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PHOSPHORIC ACID-MODIFIED AMINO BONDED STATIONARY PHASE FOR HIGH-PERFORMANCE LIQUID CHROMATOGRAPHIC CHEMICAL CLASS SEPARATION

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SUMMARY

A new stationary phase for high-performance liquid chromatography was produced by reacting a conventional amino bonded phase with phosphoric acid. Characterization of the modified amino phase indicated that ammonium phosphate salt groups and "free" phosphoric acid were present on the stationary phase surface. The column's capability for separating complex sample mixtures into chemical classes was tested using model compounds representing those typically found in fossil fuels. A solvent gradient program was developed which effectively separated hydrocarbon standards into aliphatic, non-polar aromatic, neutral/acidic polar aromatic, and basic polar aromatic classes.

INTRODUCTION

The direct characterization of complex mixtures, such as fossil fuels or other environmental samples, is often difficult even when high-resolution chromatography is combined with universal and selective detection. However, preliminary fractionation of complex mixtures simplifies their analysis and provides partial characterization of the samples. The most effective prefractionation methods are those which separate sample components rapidly and reproducibly into well-defined chemical classes. In the case of fossil fuels, solvent extraction and liquid column chromatography are the prefractionation methods used most often. Extraction procedures have major drawbacks, however; solvent–solvent partition is poorly class selective while acid–base extraction often forms emulsions, polymerizes components, or produces artifacts in samples.

Numerous liquid chromatographic (LC) stationary phases have been used to fractionate fossil fuels and many have been discussed in recent papers[1–6]. Although new bonded phases have often displaced stationary phases such as liquid–solid

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chromatography (LSC) adsorbents, coordination chromatography adsorbents, and gel permeation chromatography (GPC) gels, for this application, silica[1,7] and alumina[2,3,8] LSC adsorbents are still widely used. Because of the excellent class selectivity of these materials for compounds of widely ranging polarity, quite extensive fractionation can be achieved in a single chromatographic run. However, primary disadvantages of silica and alumina include poor recovery of some polar species[8] and bioactivity[9], and sensitivity to water in the mobile phase[8]. Varying moisture content impairs reproducibility and increased water levels reduce adsorbent activity such that overlap of chemical classes occurs. An elaborate system for maintaining constant moisture levels in solvents has been constructed[3], but is not generally available. GPC gels are also still used[10,11]; although these materials efficiently separate aliphatic hydrocarbons from aromatics with high sample recovery, they demonstrate little selectivity for polar-substituted aromatic hydrocarbon classes.

Bonded stationary phases are popular for fossil fuel fractionations because they offer improved efficiency, reproducibility, insensitivity to mobile phase moisture content (normal-phase packings), and better recovery of polar compounds and biological activity than LSC adsorbents. Four studies comparing various stationary phases for chemical class separation have been conducted[4,5,12,13]. In the first investigation[12], dimethylamino and tetranitrofluorenone packings used under normal-phase conditions, and octadecylsilane (ODS) and inorganic anion-exchange packings used under reversed-phase conditions, provided the most effective chemical class separations. Nitroaromatic, diol, polyamide, amino, cyano, alumina, silica, octyl, and other ion-exchange columns fractionated the coal liquid sample less efficiently. However, none successfully separated all classes under consideration (saturates, aromatics, neutral heterocycles, acids, and bases). The use of reversed-phase conditions is limited further by solubility problems encountered when aqueous eluents are employed with hydrocarbon based fossil fuel samples.

In the second comparison study[4], a mixed amino/cyano packing resolved saturated, olefinic, aromatic, and polar classes more efficiently than amino, cyano, diol, or phenyl bonded phases. Although this bonded phase has not been fully characterized, other work[14] suggests that resolution between polar compound classes would probably be incomplete.

Nitrophenyl, amino, cyano, and sulfonic acid bonded phases, silica and alumina adsorbents, and a porous styrene–divinylbenzene copolymer phase have been compared[13]. None of the columns could efficiently separate all the chemical classes represented by over 40 model compounds. An interesting observation was the effective separation of polar acidic, polar basic, and non-polar aromatic classes by a sulfonic acid column under normal-phase conditions. However, non-polar aromatic and polar fractions overlapped when shale oil and petroleum samples were chromatographed. Ion-exchange materials must also be prepared and used carefully to avoid artifacts[15]. Despite these limitations, their use for class separations of fossil fuels is increasing[6,15–17].

The fourth study[5], comparing silica, alumina, nitrophenyl, amino, cyano, and ODS columns emphasizing the separation of nitrogen heterocycles and non-polar aromatics, showed silica to be superior. Other studies using tetranitrofluorenone[12] and tetrachlorophthalimide[18] charge-transfer phases also gave incomplete resolution of chemical classes.

The limitations of existing stationary phases encourage the development of new materials, especially normal bonded phases, for class separation of fossil fuels. Recently, we developed a phosphoric acid-modified amino bonded phase for this application[19]. The acidic nature of this packing is promising because acidic silica and cation-exchange columns have provided some of the best class separations. The modified amino bonded phase was used successfully with rapid solvent switching to fractionate shale oils[19]. In the present study, model organic compounds encompassing a wide range of functionalities and polarities have been separated using a continuous solvent gradient, providing a basis for comparison with other prefractionation schemes. The substrate (and a compound modelling it) was characterized by elemental analysis, IR spectrometry, inductively coupled plasma (ICP) spectrometry, and acid–base titration.

EXPERIMENTAL

Reagents

Chemical standards were obtained from Aldrich (Milwaukee, WI, U.S.A.) and Eastman Kodak (Rochester, NY, U.S.A.) and were purified by distillation or recrystallization. HPLC-grade hexane and dichloromethane, 85% phosphoric acid, and silver nitrate (Fisher Scientific, Fair Lawn, NJ, U.S.A.), HPLC-grade isopropanol (J. T. Baker, Phillipsburg, NJ, U.S.A.), *n*-butylamine (Aldrich), and glacial acetic acid and sodium hydroxide (MCB Manufacturing Chemists, Cincinnati, OH, U.S.A.) were used.

Column preparation

The 10-μm aminopropyl bonded stationary phase (Chromosorb LC-9, Johns Manville, Denver, CO, U.S.A.) was packed into two 25 cm × 4.0 mm stainless-steel columns equipped with zero dead volume fittings. Column packing was in a downward configuration using a constant pressure Haskel Engineering and Supply (Burbank, CA, U.S.A.) Model DST-122 pump operated at 6000 p.s.i.g. Isopropanol was slurry and packing solvent. One of the packed columns was connected to an HPLC pump and 25–30 ml of 1% phosphoric acid in isopropanol were pumped through, followed by 50 ml each of isopropanol and dichloromethane prior to equilibration with hexane. The unmodified amino bonded phase column was washed with 50 ml dichloromethane prior to equilibration with hexane.

HPLC methods

Gradient HPLC was performed with a modular Laboratory Data Control/ Milton Roy (Riviera Beach, FL, U.S.A.) liquid chromatograph consisting of Constametric I and II pumps, a Spectromonitor II variable-wavelength UV detector, and a Rheodyne (Cotati, CA, U.S.A.) Model 7125 injector equipped with a 20-μl loop. Various binary solvent gradients employing hexane, or acetic acid in hexane, and isopropanol were evaluated. An IBM Instruments (Wallingford, CT, U.S.A.) Model 9533 instrument (254 nm detection) was used for the ternary solvent studies using hexane, 3.5% acetic acid in hexane, and isopropanol. Standard compounds used to evaluate the various solvent programs were prepared in hexane at concentrations ranging from 1 to 50 μg/ml. Solvent flow-rate was maintained at 2.0 ml/min.

Characterization of the modified amino bonded phase

The product of *n*-butylamine reacted with phosphoric acid was examined as a model system for the modified amino bonded phase. Approximately 5 ml *n*-butylamine was added dropwise to 50 ml of 5% phosphoric acid in isopropanol. A white precipitate formed immediately, was filtered, washed with isopropanol, and dried at 100°C for 48 h. It was insoluble in ethanol, isopropanol, dichloromethane, and hexane but was soluble in methanol and water. The product was also soluble in basic solution (with release of the free amine) and formed yellow silver phosphate on addition of silver nitrate. The original reaction product was further characterized by elemental and IR spectrometric (potassium bromide pellet) analyses.

The modified amino bonded phase was characterized by titration with sodium hydroxide. The sample for titration was produced by stirring a mixture of 1.5 g Chromosorb LC-9 amino-propyl bonded phase in 35 ml of 0.5% phosphoric acid in isopropanol solution for 15 min. The phase was filtered, washed with 2 × 20 ml isopropanol, and dried overnight at 100°C. After drying, 1.0753 g was added to 20 ml distilled, deionized water stirred with a magnetic stirrer in a 100-ml beaker. The solution was titrated with standard 0.03732 *M* sodium hydroxide solution; pH was measured with a Fisher combination electrode connected to a Corning Glass Works (Corning, NJ, U.S.A.) Model 7 pH meter.

Phosphorus analysis was conducted by ICP spectrometry for comparison with the titration results. A weighed modified amino bonded phase sample (58.4 mg) was placed in a 50-ml volumetric flask. To the sample was added 2 ml concentrated sulfuric acid and 20 drops concentrated nitric acid. The mixture was first warmed gently on a hot plate for 1 h and then heated at maximum hot plate temperature for 2 h. Finally, the digested sample was cooled to room temperature and diluted to 50.00 ml with distilled, deionized water prior to ICP analysis.

RESULTS AND DISCUSSION

Characterization of the modified amino bonded phase

Studies were undertaken to characterize three aspects of the amino bonded phase modification: (1) the nature of the bonding of phosphoric acid to the amino bonded phase; (2) the stoichiometry of the bonding; (3) the quantity of bonded phosphoric acid. It was considered that phosphoric acid reacted with the amino groups of the bonded stationary phase to form either a phosphoramide (eqn. 1) or an amine phosphate salt (eqn. 2).

$$\text{R-NH}_2 + \text{H}_3\text{PO}_4 \rightarrow \text{R-NH-PO(OH)}_2 + \text{H}_2\text{O} \quad (1)$$

$$\text{R-NH}_2 + \text{H}_3\text{PO}_4 \rightarrow \text{R-NH}_3^+ {}^-\text{OPO(OH)}_2 \quad (2)$$

The experiments using *n*-butylamine as a model for the amino bonded phase indicated that the amine phosphate salt was the predominant reaction product. First, phosphoramides are most readily formed from chloro-acids[20] which are more reactive than free phosphoric acid; they decompose readily to amine-phosphate salts in the presence of trace amounts of water[20]. The facile reaction of free phosphoric acid with *n*-butylamine to form a stable product in the presence of water (85% phosphoric acid

was used) therefore suggested formation of a phosphate salt. In addition, the product decomposed in basic solution, producing free *n*-butylamine and PO_4^{3-}. The amine was confirmed by its odor and gas chromatographic analysis of an diethyl ether extract of the basic solution. The PO_4^{3-} was confirmed by the formation of the silver phosphate salt on addition of a few drops 10% silver nitrate solution. This decomposition is characteristic of amine phosphate salts; phosphoramides, on the other hand, are stable at high pH[20].

Elemental carbon, hydrogen, and nitrogen analyses of the *n*-butylamine–phosphoric acid reaction product are given in Table I. Comparison with theoretical percentages calculated for phosphoramide and amine phosphate salt products support amine–phosphate salt formation. The IR spectrum was dominated by broad, intense peaks corresponding to strongly hydrogen bonded –OH groups, a feature consistent with either product. However, the conspicuous lack of a P–N stretching band at 850 cm^{-1} and an N–H bending band at 1400 cm^{-1} contraindicated phosphoramide formation. Furthermore, weak bands observed at about 1500 cm^{-1} and 1600 cm^{-1} were within the ranges of N–H bending vibrations of amine phosphate salts.

An amine phosphate salt similar to that obtained with *n*-butylamine should have been formed when phosphoric acid in isopropanol solution was pumped through the amino bonded phase column. Elemental analysis results for the *n*-butylamine–phosphoric acid reaction product also demonstrated that each molecule of phosphoric acid reacted with one amino group. However, the possibility that phosphoric acid molecules reacted with two amino groups on the bonded phase was considered. The two reaction products are shown in Fig. 1. In the one-to-one bonding scheme (Fig. 1a), two protons of the triprotic ($pK_1 = 2.12$, $pK_2 = 7.21$, $pK_3 = 12.67$) phosphoric acid remain unbound on the product, whereas in the two-to-one bonding scheme (Fig. 1b) only one proton remains free. The relative contributions of the two reaction products could be determined by base titration of the unbound protons, the predicted titration curves being shown in Fig. 1. In the two-to-one scheme, no equivalence point is expected for the single weakly acidic proton. A single equivalence point is expected for the one-to-one scheme. The weak acidity of the ammonium ion precludes interference from protonated amino bonded phase during the titration.

The experimental titration curve is shown in Fig. 2. Surprisingly, two equivalence points were observed during the titration, one at pH 4.7 and the other at pH 9.7. This indicated that free phosphoric acid (no proton loss) was bound to the stationary phase surface, probably by hydrogen bonding with amino groups, silanol groups, ionically bonded phosphate groups, or combinations of these. However, based upon the volume of titrant used to reach the two equivalence points, it could be concluded

TABLE I

ELEMENTAL ANALYSES (%) OF THE PHOSPHORIC ACID–*n*-BUTYLAMINE REACTION PRODUCT

Product	*C*	*H*	*N*
Phosphoramide	31.4	7.90	9.15
Amine phosphate salt	28.1	8.25	8.18
Experimental	27.8	8.34	7.96

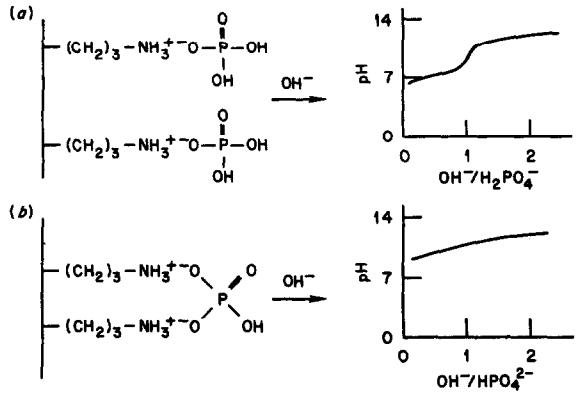

Fig. 1. Possible products of the reaction between phosphoric acid and amino stationary phase groups, and predicted titration curves for the products. (a) One-to-one reaction product, (b) two-to-one reaction product.

that most of the phosphoric acid was bonded to the stationary phase as amine dihydrogenphosphate salt groups (Fig. 1a). The $[H_2PO_4^-]/[H_3PO_4]$ ratio was 2.3:1. Furthermore, the total hydrogen bonded phosphoric acid plus dihydrogenphosphate salt on the stationary phase surface comprised 0.97 mmol phosphorus groups/g bonded phase. Nitrogen content of the original amino bonded phase was 1.38% which corresponded to a loading of 0.99 mmol amino groups/g bonded phase. Assuming that the free phosphoric acid was preferentially hydrogen bonded to amino groups (this assumption is reasonable, considering that the strength of a hydrogen bond is enhanced by acid–base interaction), the coverage of amino groups by phosphoric acid or dihydrogenphosphate was nearly complete (98% maximum coverage) and no appreciable amount of hydrogen phosphate was bonded to amino groups as suggested in Fig. 1b.

ICP spectrometry was used to quantitate directly the amount of phosphoric acid reacted with amino bonded phase groups. The result for a single phosphorus determination was 0.74 mmol phosphorus groups/g bonded phase, which agrees reasonably with the titration result. The lower value obtained by ICP may have been due in part to incomplete digestion of the bonded phase sample.

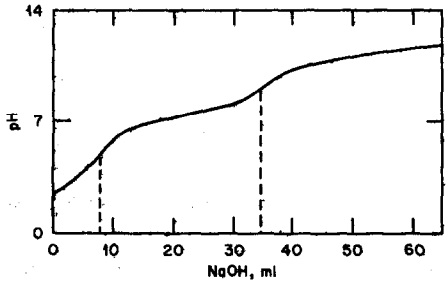

Fig. 2. Experimental titration curve for the product of the reaction between phosphoric acid and amino bonded stationary phase.

HPLC separations

Binary solvent gradients. Initial chromatographic study was directed toward developing effective separation of aromatic hydrocarbon (AH) classes in fossil fuels. These classes included unsubstituted AH, neutral nitrogen-containing AH (neutral NAH), hydroxy-AH (HAH), and basic nitrogen-containing AH (basic NAH) compounds. Overlap between polar compound classes was frequently observed when conventional HPLC stationary phases were employed in prefractionation schemes. A second goal of the preliminary studies was to determine if the extent of phosphoric acid modification was enough to produce separations significantly different from the unmodified amino bonded phase. This was accomplished by direct comparison of modified and unmodified columns.

Using simple hexane–isopropanol gradients, separations using both the modified and unmodified amino bonded phase columns were achieved on the basis of polarity. Polar compound classes were poorly resolved with both columns, as shown in Fig. 3a for the phosphoric acid-modified amino bonded phase. However, the selectivities of the columns for polar compounds were markedly different, confirming

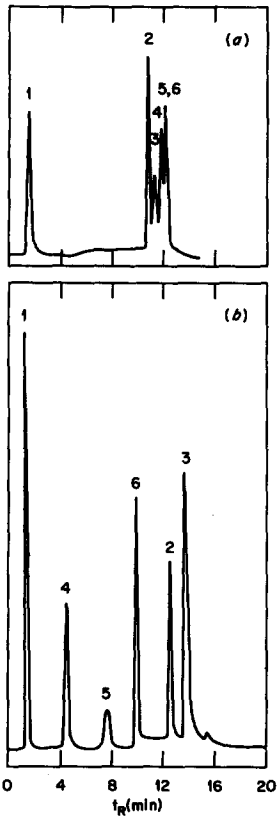

Fig. 3. Separation of model fossil fuel compounds on the phosphoric acid-modified amino bonded phase column. (a) Exponential gradient of 15 min from hexane to isopropanol, (b) 6 min isocratic elution with 5% acetic acid in hexane followed by a 15-min linear gradient to isopropanol. Peak identities: 1 = phenanthrene, 2 = pyridine, 3 = acridine, 4 = carbazole, 5 = phenol, and 6 = 1-naphthol.

that phosphoric acid had modified the amino bonded phase surface considerably. Using the same solvent conditions, acidic compounds such as phenol eluted last from the amino column whereas basic species (*e.g.* pyridine) were preferentially retained on the modified amino column. More subtle selectivity differences were noted for compounds of the same chemical class and will be reported in detail later.

In order to improve separation of the polar classes with the modified amino bonded phase column, glacial acetic acid was added at 5% (v/v) to the hexane mobile phase. The acetic acid displaced neutral NAH and HAH compounds from the column earlier in the gradient while only minimally reducing the retention of basic NAH species (Fig. 3b). Under these conditions, non-polar AH compounds such as phenanthrene eluted first from the column, followed by the displaced neutral NAH and HAH compounds. Basic NAH species eluted last from the modified amino column because of their strong hydrogen bonding interactions with the acidic dihydrogenphosphate and phosphoric acid hydroxyl groups. The separation of neutral and basic NAH classes was better than with an unmodified amino column[21]. The basic amino groups interacted preferentially with phenolic species (as noted above) and the neutral and basic NAH compounds were relatively weakly retained and overlapped.

The amount of acetic acid added to the hexane mobile phase had a pronounced effect on the separation with the modified amino bonded phase. From Table II it is apparent that added acetic acid increased mobile phase strength, capacity factors for all species being reduced. The desired elution sequence was maintained with high acetic acid content (10%), but resolution between classes was lowered significantly. For some samples this could lead to overlap of chemical classes, particularly those eluting early in the gradient. Very low acetic acid content (1.5%) gave inadequately resolved basic NAH (phenanthridine) and HAH (phenol) species, this behavior being similar to that found in the absence of acetic acid. A level of 3.5% acetic acid was chosen for subsequent work because it eluted HAH species before the basic NAH class while maintaining adequate resolution of early eluting compounds.

Ternary solvent gradients. As many complex samples such as synthetic fuels contain high levels of non-polar and moderately polar compounds, it was necessary to weaken the solvent strength at the beginning of the binary solvent gradient in order to separate these species more effectively into chemical classes with the modified amino bonded phase. The results using a ternary solvent program (hexane added to the

TABLE II

EFFECT OF ACETIC ACID CONCENTRATION IN THE HEXANE MOBILE PHASE ON THE RETENTION (k') OF MODEL FOSSIL FUEL COMPOUNDS

Compound	*Chemical class*	*Acetic acid concentration (%)*		
		1.5	*5*	*10*
Pentacene	ALH	2.43	1.01	0.58
Carbazole	Neutral NAH	5.04	2.29	0.87
Phenol	HAH	16.4	4.45	1.53
Phenanthridine	Basic NAH	15.5	9.04	3.96

beginning of the binary gradient) and an extensive series of standard compounds covering a wide range of functionalities are shown in Fig. 4 and Table III. Inspection of the early portion of the "elution sequence profile" (Fig. 4) showed that after aliphatic hydrocarbon (ALH) species, which were not retained by the modified amino bonded phase, non-polar AH compounds eluted well before the polar compounds. Included in the non-polar AH fraction were unsubstituted AH, alkylated AH, heterocyclic sulfur AH (SAH), and heterocyclic oxygen AH (OAH) compounds. Within each of these classes, elution was according to the number of fused aromatic rings. This allows for subfractionation of the non-polar aromatic compounds according to ring number, an objective of some previous studies[1,18].

As actic acid in hexane was added to the mobile phase, the moderately polar (very weak hydrogen bonding) AH compounds, including cyano- and nitro-substituted AH species as well as quinones, eluted quickly from the column. They were followed, in turn, by neutral NAH compounds such as carbazoles, indoles, and phenazines. Although the fraction eluting between 25 and 75% of 3.5% acetic acid in hexane was enriched in neutral NAH species, *ortho*-substituted alkyl-HAH compounds also eluted in this fraction because hydrogen bonding with their hydroxyl groups was sterically hindered. Unhindered HAH compounds eluted only after the addition of acetic acid was complete.

The basic NAH class was well-separated from the HAH class, eluting only when a significant proportion of isopropanol was present in the mobile phase. Amino-substituted AH (AAH) compounds eluted close together allowing an enriched "subfraction" of these species to be isolated. This is significant since AAH compounds have been implicated as the principal mutagenic components in synthetic fuels. It is interesting that the basic NAH compounds considered here eluted approximately according to their base strengths (Table III), indicating further that the basicity of these species was primarily responsible for their selective retention on the modified amino column. However, the elution order of basic NAH compounds is also strongly dependent on structural features. It has been reported that basic NAH compounds with sterically hindered nitrogens elute from polar bonded phase columns before their unhindered isomers[22]. In the present study, 7,8-benzoquinoline, containing a nitrogen

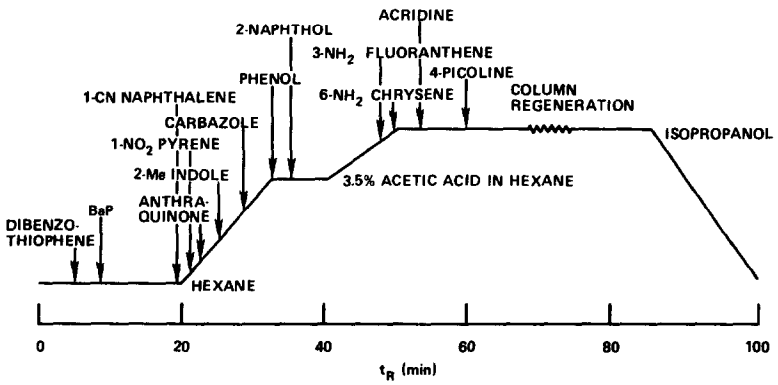

Fig. 4. Elution sequence profile of model fossil fuel compounds on the phosphoric acid-modified amino bonded phase column.

TABLE III

RETENTIONS OF MODEL FOSSIL FUEL COMPOUNDS

Compound	*Chemical class*	*k'*	*Compound*	*Chemical class*	*k'*
1-Octadecane	ALH	0.00			
1-Octadecene	ALH	0.00	2-Methylindole	Neutral NAH	10.4
Toluene	Non-polar AH	0.36	5-Methylindole	Neutral NAH	10.7
Thionaphthene	SAH	0.68	Indole	Neutral NAH	11.0
Naphthalene	Non-polar AH	0.72	Carbazole	Neutral NAH	11.1
Dibenzofuran	OAH	0.96	2,4-Dimethylphenol	HAH	11.4
Dibenzothiophene	SAH	1.24	2-Methylphenol	HAH	11.6
Fluorene	Non-polar AH	1.32	3,4-Dimethylphenol	HAH	13.1
Anthracene	Non-polar AH	1.72	3-Methylphenol	HAH	13.7
Pyrene	Non-polar AH	2.24	Phenol	HAH	14.0
Chrysene	Non-polar AH	4.40	1-Naphthol	HAH	14.1
Benzo[*a*]pyrene	Non-polar AH	5.76	2-Naphthol	HAH	15.3
Perylene	Non-polar AH	7.57	9-Phenanthrol	HAH	15.3
1-Nitronaphthalene	Nitro-AH	9.64	7,8-Benzoquinoline	Basic NAH (9.8)*	19.4
9-Nitroanthracene	Nitro-AH	9.68	3-Aminofluoranthene	Basic NAH	19.8
1-Cyanonaphthalene	Cyano-AH	9.72	6-Aminochrysene	Basic NAH	19.8
9-Cyanoanthracene	Cyano-AH	9.76	9-Aminophenanthrene	Basic NAH	20.3
9-Fluorenone	Quinone	9.76	1-Aminonaphthalene	Basic NAH (10.1)	20.3
1-Nitropyrene	Nitro-AH	9.84	Phenanthridine	Basic NAH (9.5)	20.6
Anthraquinone	Quinone	9.84	Quinoline	Basic NAH (9.1)	21.7
2,4,6-Trimethylphenol	HAH	9.92	5,6-Benzoquinoline	Basic NAH (8.9)	22.0
2,3,5-Trimethylindole	Neutral NAH	9.92	7-Azaindole	Basic NAH	22.6
Benzanthrone	Quinone	10.0	Acridine	Basic NAH (8.4)	22.7
1,4-Chrysenequinone	Quinone	10.0	Quinaldine	Basic NAH (8.2)	23.3
2,3-Dimethylindole	Neutral NAH	10.0	8-Hydroxyquinoline	Basic NAH; HAH	23.9
7-Methylindole	Neutral NAH	10.4	4-Picoline	Basic NAH	24.6

* pK_b values in parentheses.

shielded in a bay region, eluted from the modified amino column before phenanthridine and 5,6-benzoquinoline, containing unhindered nitrogens. Alkylated NAH species with sterically hindered nitrogens would also be expected to elute early in the basic NAH class fraction.

Finally, polyfunctional basic compounds were most strongly retained on the modified amino column. The second polar functional group enhanced retention; thus, 8-hydroxyquinoline eluted after quinoline. Other polyfunctional compounds, especially those of high molecular weight and possibly including species without basic functional groups, would be expected to elute in the final HPLC fraction.

Column regeneration

It was observed that column activity decreased after several (3–5) repetitions of the ternary solvent gradient. In particular, the basic NAH class eluted earlier and began to overlap the HAH compounds. However, column performance never reverted to that of an unmodified amino bonded phase column. This behavior was probably due to removal of some hydrogen bonded phosphoric acid (and acetic acid, some of which may have replaced or associated with the phosphoric acid during the elution sequence), but not the ionically bonded dihydrogenphosphate, from the stationary

phase surface when isopropanol, a strong protic solvent, was passed through the column. The modified amino bonded phase was easily regenerated by pumping 15–20 ml of 0.5% phosphoric acid in isopropanol through the column. In order to maintain retention reproducibility, the reactivation procedure was repeated between chromatographic runs, thus adding about 10 min to the ternary solvent program (Fig. 4). Continuous column regeneration using a low concentration of phosphoric acid in the isopropanol mobile phase could not be used because insoluble salts are formed with strongly basic solutes such as acridine.

Practical considerations

The facile preparation procedure is an attractive feature of the phosphoric acid-modified amino bonded phase. Many other stationary phases for HPLC might be produced as easily provided that a suitably acidic functionality is present on the organic or inorganic modifier. It has been demonstrated that the efficacy of synthesis and characterization of new bonded phases can be quickly evaluated using *n*-butylamine as a model for amino bonded phase material.

The phosphoric acid-modified amino bonded phase column effectively fractionated model fossil fuel compounds into the following chemical classes: (a) aliphatic hydrocarbons, (b) non-polar aromatic hydrocarbons, (c) neutral/acidic polar aromatic hydrocarbons (including quinone, cyano-, and nitro-aromatic hydrocarbon enriched, neutral nitrogen-containing aromatic hydrocarbon enriched, and hydroxy aromatic hydrocarbon enriched subclasses), and (d) basic polar aromatic hydrocarbons (including an aminoaromatic hydrocarbon enriched subclass). The elution sequence of polar compounds is complementary to that obtained with an amino bonded phase; the columns could be used for two-dimensional fractionation of polar mixtures.

The ternary gradient method developed for the phosphoric acid-modified amino bonded phase separates complex mixtures in about 1 h, followed by a short step to reactivate the stationary phase. The method can be scaled up to semi-preparative size and used to prefractionate synthetic fuel samples as will be reported in detail elsewhere[23].

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