly identical interaction with nitrogen probe molecules.

2.3. Column packing

The modified silicas were packed into 150 mm× 2.1 mm I.D. stainless steel tubes purchased from the Handy & Harman Tube Company (Norristown, PA, USA) using a slurry packing technique described previously [18]. A slurry was prepared in 30 ml of HPLC grade 2-propanol from about 0.9 g of modified silica and transferred into the reservoir of the column packing apparatus, which was completely filled with additional 2-propanol and sealed. The column packing was performed in the upward fashion using methanol as the carrier solvent at the pressure of approximately 6000 p.s.i., maintained using a Haskel model DST-162-52 (Burbank, CA, USA) air driven fluid pump (1 p.s.i.=6894.76 Pa).

2.4. Chromatographic measurements

The packed columns were conditioned with approximately 600 ml of flowing methanol and subsequently with at least 100 ml of the flowing appropriate mobile phase prior to the measurements. Chromatographic measurements for the phenylamide and cyanophenylamide phases were carried out using a Varian HPLC system (Walnut Creek, CA, USA) consisting of a model 9012 ternary gradient pump, a model 9050 variable-wavelength detector ($\lambda=254$ nm), and a manual injector utilizing a Rheodyne model 7010 (Berkeley, CA, USA) six-port injection valve equipped with a 20 μl sample loop. The column was thermostated at 303(±1) K in a Varian Mistral oven. The flow-rate (1 ml/min) was monitored at the detector outlet using a Varian model Optiflow 1000 digital liquid flow meter. The system operation and data collection were performed on a personal computer using the vendor provided software package.

Chromatographic measurements for the phenylacetylamide and octylamide phases were performed using a liquid chromatograph which consisted of a Spectra-Physics model SP-8810 (San Jose, CA, USA) isocratic pump, a model Spectra 100 variable-wavelength detector ($\lambda=254$ nm), and a manual injector utilizing a Rheodyne model 7125 (Berkeley, CA, USA) six-port injection valve equipped with a 20 μl sample loop. The column was thermostated at 298.2(±0.1) K in a Fisher Scientific model 9500 (Pittsburgh, PA, USA) Isotemp Refrigerated Circulator. The flow-rate (1 ml/min) was monitored at the detector outlet using a Phase Separations model FL4POC (Queensferry, UK) digital liquid flow meter. The chromatographic data were collected using a Spectra-Physics DataJet integrator.

2.5. Calculations

The retention factors (k') for the test solutes studied were calculated according to the standard equation:

$$k' = \frac{t_r - t_0}{t_0} \tag{3}$$

where t_r is the retention time of the solute and t_0 is the column void time. The column void time for each column studied was taken at the point for which the mobile phase disturbance peak becomes negative and at least duplicate injections were performed for all solutes.

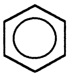
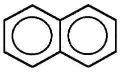
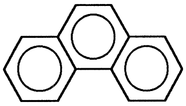
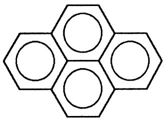
3. Results and discussion

3.1. Basic chromatographic properties

The chromatographic studies of amide bonded phases were carried out for several methanol–water binary mixtures of varying composition. The test solutes studied consisted of a homologous series of alkylbenzenes (benzene, toluene, ethylbenzene, propylbenzene, butylbenzene) and a series of aromatic hydrocarbons (benzene, naphthalene, phenanthrene, pyrene). As expected the aromatic amide phases did not show a good methylene selectivity in comparison to the octylamide bonded phase as the k' values were quite low for alkylbenzenes even for mobile phases with a relatively high water content [e.g., water–methanol (70:30, v/v)]. However, all aromatic amide phases exhibited a substantially improved selectivity for the aromatic hydrocarbon test solutes. Shown in Table 2 are the k' values for benzene naphthalene, phenanthrene and pyrene for each of the amide bonded phases under study. In comparison to the octylamide phase, the retention of aromatic hydrocarbons on the phenylamide and phenylacetylamide phases is much lower (particularly on the phenylamide phase). The presence of a nitrile group in the bonded phase (i.e. cyanophenylamide phase) results in retention of aromatic hydrocarbons with extensive π electron systems (phenanthrene and pyrene) comparable to that on the octylamide bonded phase. At the mobile phase composition investigated [water–methanol (70:30, v/v)] the separation of aromatic hydrocarbons on the phenylacetylamide phase is better and about three times faster than that on the octylamide phase.

The effects of the presence of a methylene unit and a nitrile functional group in the bonded phases on solute retention, could be evaluated by comparing the phenylacetylamide and cyanophenylamide bonded phases, respectively, to the phenylamide phase (Table 2). The surface coverage of amide ligands is essentially identical for the aromatic amide bonded phases under study (Table 1). Since the solute shape selectivity and solute conformational effects, which are often used to explain the LC separations of polyaromatic hydrocarbons [19], are

Table 2
Comparison of retention for aromatic hydrocarbons on amide bonded phases for the water–methanol (70:30, v/v) mobile phase

Test solute	Structure	$k'_{\text{Silica-Ph}}$	$k'_{\text{Silica-PhAc}}$	$k'_{\text{Silica-CNPh}}$	$k'_{\text{Silica-AA}_8}$
Benzene		0.20	0.87	0.43	1.13
Naphthalene		1.00	2.42	2.39	4.13
Phenanthrene		3.09	8.24	14.5	18.0
Pyrene		6.18	16.7	48.8	43.2

similar, the changes in retention of these solutes could be interpreted in terms of the bonded phase structure. As can be seen, the single methylene unit inserted between the aromatic ring and the carbonyl group results in about 3-fold increase in retention of the aromatic hydrocarbons (see k' values for silica-Ph and silica-PhAc in Table 2). The presence of the nitrile group in the bonded phase has an even more dramatic effect on solute retention (see k' values for silica-Ph and silica-CNPh in Table 2). The smaller aromatic hydrocarbons (benzene and naphthalene) show about 2-fold increase in retention on the cyanophenylamide phase relative to the phenylamide. However, the solutes with a larger number of aromatic rings exhibit a very significant increase in retention on the cyanophenylamide phase (approximately 5-fold and 8-fold increases were observed in k' values for phenanthrene and pyrene, respectively, in comparison to the phenylamide phase). As was already noted, phenanthrene and pyrene are retained nearly to the same extent on the cyanophenylamide and octylamide phases. It has been previously reported that cyanoalkyl bonded phases provide some selective interactions with compounds having double bonds [20] and that the nitrile group can strongly interact with aromatic rings via specific π–π interactions [21,22]. This correlates well with the current data, where the aromatic hydrocarbon solutes with extensive π electron systems were found to interact very strongly with the bonded phase containing nitrile functional groups.

3.2. Stability under hydro–organic conditions

The experimental conditions employed for the stability studies of the amide bonded phases, i.e. particular test solute used and the mobile phase composition, were adjusted for each phase so that the test solutes had initial k' values in the range of 5 to 15. The choice of the experimental conditions, for which intermediate k' values were obtained, ensured that the solute retention was high enough for any significant changes in retention to be easily detected and at the same time not too high to obtain reasonable chromatographic peak profiles.

In Table 3 the retention of selected test solutes immediately after the conditioning of the chromatographic column is compared with the retention of the same solutes after approximately 100 h of exposure of the amide phases to the flowing hydro–organic eluent. As can be seen, the k' values for the test solutes studied on the phenylacetylamide and octylamide phases decreased by about 1%. Such small changes in the k' values are within the experimental error of the measurements and in addition they can be attributed to the conditioning of the column. However, for the phenylamide and cyanophenylamide phases the percent decrease in k' values for the test solutes is approximately 20- and 30-fold greater, respectively, than for the phenylacetylamide and octylamide phases. As was previously noted, all amide phases were prepared according to the same experimental procedure from the same sample of aminopropyl silica. Moreover, these materials were conditioned in a nearly identical fashion prior to the chromatographic studies. On the basis of the chromatographic data, the phenylacetylamide and octylamide phases seem to be stable, while the phenylamide and cyanophenylamide do not. It is possible that the apparent instability is chemical in nature and is related to the bonded phase structure.

High resolution thermogravimetric studies were carried out for samples of phenylamide and cyanophenylamide silica, which were initially exposed to the flowing hydro–organic mobile phase for about 100 h and later left with the mobile phase

Table 3
Retention of selected test solutes on amide bonded phases immediately following the conditioning of the column with mobile phase and after approximately 100 h of exposure to the flowing hydro–organic eluent

Modified silica	Test solute	Mobile phase	k' initial	k' after 100 h of use	% decrease in k'
Silica-Ph	Phenanthrene	Water	12.6	10.1	19.8
Silica-CNPh	Naphthalene	Water	7.68	5.52	28.1
Silica-PhAc	Phenanthrene	Water–MeOH (70:30, v/v)	8.24	8.13	1.33
Silica-AA_8	Butylbenzene	Water–MeOH (70:30, v/v)	7.79	7.73	0.77

inside chromatographic columns for an extended period of time (approx. 9 months). The instrumentation and experimental parameters used in these studies were identical to those described previously [14]. The samples of phenylamide and cyanophenylamide phases unpacked from the chromatographic columns were twice washed with about 5 ml of pure methanol and dried at 393 K for 2 h. The thermogravimetric studies suggest that a small amount of amide groups present on the surface may be undergoing hydrolysis, which could account for the gradual decreases in the retention of test solutes studied. The derivative mass-loss curves are compared in Fig. 3 for aminopropyl silica and phenylamide silica as-synthesized (silica-Ph) and after long-term exposure to hydro–organic conditions (silica-Ph*). The large peak at about 650 K for the phenylamide silica samples indicates a maximum rate of mass-loss at this temperature, and it can be attributed to the thermal decomposition of the amide portion of the bonded ligands. The smaller peak at about 750 K for aminopropyl silica, and both samples of the phenylamide phase can be attributed to the decomposition of the aminopropyl portion of the bonded ligands and/or residual amine groups. The area under a peak is directly related to the amount of species undergoing thermal decomposition. As can be seen in Fig. 3, the profile of the peak corresponding to the decomposition of the aminopropyl portion of the ligands remains essentially unchanged for the as-synthesized phenylamide silica sample and the sample exposed to hydro–organic conditions. However, the area of the peak corresponding to the decomposition of the amide portion of the bonded ligands decreases for the exposed sample. It must be pointed out that if hydrolysis of amide groups of the phenylamide and cyanophenylamide bonded phases does indeed occur, the extent of hydrolysis is not very large. However, these small changes in the bonded phase composition are apparently large enough to produce the observed gradual changes in the solute retention.

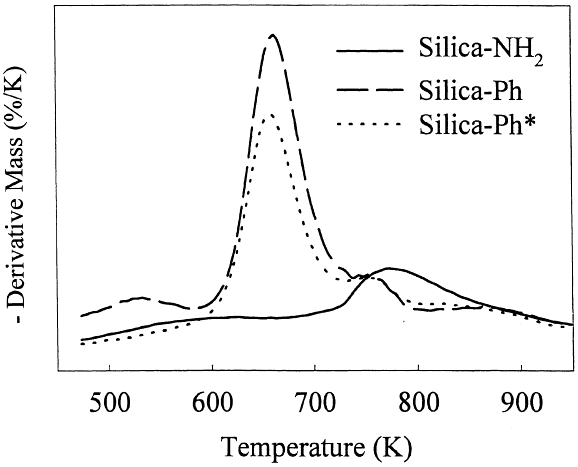


Fig. 3. Derivative thermogravimetric mass-loss curves for aminopropyl silica, and phenylamide bonded phase as-synthesized (silica-Ph) and following long-term exposure to hydro–organic conditions (silica-Ph*).

4. Conclusions

The surface properties of three silica-based amide-bonded phases containing unsubstituted and substituted aromatic rings were studied by HPLC and compared to the properties of an octylamide phase. Comparative studies on the phenylamide, cyanophenylamide and phenylacetylamide phases were used to evaluate the effects of the presence of a methylene unit and nitrile functional group in the bonded phase on the retention of aromatic hydrocarbons. Also, it was found that the amide-bonded phases under study exhibited very different stabilities under hydro–organic conditions. Supporting thermogravimetric studies suggested that the apparent instability of the phenylamide and cyanophenylamide bonded phases may be related to the partial hydrolysis of amide linkages in these materials.

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