Preparation and Characterization of Modified 3-Aminopropylsilyl Silica Phases with 1,8-Naphthalic Anhydrides in HPLC

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Abstract

Modified 3-aminopropylsilyl silica stationary phases with 1,8-naphthalic anhydrides [3-(1,8-naphthalimido)propyl (NAIP), 3-(4-amino-1,8-naphthalimido)propyl (4A-NAIP), 3-(3-nitro-1,8-naphthalimido)propyl (3N-NAIP), and 3-(4-nitro-1,8-naphthalimido)propyl modified silyl silica gel (4N-NAIP)] are prepared in a single reaction. Chromatographic properties of these phases are characterized using various solutes including monosubstituted benzenes, alkyl benzenes, and polycyclic aromatic hydrocarbons (PAHs) in high-performance liquid chromatography. Even though the retention in these NAIPs is mainly based on the reversed-phase mode, 3N- and 4N-NAIP show a remarkable recognition toward aromatic hydrocarbons, which is attributed to their strong π–π interaction. Furthermore, the recognition ability is according to the molecular planarity and non-linearity of PAHs. Subsequently, 3N- and 4N-NAIP has a distinct selectivity from octadecyl silica and, therefore, will be a valuable alternative for a separation or a solid-phase extraction of aromatic compounds.

Introduction

In recent years, we have prepared and evaluated a series of derivatized aminopropyl-modified silyl silica gels (APS) with organic dyes (1) and aromatic dicarboxylic anhydrides (2–4) as new stationary phases for high-performance liquid chromatography (HPLC). Among them, 3-(1,8-naphthalimido)propyl-modified silyl silica gel (NAIP) (Figure 1) was successfully applied to the separation of purine derivatives (i.e., xanthine, hypoxanthine, uric acid, theobromine, theophylline, and caffeine) (2) and barbiturates (3) in HPLC. More recently, NAIP was proved to be a useful stationary phase for capillary electrochromatography (CEC) of these analytes (4,5). The retention behaviors of the mentioned analytes in HPLC and CEC suggested that the retention mechanism in NAIP was based on the reversed-phase mode with π–π

interaction. To confirm the π–π interaction, 3-maleimidopropyl-modified silyl silica gel (MAIP) and 3-(1,2-phthalimido)propyl-modified silyl silica gel (PHIP) have been also prepared, and their retention behaviors in the separation of aromatic compounds were compared with those of NAIP and APS when applied for the separation of some aromatic hydrocarbons (6). As a result, it was observed that the retention of the aromatic hydrocarbons increased according to the total π-electron density for C=C double bond of stationary phases with the order of NAIP > PHIP > MAIP > APS. Actually, aromatic hydrocarbons were not retained by APS having no π-electron. It was thus concluded that π–π interaction contributed to the retention of the aromatic solutes (6).

The contribution of π–π interaction in the retention behavior is generally smaller than that of hydrophobic and electrostatic interactions (7). However, π–π interaction has an important role in chiral recognition mechanisms with Pirkle's type stationary phases (8,9). The stationary phases with π–π interaction have also been developed using metal phthalocyanine (10,11), anthracene (12), and porphyrin derivatives (13,14) as modifiers of silica gels. Among them, copper (II)-phthalocyanine sulfonyl aminopropyl silica gel (10) showed a high planarity recognition toward a mixture of planar triphenylene and nonplanar o-terphenyl. Silica-
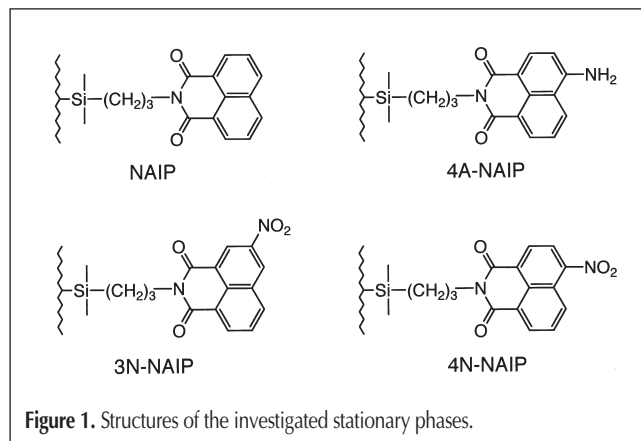
Figure 1. Structures of the investigated stationary phases.

supported linear polystyrene has also been reported to provide a good separation for polycyclic aromatic hydrocarbons (PAHs) (15). However, most of them required troublesome procedures for their preparation, and the development of a novel stationary phase with π–π interaction is still challenging.

For the analyses of biological and environmental compounds, both selectivity and sensitivity, which are closely related to separation and detection technologies, are important factors. Potential stationary phases with prominent π–π interaction should be useful for a separation or a solid-phase extraction of biologically important aromatic analytes. Furthermore, these stationary phases should also be valuable for the analytes labeled with reagents possessing strong UV absorption and fluorescence for highly sensitive detection because all of these reagents have π-electrons.

In this study, 3-(4-amino-1,8-naphthalimido)propyl (4A-NAIP), 3-(3-nitro-1,8-naphthalimido)propyl (3N-NAIP), and 3-(4-nitro-1,8-naphthalimido)propyl modified silyl silica gel (4N-NAIP) were prepared in a single reaction and evaluated as an HPLC stationary phases (Figure 1). The retention behavior of various aromatic compounds on these stationary phases was investigated, and the knowledge obtained here will contribute to further development of the stationary phase with high recognition toward aromatic compounds.

Experimental

Preparation of stationary phases

NAIPs were prepared by modifying APS (Daisogel SP-120-APS, 5-μm particle size, 120-Å pore diameter) (Daiso, Osaka, Japan) with the corresponding 1,8-naphthalic anhydrides. Briefly, dried APS (5.0 g) was suspended in N,N-dimethylformamide (DMF, 40 mL), and to this was added each 1,8-naphthalic anhydride (ca. 7 mmol). The mixture was stirred at 120°C for 2 h, and the modified APS was collected and thoroughly washed with DMF and methanol. The modification ratios of APS with 1,8-naphthalic anhydrides were as follows: 57% for NAIP, 58.2% for 4A-NAIP, 43% for 3N-NAIP, and 41.4% for 4N-NAIP. They were calculated from the percentage of carbon obtained by elemental analysis. In addition, we confirmed that 1,8-naphthalic anhydrides easily react with a primary amine to form a heterocyclic ring by IR spectra using model reaction of cyclohexylamine and 4-nitro-1,8-naphthalic anhydride. After drying under reduced pressure, these phases were packed by a slurry method using a mixture of glycerol and methanol (50:50, v/v) in stainless steel columns (250- × 4.6-mm i.d.), according to our previous method (16). All 1,8-naphthalic anhydrides were purchased from Aldrich (Milwaukee, WI).

HPLC apparatus and conditions

The HPLC system consisted of a Shimadzu LC9A pump (Kyoto, Japan), a Rheodyne 7125 injector (Cotati, CA) with a 20-μL sample loop, an analytical column packed with NAIPs or a Daisopak-SP-120-5-octadecyl silica (ODS) commercial ODS packed column from Daiso (250- × 4.6-mm, 5-μm particle size, 120-Å pore diameter) placed in a Tosoh CO 8010 column oven,

a Shimadzu SPD6A UV detector, and a Rikadenki R-01 recorder (Tokyo, Japan).

The column temperature was maintained at 30°C. The elution was performed with aqueous acetonitrile solutions at a flow rate of 1.0 mL/min, and the detection wavelength was set at 260 nm.

Results and Discussion

Separation properties of monosubstituted benzenes

In biological and environmental samples and pharmaceutical preparations, there are many biologically active compounds with π-electrons. In addition, the chromophore or fluorophore of derivatizing reagents used for highly sensitive analysis in HPLC also posses π-electrons. From this point of view, the separation properties of various monosubstituted benzenes (i.e., benzene, acetophenone, aniline, benzonitrile, chlorobenzene, nitrobenzene, and toluene) on the NAIPs were first investigated. The retention data on NAIPs were compared with those on a commercial ODS-packed column.

The retention factor (k) of NAIPs for each monosubstituted benzene decreased with an increase in acetonitrile contents from 50% to 80% in the mobile phase, which was also the case when ODS was used. However, the k values of monosubsti-
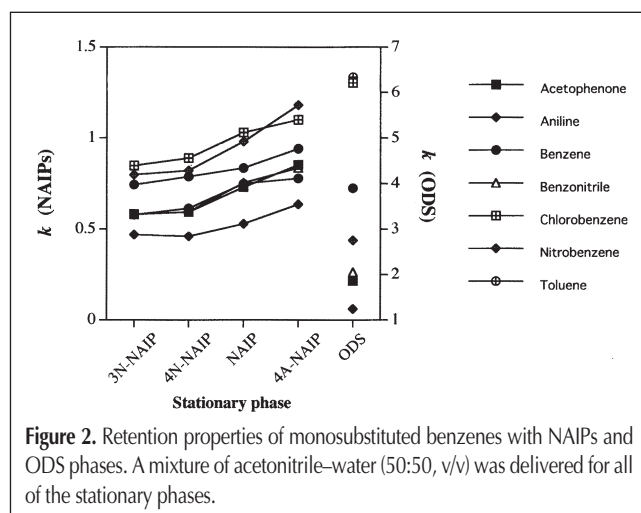

Figure 2. Retention properties of monosubstituted benzenes with NAIPs and ODS phases. A mixture of acetonitrile–water (50:50, v/v) was delivered for all of the stationary phases.
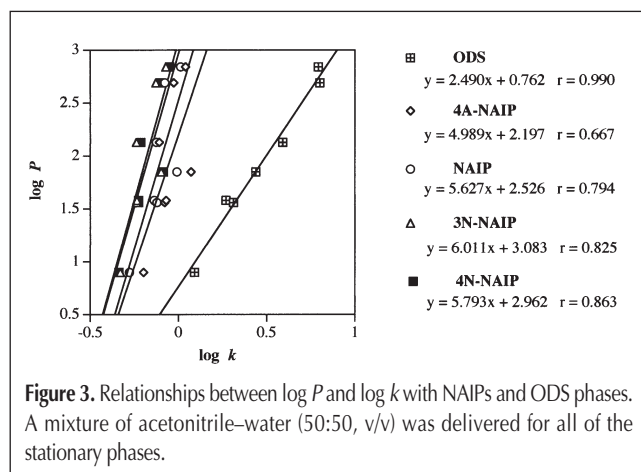

Figure 3. Relationships between log P and log k with NAIPs and ODS phases. A mixture of acetonitrile–water (50:50, v/v) was delivered for all of the stationary phases.

tuted benzenes obtained by NAIPs were much smaller than those with ODS (Figure 2). The order of k values on NAIPs was 4A-NAIP > NAIP > 4N-NAIP ≥ 3N-NAIP. The relationship between the logarithmic retention factor (log k) and the log P value for each solute is shown in Figure 3. The linear relationships were obtained with good correlation coefficients (r) ranging from 0.665 to 0.867, though the retention orders on NAIPs were slightly different than that on ODS. This result suggests that a reversed-phase mode retention is a part of the retention mechanism in NAIPs.

Separation properties of alkylbenzenes and aromatic hydrocarbons

The effects of the length of alkylchain of alkylbenzenes and the number of aromatic rings on the retention by NAIPs were then investigated. Toluene, ethylbenzene, propylbenzene, and butylbenzene were employed as alkylbenzenes. As shown in Figure 4, the length of alkylchain slightly affected the k values of the solutes on NAIPs, which were very small compared with that on ODS. As in the case of the monosubstituted benzenes, among NAIPs, alkyl benzenes were most retained by 4A-NAIP.

On the other hand, the retention behaviors of the aromatic hydrocarbons (benzene, naphthalene, phenanthrene, and naphthacene) on NAIPs were remarkably influenced by the number of
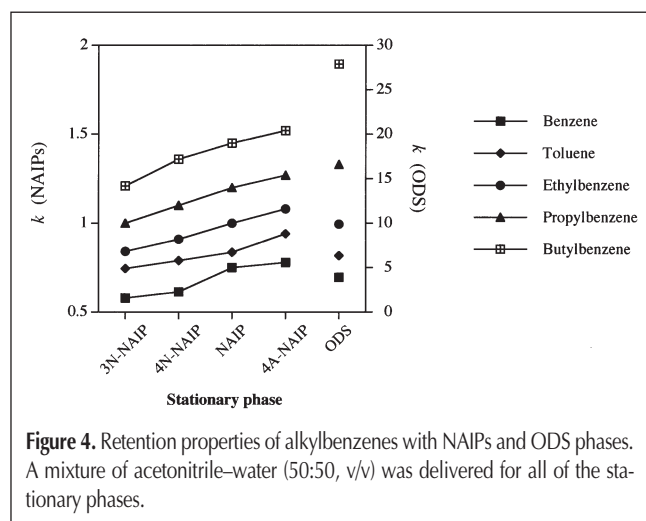
their aromatic rings (Figure 5). The greater impact was on nitro-substituted NAIPs (i.e., 3N- and 4N-NAIP) rather than ODS: the k value of naphthacene on 4N-NAIP was 86.8-fold more than that of benzene, but the k value of naphthacene on ODS was 19.5-fold more than that of benzene. Moreover, on 3N-NAIP, naphthacene was too strongly retained to be eluted under the HPLC conditions examined.

The log k values on 4N-NAIP were plotted against those on ODS in order to evaluate the overall retention order of alkylbenzenes and aromatic hydrobarbones on these phases (Figure 6). In Figure 6, it was clearly found that 4N-NAIP highly recognized aromatic hydrocarbons. To take phenanthrene as an example, it was retained much stronger than all the alkylbenzenes on 4N-NAIP, although it was eluted between propylbenzene and butylbenzene on ODS.

These differences in the retention behavior between nitrosubstituted NAIPs and ODS should be attributed to the stronger π–π interaction based on the π-acidic nature of nitrosubstituted NAIPs. 3N- and 4N-NAIP will be valuable for the selective recognition toward aromatic compounds in a mixture of aliphatic and aromatic compounds that possess similar hydrophobicity.

Separation properties of PAHs

The higher recognition toward aromatic compounds on nitro-

Figure 4. Retention properties of alkylbenzenes with NAIPs and ODS phases. A mixture of acetonitrile–water (50:50, v/v) was delivered for all of the stationary phases.
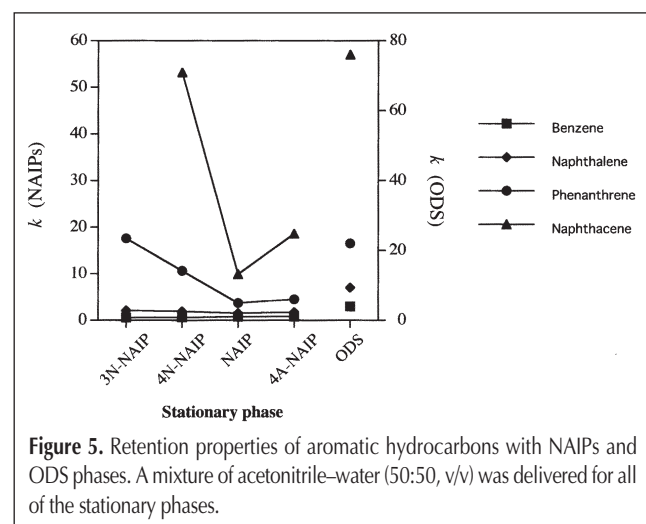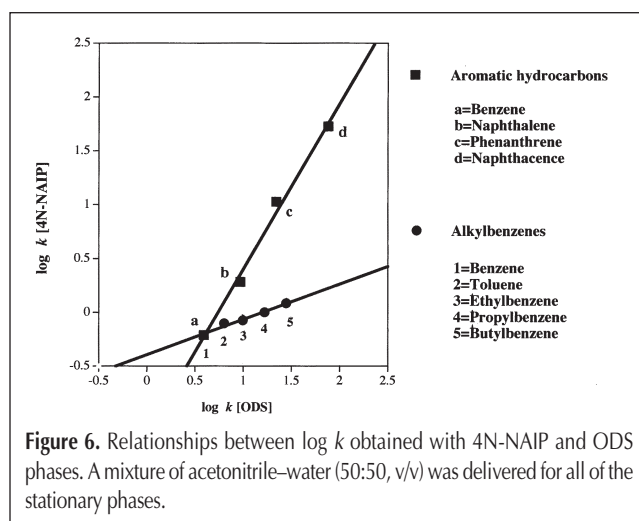

Figure 6. Relationships between log k obtained with 4N-NAIP and ODS phases. A mixture of acetonitrile–water (50:50, v/v) was delivered for all of the stationary phases.

Figure 5. Retention properties of aromatic hydrocarbons with NAIPs and ODS phases. A mixture of acetonitrile–water (50:50, v/v) was delivered for all of the stationary phases.

Table I. k for Various PAHs Obtained with NAIPs and ODS Phases

Sample	k				
	3N-NAIP	4N-NAIP	NAIP	4A-NAIP	ODS
Benzene	0.02	0	0.15	0	0.10
Naphthalene	0.09	0.07	0.18	0.05	0.24
Anthracene	0.68	0.36	0.35	0.23	0.45
Phenanthrene	0.75	0.41	0.35	0.25	0.43
Naphthacene	2.73	1.45	1.00	0.86	0.91
Benzo[a]anthracene	2.77	1.41	0.58	0.68	0.79
Chrysene	3.23	1.55	0.58	0.71	0.74
Pyrene	4.50	1.91	0.55	0.50	0.67
Triphenylene	6.27	2.23	0.73	0.55	0.71
o-Terphenyl	0.02	0.02	0.18	0.07	0.43

substituted NAIPs than that on ODS was found. Retention behaviors of NAIPs were further examined using extended aromatic compounds. The k values of various PAHs are summarized in Table I. A higher organic mobile phase (acetonitrile–water, 80:20 v/v) was employed here to minimize the reversed-phase mode retention and distinguish the retention based on π–π interaction. Actually, in this condition, 3N-NAIP much more strongly retained most of the PAHs than ODS, which also supported the existence of π–π interaction on nitrosubstituted NAIPs. Furthermore, the characteristics of NAIPs on the recoginition toward PAHs were described in the following.

It was reported that a typical ODS phase strongly retains planar PAHs compared with nonplanar ones (17). Similarly, in this study, o-terphenyl eluted earlier than triphenylene and coeluted with phenanthrene and anthracene, which have the same numbers of rings as o-terphenyl. However, 3N- and 4N-NAIP much more strongly retained triphenylene than o-terphenyl, and, furthermore, the elution time of o-terphenyl was the same as the lower aromatic ring compound (benzene). Nitrosubstituted NAIPs showed superior planarity recognition to ODS, as in the case with

other stationary phases having π–π interaction (10).

Molecular linearity of PAHs also affects their retention on the ODS phase. This fact was supported by Jinno et al. (17). In this study, linear PAHs were more strongly retained than nonlinear ones on ODS. As starkly contrasted to the results on ODS, the retention of nonlinear PAHs was stronger than that of linear ones on 3N- and 4N-NAIP. Specifically, for the retention of naphthacene and pyrene on 3N-NAIP, the k value of pyrene was 1.6-fold larger than that of naphthacene, but the opposite was found in ODS. For further confirmation of this unique recognition on NAIPs, a relationship between the k value and the length-to-breadth ratio (L/B), which is a molecular shape parameter, was investigated on NAIPs and ODS with respect to naphthacene, benzo[a]anthracene, chrysene, and triphenylene (Figure 7). A large L/B ratio of PAHs means more linear shape (17), and the value for each PAH is reported as follows: 1.12 for triphenylene, 1.58 for benzo[a]anthracene, 1.72 for chrysene, and 1.89 for naphthacene. The 3N- and 4N-NAIP provided a negative correlation between k value and L/B ratio, whereas a positive correlation was obtained with ODS and 4A-NAIP. The negative correlations proved that the selectivity on 3N- and 4N-NAIPs was completely different from that on ODS and was based on the recognition toward the non-linearity of compounds. Representative chromatograms of PAHs on ODS and 4N-NAIP under same conditions are presented in Figure 8.

As a result, although the reversed-phase mode retention was dominant in the retention mechanism of NAIPs, 3N-, and 4N-NAIP realized more of a distinct selectivity on the separation of PAHs than ODS, which should be because of the strong π–π interaction provided by their π-acidic nature. The high-planarity and nonlinearity recognition toward PAHs in highly organic mobile phase were remarkable.
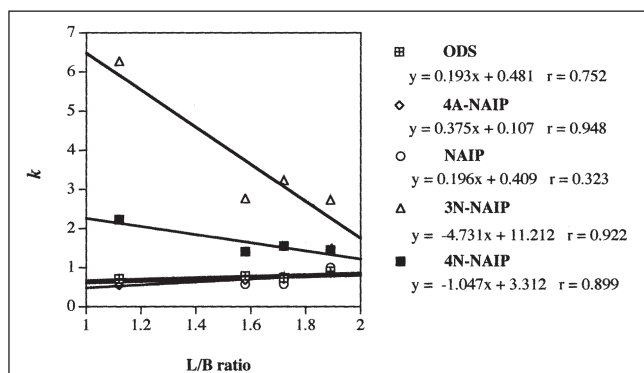

Figure 7. Relationships between k and L/B ratio with NAIPs and ODS phases. A mixture of acetonitrile–water (80:20, v/v) was delivered for all of the stationary phases.

Conclusion

The modified 3-aminopropylsilyl silica phases with 1,8-naphthalic anhydrides were prepared and characterized on their separation properties of various solutes in HPLC. The retention mechanism in all of the NAIPs was dominated by a reversed-phase retention mode. However, 3N- and 4N-NAIP demonstrated high recognition toward the number of aromatic rings rather than the length of alkyl chain and, in addition, presented the planarity and nonlinearity recognition toward PAHs. These characteristics could be explained by the stronger π–π interaction, which was based on the substitution with electron acceptor (i.e., nitro group).

The 3N- and 4N-NAIP provided a dramatically different selectivity for PAHs and, therefore, will be a valuable alternative for a separation of aromatic compounds, including drugs, pesticides, PAHs, and analytes derivatized with fluorescence reagents, or a solid-phase extraction of those compounds, especially in lipophilic samples. Moreover, the modification of a primary amino group with 1,8-naphthalic anhydride is quite simple, and NAIPs substituted by a nitro group will be a useful modification to a Pirkle's type stationary phase with the strong π–π interaction.
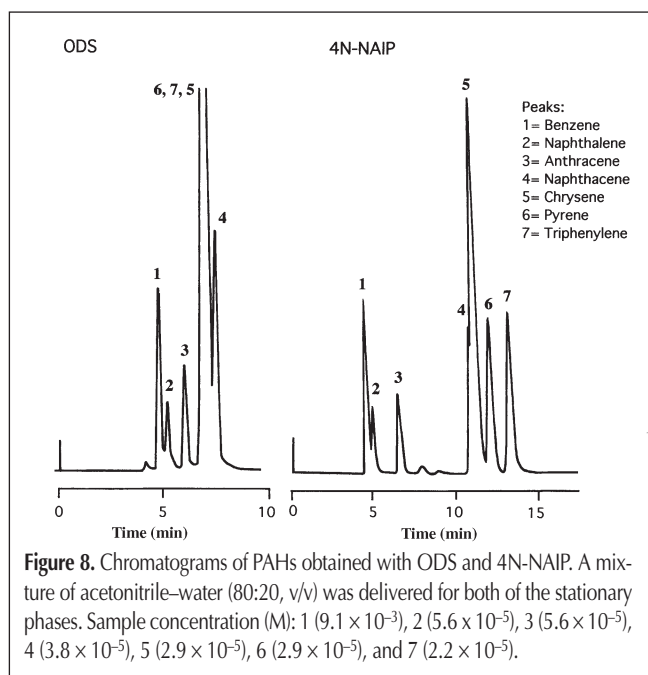

Figure 8. Chromatograms of PAHs obtained with ODS and 4N-NAIP. A mixture of acetonitrile–water (80:20, v/v) was delivered for both of the stationary phases. Sample concentration (M): 1 (9.1×10^{-3}), 2 (5.6×10^{-5}), 3 (5.6×10^{-5}), 4 (3.8×10^{-5}), 5 (2.9×10^{-5}), 6 (2.9×10^{-5}), and 7 (2.2×10^{-5}).

Acknowledgments

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