

See discussions, stats, and author profiles for this publication at: <https://www.researchgate.net/publication/11997763>

# Covalently Bound Ionene Polyelectrolyte–Silica Gel Stationary Phases for HPLC

ARTICLE *in* ANALYTICAL CHEMISTRY · MAY 2001

Impact Factor: 5.64 · DOI: 10.1021/ac001003x · Source: PubMed

CITATIONS

13

READS

26

7 AUTHORS, INCLUDING:



**Frank Quina**

University of São Paulo

165 PUBLICATIONS 4,409 CITATIONS

SEE PROFILE



**Alain Berthod**

Claude Bernard University Lyon 1

260 PUBLICATIONS 5,852 CITATIONS

SEE PROFILE



**Mustafa Culha**

Yeditepe University

96 PUBLICATIONS 1,520 CITATIONS

SEE PROFILE



**Willie L Hinze**

Wake Forest University

82 PUBLICATIONS 3,716 CITATIONS

SEE PROFILE

# Covalently Bound Ionene Polyelectrolyte-Silica Gel Stationary Phases for HPLC

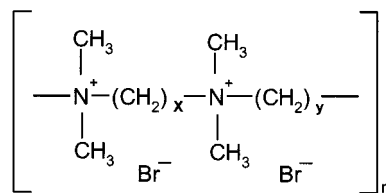
Yasuzo Suzuki,<sup>†</sup> Frank H. Quina,<sup>‡</sup> Alain Berthod,<sup>§</sup> Ronald W. Williams, Jr.,<sup>||</sup> Mustafa Culha,<sup>⊥</sup> Imdad Ullah Mohammadzai,<sup>#</sup> and Willie L. Hinze\*

Department of Chemistry, Wake Forest University, P.O. Box 7486, Winston-Salem, North Carolina, 27109

**Micelle-mimetic ionene-based stationary phases for high-performance liquid chromatography (HPLC) are prepared by attaching [3,16]- and [3,22]-ionenes to aminopropyl silica through a carbon–nitrogen bond. These [x,y]-ionenes are polyelectrolytic molecules consisting of dimethylammonium charge centers interconnected by alternating alkyl chain segments containing x and y methylene groups, some of which can form aggregate species whose properties mimic those of conventional surfactant micelles. These ionene-bonded stationary phases were characterized using different recommended HPLC test mixtures. Test solute chromatographic behavior on the ionene phases was found to be similar to that of intermediate oligomeric or polymeric C-18 and/or phenyl phases, depending upon the specific test mixture employed. In addition, the phases exhibit significant solute shape recognition ability. The ionene stationary phases were successfully employed for the separation of the components of the recommended ASTM reversed-phase test mixture, as well as for ortho-, meta- and para-disubstituted benzenes and other positional or geometric isomeric compounds. The ionene materials allow for chromatographic separations under either reversed-phase or ion-exchange conditions. The retention mechanism on these multimodal phases can occur by hydrophobic partitioning or electrostatic interactions, depending upon the characteristics of the components of the analyte mixture (neutral or anionic). The effects of alteration of the percent organic modifier, flow rate and temperature of the mobile phase on chromatographic retention and efficiency on these phases were briefly examined.**

Chemically modified silicas have been widely employed as stationary-phase supports in chromatography. Although a variety

of stationary phases for HPLC have been prepared by immobilization of organic ligands or polymers on silica gels, there appear to be no prior reports concerning immobilization of micelle-mimetic ionenes onto silica.<sup>1–6</sup> [x,y]-ionenes are linear cationic polyelectrolytes consisting of dimethylammonium charge centers interconnected by alternating chain segments containing x and y CH<sub>2</sub> groups, respectively (see structure I). Previous reports indicate



[x,y]-Ionene Bromide (I)

that ionenes containing short segments, that is, the [3,6]-, [6,6]-ionenes, and the [3,y]-ionenes in which y < 14, exhibit behavior typical of polyelectrolytes with rodlike or extended conformations.<sup>7</sup> In contrast, the [3,y]-ionenes in which y is increased beyond 14 progressively form more compact (globular or micelle-like) aggregate structures and exhibit many of the properties (substrate solubilization, catalytic rate enhancements, counterion binding, etc.) reminiscent of those observed for aqueous cationic micellar solutions.<sup>7–9</sup> As a consequence, such ionenes have been dubbed micelle-mimetic agents.

When compared to aqueous micellar media, the ionenes possess several advantages. For example, ionenes are less tensoactive, and their resulting solutions do not foam. In addition,

\* Corresponding author: hinze@wfu.edu.

<sup>†</sup> Present address: National Institute of Materials and Chemical Research, Tsukuba, Japan.

<sup>‡</sup> Present address: Instituto de Quimica, Universidade de São Paulo, São Paulo, Brazil.

<sup>§</sup> Present address: Laboratoire des Sciences Analytiques, Université Claude Bernard, 69622 Villeurbanne cedex, France.

<sup>||</sup> Present address: Syngenta Crop Protection, P.O. Box 18300, Greensboro, NC.

<sup>⊥</sup> Present address: Department of Chemistry, University of Tennessee, Knoxville, TN.

<sup>#</sup> Present address: Department of Chemistry, University of Peshawar, Peshawar, Pakistan.

- (1) Scott, R. P. W. *Silica Gel and Bonded Phases, Their Production, Preparation and Use in LC*; Separation Science Series; John Wiley: Chichester, 1993; Vol. 4.
- (2) *Packing and Stationary Phases in Chromatographic Techniques*; Chromatographic Science Series; Unger, K. K., Ed.; Marcel Dekker: New York, 1990; Vol. 47.
- (3) Buszewski, B.; Jezierska, M.; Weiniak, M.; Berek, D. *J. High Resol. Chromatogr.* **1998**, *21*, 267–281.
- (4) Petro, M.; Berek, D. *Chromatographia* **1993**, *37*, 549–561.
- (5) Majors, R. E. *LC-GC* **1993**, *11*, 778–888.
- (6) Hanson, M.; Unger, K. K. *Trends Anal. Chem.* **1992**, *11*, 368–373.
- (7) Soldi, V.; Erimann, N. M.; Quina, F. H. *J. Am. Chem. Soc.* **1988**, *110*, 5137–5143.
- (8) Hinze, W. L.; Moreno, B.; Quina, F. H.; Suzuki, Y.; Wang, H. *Anal. Chem.* **1994**, *66*, 3449–3457.
- (9) Hinze, W. L.; Feng, L. W.; Moreno, B.; Quina, F. H.; Suzuki, Y.; Wang, H. *Anal. Sci.* **1995**, *11*, 183–187.

organic solvents can be used to extract solutes from aqueous ionene solutions without the formation of troublesome emulsions. Because they are intrapolymeric aggregate species, ionenes possess no critical micelle concentration (cmc), thus allowing for their use under a greater variety of solution conditions (i.e., organic cosolvents and other additives do not impact the aggregate structure as severely as that of conventional detergent micelles).<sup>7-9</sup> Perhaps the most important distinction arises from the fact that micelles are in equilibrium with free surfactant monomer and cannot be immobilized on solid supports. In contrast, ionenes, which are intramolecular species, should be amenable to chemical attachment to supports.

Previously, the advantageous use of such micelle-mimetic ionenes in chemical analysis had been reported.<sup>8,9</sup> As in conventional micellar liquid chromatography,<sup>10</sup> where surfactant micelles are present in the mobile phase, ionenes can also be successfully used in a similar fashion as a mobile phase component.<sup>9</sup> In addition, some ionenes (of the type that presumably do not form micelle-like entities) have been electrostatically coated or sorbed onto silica, Silasorb C<sub>8</sub>, or polymeric supports and been shown to function as ion chromatographic stationary phases for the separation of anions.<sup>11-13</sup> Given that the [3,16]- or [3,22]-ionenes are intramolecular aggregate-forming species, it was interesting to attempt to link them covalently to aminopropyl silica stationary phases in the hopes of obtaining micelle-like, or perhaps some other novel, characteristics. Herein, the synthesis, characterization, and an evaluation of the chromatographic performance of a series of chemically bonded micelle-mimetic ionenes attached to a high purity, spherical silica gel are described. The chromatographic performance of the two new ionene-derivatized silica stationary phases based upon [3,16]- and [3,22]-ionene was evaluated by employing a wide selection of test mixtures, and the results were compared to those obtained using commercially available C-18, phenyl, or amine stationary phases.

## EXPERIMENTAL SECTION

**Instrumentation.** Three modular liquid chromatographic systems (Waters Associates) were utilized. The first consisted of two model 510 pumps, a U6K injector, a model 680 automatic gradient controller, and a model 484 Lambda-Max LC spectrophotometric detector (14- $\mu$ L cell, 1-cm path length) with the detector response monitored by a Fisher Recordall series 5000 strip chart recorder. The second LC system was the same, except that two model 501 pumps were employed in conjunction with a model 991 photodiode array detector (8- $\mu$ L cell, 1-cm path length) and a 5200 Printer Plotter. The third chromatographic system was as described above, except that the data was acquired on a DataQ DI150-RS system operated with Windaq Acq software under Microsoft Windows 3.1 on an IBM-compatible computer. For chromatographic runs at elevated temperature, a Timberline Instruments model H-500 aluminum block HPLC column heater/controller was used in conjunction with a Timberline solvent

preheater (both obtained from Rainin Instruments Co., Woburn, MA) to control the column and mobile phase temperature. For some comparative studies, a 100 (or 150)  $\times$  4.6 mm, 5  $\mu$ m C-18 or amine column (Advanced Separation Technologies, Inc., Whippany, NJ) was employed.

Elemental analyses were performed by Atlantic Microlab, Inc. (Norcross, GA).

**Materials. Reagents and Solutions.** The test mixture for base deactivation was composed of phenol (Mallinckrodt) and pyridine (Aldrich). A test mixture for evaluation of reversed phases composed of naphthalene and 1-nitronaphthalene (Aldrich) was prepared as described in the literature.<sup>14</sup> The column selectivity test mix (SRM 869; mixture composed of the polycyclic aromatic hydrocarbons benzo[a]pyrene (BAP), tetrabenzonaphthalene (TBN) and phenanthro[3,4c]phenanthrene (PhPh) dissolved in acetonitrile) was obtained from the National Institute of Standards & Technology (Gaithersburg, MD).

The recommended six-component ASTM test mix<sup>15</sup> composed of the solutes benzyl alcohol, acetophenone, methyl benzoate, benzaldehyde, benzene, and dimethyl terephthalate (all either Fisher Scientific or Aldrich materials) was also employed in this work. This test-mix solution was prepared by adding the indicated amounts of each solute, benzyl alcohol (10  $\mu$ L), benzaldehyde (10  $\mu$ L), benzene (10  $\mu$ L), methyl benzoate (10  $\mu$ L), acetophenone (2  $\mu$ L), and dimethyl terephthalate (1–2 mg), to 10.0 mL of HPLC grade methanol. An ortho-, meta-, and para- positional isomeric test mixture composed of 2-phenylphenol (Eastman) and 3- and 4-phenylphenol (Aldrich) was prepared by dissolution of  $\sim$ 4 mg of each component in 10.0 mL of methanol or acetonitrile.

All of the alkylbenzene homologous series test solutes, benzene, toluene, ethylbenzene, *n*-propylbenzene, *n*-butylbenzene, and *n*-pentylbenzene were used as received (Aldrich). The other test solutes included 1-nitroso-2-naphthol (Sigma); 2-nitroso-1-naphthol (Sigma); triphenylene (Aldrich); *o*-terphenyl (Aldrich); *o*-, *m*-, and *p*-cyanophenol (Aldrich); and *cis*- and *trans*-stilbene (Aldrich). Either HPLC grade water (Fisher Scientific Co.) or in-house distilled, purified (Milli-Q Reagent Water System from Millipore Corp.) water was used to prepare all of the aqueous solutions and mobile phases.

**Preparation of 3-Aminopropyl-Bonded Silica Gel.** The aminopropylated silica gel (3-APS) was prepared as previously described in the literature.<sup>16</sup> The stationary-phase base material used in this preparation was 5.1  $\mu$ m spherical acid-washed silica (lot no. 890109) as obtained from Advanced Separation Technologies, Inc. (Whippany, NJ). This silica had a pore size of 80 Å, pore volume of 0.49 cm<sup>3</sup>/g and a surface area of 180 m<sup>2</sup>/g, according to data provided by the supplier. 3-Aminopropyltriethoxysilane, APTES (Aldrich), was purified by vacuum distillation. The silica gel (6.75 g) was activated by heating overnight at 150 °C. This dried silica was added to 200 mL of dry toluene and refluxed. After residual water had been azeotropically removed, 2.0 mL of 3-aminopropyltriethoxysilane was added, and this mixture refluxed. After 3 h,  $\sim$ 40 mL of ethanol containing toluene

(10) Berthod, A.; Garcia-Alvarez-Coque, M. C. *Micellar Liquid Chromatography*; Chromatographic Science Series, Vol. 83, Marcel Dekker: New York, 2000.

(11) Pirogov, A. V.; Krokhin, O. V.; Platonov, M. M.; Deryugina, Y. I.; Shpigun, O. O. *J. Chromatogr.* **2000**, *884*, 31–39.

(12) Krokhin, O. V.; Smolenkov, A. D.; Svintsova, N. V.; Obrezkov, O. N.; Shpigun, O. A. *J. Chromatogr.* **1995**, *706*, 93–98.

(13) Pirogov, A. V.; Platonov, M. M.; Shpigun, O. A. *J. Chromatogr.* **1999**, *850*, 53–63.

(14) Verzele, M.; Dewaele, C. *Chromatographia* **1984**, *18*, 84–86.

(15) Subcommittee E-19.08 Task Group on Liquid Chromatography of the American Society for Testing and Materials, *J. Chromatogr. Sci.* **1981**, *19*, 338–348.

(16) Normura, A.; Yamade, J.; Tsunoda, K. *Anal. Sci.* **1987**, *3*, 209–212.

Table 1. Preparation Conditions and Elemental Analyses of 3-Aminopropyl-Bonded Silica

run	silica gel, g	refluxing time, h	elemental analysis, %			C/N ratio	coverage <sup>a</sup> , $\mu\text{mol}/\text{m}^2$
			C	H	N		
1	6.75	5.0	2.41	0.54	0.61	3.5	2.51 (2.57) <sup>b</sup>
2	3.25	5.5	2.80	0.79	0.81	3.5	3.39 (3.41) <sup>b</sup>
3 <sup>c</sup>	<i>d</i>	6.0	3.84	0.97	1.07	3.6	4.56 (4.79) <sup>b</sup>

<sup>a</sup> In terms of aminopropyl groups. <sup>b</sup> Based upon % nitrogen; number in parentheses based upon % carbon. <sup>c</sup> The product obtained from this run was subsequently utilized for immobilization of the ionenes. <sup>d</sup> Silica obtained from run 2 was used.

was removed via distillation, and the reaction mixture refluxed for another hour. This procedure was repeated twice. The cooled silica material was filtered and washed with hexane and then methanol, followed by drying over  $\text{P}_2\text{O}_5$  in vacuo. The yield obtained for this specific run was 6.40 g. Table 1 summarizes the reaction conditions, yields, and elemental analyses for several typical runs.

**Immobilization of the Ionenenes on the Aminopropylated Silica Gel.** The two ionenes, that is, [3,16]- and [3,22]-ionene, were synthesized as previously described.<sup>7,8,17</sup> Immobilization via coupling to the bonded 3-APS was accomplished using the following two methods:

**Method A.** To a methanol solution of [3,16]-ionene (1.77 g in 5.0 mL) was added 1.55 g of 3-aminopropylated silica, and the resulting slurry rotatory-mixed at 40 °C. After 140 h, an additional 0.54 g of 3-aminopropylated silica and 0.39 g of the ionene were added, and the reaction continued for an additional 280 h at 40 °C. After reaction, the silica material was filtered, washed with methanol and ethyl ether, and then dried over  $\text{P}_2\text{O}_5$  in vacuo. The final yield was 2.05 g from this specific preparation.

**Method B.** To 10.0 mL of methanol containing 0.83 g of [3,22]-ionene was added 1.80 g of 3-aminopropylated silica. The mixture was reacted for 140 h at room temperature under sonication. A few milliliters of a methanol solution of the same composition as noted above were added every 24 h in order to maintain the initial volume. After reaction, the silica material was filtered, washed with methanol and ethyl ether, and dried over  $\text{P}_2\text{O}_5$  in vacuo. The yield was 2.13 g.

These ionene-bonded phases were not end-capped. Thus, residual unreacted silanol groups from the base silica, the unreacted primary amino moieties from the intermediate aminopropyl silica material, and the terminal tertiary amine group of each of the ionene molecules immobilized are present in these stationary phase materials. Both of the bonded ionene stationary phases were packed using the slurry method into 10 cm  $\times$  4.6 mm stainless steel columns by Advanced Separation Technologies, Inc. (Whippany, NJ).

**Procedures.** Mobile phases were prepared at room temperature and filtered through a 0.45- $\mu\text{m}$  membrane prior to use. The test mixtures were prepared by dissolution of the individual components in methanol or ethanol and were stored refrigerated in the dark when not in use. Unless otherwise noted, the chromatographic runs were performed at room temperature (21–25 °C) using a mobile-phase flow rate of 1.00 mL/min. Typically, 5–20  $\mu\text{L}$  of the individual test solute (or mixture), at a concentration of 0.1–10 mg/mL in methanol, ethanol, or the mobile phase,

were injected. All chromatographic peaks were assigned by the injection of single solutes under identical elution conditions. The retention factors reported for each test solute were based on the average of at least two (in most instances, three) determinations. The specific chromatographic analysis conditions for the different test mixtures are listed in the respective Tables and Figures. Chromatographic parameters, such as retention, selectivity, and resolution factors were calculated in the usual manner. The column void volume (or time) was taken as the time from injection to the baseline disturbance provoked by the solvent in which the sample was dissolved (or by the injection of distilled water,  $\text{H}_2\text{O}$ , or  $\text{D}_2\text{O}$ ). Depending upon the specific mobile phase examined the void time,  $t_0$ , was in the range of ~60 to 78 s at a flow rate of 1.00 mL/min for both the [3,16]- and [3,22]-ionene columns. The number of theoretical plates,  $N$ , and the peak asymmetry factor,  $A_{0.1}$ , were calculated as described by Foley and Dorsey.<sup>18</sup> The reduced plate height,  $h$ , was calculated as

$$h = H/d_p \quad (1)$$

where  $d_p$  is the particle size and  $H$  (or HETP) is the height equivalent to a theoretical plate ( $= L/N$ ), and the linear velocity,  $\mu$ , is calculated as

$$\mu = L/t_0 \quad (2)$$

where  $L$  is the column length and  $t_0$  the retention time of the void marker.<sup>19</sup>

## RESULTS AND DISCUSSION

**Preparation of the Ionene-Bonded Phases.** A two-step reaction scheme was used to attach the [3,16]- and [3,22]-ionenes covalently onto silica. First, a reactive aminopropyl spacer was bonded directly to the silica surface, followed by the linking of the ionene polymer to this spacer. The required [3,16]- and [3,22]-ionenes can be easily prepared in good yields as previously reported.<sup>7,8</sup> The reaction of 3-aminopropyltriethoxysilane (APTES) with silica was carried out in refluxing toluene,<sup>16</sup> and the results are summarized in Table 1. In this reaction, if each aminopropylsilyl (APS) group bonds onto the silica surface by elimination of three ethanol molecules from the ethoxy group of APTES, then the carbon/nitrogen ratio in the APS-bonded phase should be equal to three.<sup>16</sup> On the basis of the C/N ratio of the APS (Table 1), ~0.50 ethoxy carbon atoms, or roughly 0.25 ethoxy groups,

(18) Foley, J. P.; Dorsey, J. G. *Anal. Chem.*, **1983**, *55*, 730–737.

(19) Pryde, A.; Gilbert, M. T. *Applications of High Performance Liquid Chromatography*; Chapman and Hall: London, 1979, Chapter 2.

(17) Kunitake, T.; Tsuge, A.; Takarabe, K. *Polymer J.* **1985**, *17*, 633–640.



remain per bonded aminopropyl group. If this is taken into account, then the surface concentration of bonded 3-aminopropyl groups on the silica can be calculated from eq 3

$$\text{coverage } (\mu\text{mol}/\text{m}^2) = [10^6/S_{\text{bet}}] [(100 \text{ AM } (n_{\text{N or C}})/P_{\text{N or C}}) - \text{MM} + \delta]^{-1} \quad (3)$$

where  $P_{\text{N or C}}$  is the percent nitrogen or carbon increase in the bonded phase as determined by elemental analysis, AM is the atomic mass of nitrogen or carbon,  $n_{\text{N or C}}$  is the number of nitrogen or carbon atoms present in the bonded group, MM is the molecular mass of the bonded group,  $S_{\text{bet}}$  is the specific surface area of the unbonded base silica in  $\text{m}^2/\text{g}$ , and  $\delta$  is the correction factor for desorbed water (usually taken as 5)<sup>21,24</sup> [note: the original Berendsen and De Galan equation results if  $\delta = 0$ ].<sup>25</sup> Assuming an intrinsic coverage of silanol groups on the silica gel surface of  $\sim 8 \mu\text{mol}/\text{m}^2$ <sup>26,27</sup> the observed aminopropyl group coverage of 2.5–4.5  $\mu\text{mol}/\text{m}^2$  (Table 1) corresponds to the reaction of about 30–60% of the surface silanol groups. The coverage of the 3-aminopropyl moiety on the silica can be increased by repetition of the silanization reaction (Table 1, run 3). The coverage values that were obtained appear to be in the range previously reported in the literature for 3-aminopropylsilica materials (2.3–5.6  $\mu\text{mol}/\text{m}^2$ ).<sup>28–31</sup>

The ionenes were then covalently bonded to this 3-aminopropyl-bonded silica material. The extent of ionene coverage on the aminopropyl silica was estimated from the increase in the nitrogen content of the silica phase following ionene immobilization. The difference in nitrogen content between the ionene-based silica and the aminopropyl silica was 0.34% and 0.10% for the [3,16]- and [3,22]-ionene phases, respectively. This corresponds to an ionene coverage of roughly 0.041 and 0.014  $\mu\text{mol}/\text{m}^2$ , respectively. This calculation of the ionene coverage using eq 3 requires knowledge of the molecular mass of the respective ionenes. The ionene coverage calculation was based on an average number of repeat units of 17.5 in the bonded ionene polymers.<sup>7</sup> These particular ionene molar masses (9000–10 300 Da) are in the range of values (8000–15 000 Da) reported in the literature for such or similar ionene materials.<sup>13,32</sup>

Fluorescence probe studies have shown that in water, the [3,16]- and [3,22]-ionenes spontaneously adopt globular conformations via intrapolymeric aggregation of the long methylene segments.<sup>7</sup> Bound probe molecules (such as pyrene) experience a partially nonaqueous solubilization microenvironment, with a modest decrease in aqueous character upon going from the [3,

16]- to the [3,22]-ionene.<sup>7</sup> When utilized with highly aqueous mobile phase compositions, the surface-immobilized ionenes can, thus, be expected to adopt an aggregated conformation, which exhibits a high degree of orientation of the long alkyl chain repeat units of the ionene on the stationary phase. Such micelle-mimetic aggregate structures should interact with a variety of functionalized organic molecules through a partition mechanism like that involved in the binding of solute molecules to cationic surfactant micelles or vesicles.

## GENERAL CHARACTERIZATION OF THE IONENE-BONDED PHASES

**Test for Residual Silanols.** Various test mixtures were utilized in order to evaluate the general chromatographic characteristics of these ionene-bonded phases. First, an analyte mixture composed of pyridine ( $pK_b$  8.81)<sup>33</sup> and phenol ( $pK_a$  10.0)<sup>33</sup> was employed in a neutral mobile phase to test for base deactivation, which is an indicator of residual exposed silanol groups on the surface of the silica material.<sup>24,27,34</sup> With this test mixture, a well-deactivated stationary phase should exhibit good peak shape for both pyridine and phenol, with phenol eluting later than pyridine. Pyridine will be retained proportionately longer than phenol on packing materials that have a greater number of exposed silanol sites. The results obtained with this test mixture on the [3,16]- and [3,22]-ionene-bonded-phase columns are summarized in Table 2. For the ionene-bonded phases, the behavior is similar to that seen on well-end-capped C-18 phases, that is, pyridine elutes prior to phenol, even though unreacted residual silanols are presumably present on the silica surface of these ionene materials. This suggests that both the acidic and basic silanol sites are shielded by the ionenes and do not contribute to the separation process for these analytes. This unusual behavior may be due to the interaction of the large, polymeric ionene molecules with the free silanols, internally deactivating them or blocking the access of small analytes to the active silanols. A similar explanation has been used to rationalize the fact that cyclodextrin and amide-functionalized bonded (or polymer-coated alumina) phases, which also contain unreacted silanols (or free  $-\text{OH}$  groups of the alumina support), nonetheless exhibit chromatographic performance typical of well-end-capped C-18 phases.<sup>24,34,35</sup> It should be noted that in control experiments on a commercial aminopropyl silica column (with no bonded ionene), the opposite behavior was observed, that is, pyridine eluted after phenol.

**Test for Lewis Acidity and Metal Traces.** Next, the test mixture composed of naphthalene, 1-nitronaphthalene, and acetylacetone proposed by Verzele and Dewaele<sup>14</sup> was utilized to evaluate the ionene-bonded phases. For properly endcapped octadecylsilica (ODS) phases, the selectivity factor for naphthalene relative to 1-nitronaphthalene,  $\alpha_{\text{N/NN}} > 1.4$ , but for nonendcapped ODS phases,  $\alpha = 1.1$ –1.2. Reversal of the elution order of naphthalene and 1-nitronaphthalene is observed on phenyl-bonded silica phases (i.e.,  $\alpha_{\text{N/NN}} = 0.50$ –0.90, depending upon whether they are endcapped or not).<sup>14</sup> The chromatographic results obtained on the ionene phases are tabulated in Table 3. In all

(20) Unger, K. K.; Becker, N.; Roumeliotis, P. J. *J. Chromatography*, **1976**, *125*, 115–127.

(21) Amati, D.; Kovats, E. *Langmuir*, **1987**, *3*, 687–695.

(22) Pesek, J. J.; Matyska, M. T.; Soczewinski, E.; Christensen, P. *Chromatographia*, **1994**, *39*, 520–528.

(23) Sandoval, J. E. *J. Chromatogr.*, **1999**, *852*, 375–381.

(24) Ascar, T. L.; Kallury, K. M. R.; Szafranski, C. A. *J. Liq. Chromatogr.*, **1996**, *19*, 3049–3073.

(25) Berendsen, G. E.; DeGalan, L. *J. Liq. Chromatogr.*, **1978**, *1*, 561–586.

(26) Cheng, W.; McCown, M. *J. Chromatogr.*, **1985**, *318*, 173–176.

(27) Feibush, B. *J. Liq. Chromatogr. Rel. Technol.*, **1996**, *19*, 2315–2328.

(28) Hargitai, T.; Kaida, Y.; Okamoto, Y. *J. Chromatogr.*, **1993**, *628*, 11–22.

(29) Kibbey, C. E.; Meyerhoff, M. E. *Anal. Chem.*, **1993**, *65*, 2189–2196.

(30) Xiao, J.; Meyerhoff, M. E. *Anal. Chem.*, **1996**, *68*, 2818–2825.

(31) Buszewski, B.; Lodkowski, R. *J. Liq. Chromatogr.*, **1991**, *14*, 1185–1201.

(32) Frommel, J.; Wolff, T. *J. Colloid Interface Sci.*, **1998**, *201*, 86–92.

(33) *The Merck Index*, 11th ed.; Budavari, S., Ed.; Merck & Co., Inc.: Rahway, NJ, 1989; pp 104, 1150, 1267.

(34) Arenas, R. V.; Foley, J. P. *Am. Lab.*, **1994**, *32*, 32CC–32JJ.

(35) *Cyclobond Handbook*; Advanced Separation Technologies, Inc.: Whippany, NJ, 1995; p 2.

Table 2. Summary of Chromatographic Parameters Obtained for the Base Deactivation Test Mixture<sup>a</sup>

column	mobile phase	$K_{\text{pyridine}}$	$K_{\text{phenol}}$	$\alpha_{\text{phenol/pyridine}}^b$
[3,16]-ionene	50:50 (v/v) MeOH:H <sub>2</sub> O	0.22	1.26	5.73
	25:75 (v/v) MeOH:H <sub>2</sub> O	0.39	3.33	8.54
		0.33 <sup>c</sup>	2.72 <sup>c</sup>	8.24
	15:85 (v/v) MeOH:H <sub>2</sub> O	0.47 <sup>c</sup>	3.12 <sup>c</sup>	6.63
[3,22]-ionene	50:50 (v/v) MeOH:H <sub>2</sub> O	0.21	1.33	6.33
	40:60 (v/v) MeOH:H <sub>2</sub> O	0.27	2.08	7.70
		0.30 <sup>d</sup>	1.95 <sup>d</sup>	6.50 <sup>d</sup>

<sup>a</sup> Conditions: 10 cm × 4.6 mm column; mobile phase flow rate, 1.00 mL/min; detector wave wavelength, 254 nm; 25.0 °C;  $P$  = 1933 psi; chromatographic system 1. <sup>b</sup> Selectivity (or separation) factor. <sup>c</sup> Conditions as in footnote a, except that  $T$  = 20.4 °C,  $P$  = 1500 psi, chromatographic system 2. <sup>d</sup> Results obtained on chromatographic system 3 on a 5-year-old column.

Table 3. Summary of Chromatographic Parameters for Reversed-Phase Test Mixture<sup>a</sup>

column	mobile phase	$K_N^b$	$K_{NN}^b$	$\alpha_{N/NN}^c$
[3,16]-ionene	50:50 (v/v) MeOH:H <sub>2</sub> O	1.53	1.92	0.80
	25:75 (v/v) MeOH:H <sub>2</sub> O	6.28	9.70	0.65
[3,22]-ionene	50:50 (v/v) MeOH:H <sub>2</sub> O	1.79	2.06	0.87
	40:60 (v/v) MeOH:H <sub>2</sub> O	3.92	4.64	0.85

<sup>a</sup> Conditions: chromatographic system 1; flow rate, 1.00 mL/min; 25.0 °C; column back-pressure, 1680–1865 psi; UV detection at 254 nm. <sup>b</sup>  $N$  refers to naphthalene, and  $NN$  refers to nitronaphthalene. <sup>c</sup> Selectivity factor for naphthalene/nitronaphthalene.

cases, naphthalene eluted prior to nitronaphthalene, with the selectivity factor,  $\alpha_{N/NN}$ , ranging from 0.65 to 0.87 (Table 3). On the basis of this test-mix criterion, the ionene phases appear to function in a manner akin to that of some phenyl-bonded phases. For comparison, several runs were also performed on an amino-propyl silica column using 40:60 (v/v) methanol (or acetonitrile): water as the mobile phase. Although the retention times for naphthalene and nitronaphthalene were close to the column void time and their peaks overlapped under these conditions, naphthalene did elute slightly prior to nitronaphthalene, which is the same order as observed on the ionene-bonded phases.

This test mixture also contains acetylacetone to test for metal activity.<sup>14</sup> However; no results with this probe are presented for the ionene phases, due to the fact that Engelhardt and Lobert questioned the use of such 1,3-diketones as markers for trace metals in a recent publication.<sup>36</sup>

**Monomeric Versus Polymeric Character.** The ionene phases were next characterized using the NIST Column Selectivity (SRM 869) test mixture as proposed by Sander and Wise<sup>37–42</sup> (for recent overviews and comparisons based on this standard, see refs 43 and 44). This test mixture contains three polycyclic aromatic hydrocarbons that differ in their three-dimensional shape, that is, benzo[a]pyrene (BaP, a planar molecule), tetrabenzonaph-

thalene (TBN, saddle-like shape), and phenanthro[3,4-*c*]phenanthrene (PhPh, helical-spiral-shaped and the least planar molecule) and serves to classify the C-18 surface bonding as monomeric-like, polymeric-like or intermediate, on the basis of the elution order observed. For monomeric-like phases, the elution order is usually BaP < PhPh < TBN, whereas for polymeric-like phases, it is PhPh < TBN < BaP. Thus, on polymer-type phases, bulky solutes elute prior to planar molecules and narrow, long species elute after square-shaped molecules.<sup>41</sup> The selectivity factors,  $\alpha_{\text{TBN/BaP}}$  and  $\alpha_{\text{PhPh/BaP}}$ , are indicative of the relative monomeric or polymeric nature of the bonded phase and can be utilized to predict column selectivity toward more complex polyaromatic hydrocarbon mixtures on the basis of their molecular planarity.<sup>41</sup> Values of  $\alpha_{\text{TBN/BaP}} < 1.0$  are typical of polymeric-like phases, possessing a higher degree of surface chain ordering, that exhibit greater selectivity toward PAHs, and those with  $\alpha_{\text{TBN/BaP}} > 1.7$  are typical of monomeric-like phases with less selectivity. Phases whose selectivity values are between these values ( $1.0 < \alpha_{\text{TBN/BaP}} < 1.7$ ) are classified as intermediate.<sup>37–44</sup>

On both of the ionene phases, the elution order observed was PhPh < BaP < TBN (Table 4), which corresponds to an oligomeric or intermediate-type bonded-phase classification for C-18 supports.<sup>37–44</sup> The selective retention of the planar BaP molecule relative to that of the larger, helically shaped PhPh molecule demonstrates the ability of the ionene phases to discriminate different PAH molecules on the basis of their shape. Based upon this test mixture and the  $\alpha_{\text{TBN/BaP}}$  values, the ionene phases exhibit behavior that is intermediate between that displayed by densely loaded monomeric or lightly loaded di- or trifunctional polymeric C-18 phases.<sup>39</sup> For instance, this elution order is similar to that previously reported for a monomeric phase with a C-22 alkyl-chain moiety<sup>39</sup> or that of intermediate oligomeric phases.<sup>40</sup> Based upon comparison of  $\alpha_{\text{TBN/BaP}}$  and  $\alpha_{\text{PhPh/BaP}}$ , the ionene phases are also similar to 4-[4-(allyloxy)benzoyl]oxy-4'-methoxybiphenyl or cholesteryl-10-undecenoate liquid-crystalline-bonded phases.<sup>45,46</sup> In addition, C-30- and C-22-bonded stationary phases produced similar  $\alpha_{\text{TBN/BaP}}$  values.<sup>47</sup> It should be noted that, as the percentage of water in the mobile phase was increased, the selectivity factor ( $\alpha_{\text{TBN/BaP}}$ ) remained essentially constant, for

(36) Engelhardt, H.; Lobert, T. *Anal. Chem.* **1999**, *71*, 1885–1892.

(37) Sander, L. C.; Wise, S. A. *Certificate of Analysis, SRM 869*; Standard Reference Materials Program, NIST: Gaithersburg, MD, 1990.

(38) Sander, L. C.; Wise, S. A. *Anal. Chem.* **1987**, *59*, 2309–2313.

(39) Sander, L. C.; Wise, S. A. *LC-GC* **1990**, *8*, 378–388.

(40) Sander, L. C.; Wise, S. A. *Anal. Chem.* **1984**, *56*, 504–510.

(41) Sander, L. C.; Wise, S. A. *Anal. Chem.* **1995**, *67*, 3284–3292.

(42) Sander, L. C. *J. Chromatogr. Sci.* **1985**, *26*, 380–387.

(43) Sander, L. C.; Pursch, M.; Wise, S. A. *Anal. Chem.* **1999**, *71*, 4821–4830.

(44) Engelhardt, H.; Nikolov, M.; Arangio, M.; Scherer, M. *Chromatographia* **1998**, *48*, 183–189.

(45) Saito, Y.; Jinno, K.; Pesek, J. J.; Chen, Y. L.; Luehr, G.; Archer, J.; Fetzer, J. C.; Biggs, W. R. *Chromatographia* **1994**, *38*, 295–303.

(46) Pesek, J. J.; Matyska, M. T.; Williams, E. J.; Tam, R.; Wang, Z. *J. Liq. Chromatogr. Relat. Technol.* **1998**, *21*, 2747–2762.

(47) Pesek, J. J.; Matyska, M. T.; Takhar, S. *Chromatographia* **1998**, *48*, 631–636.

Table 4. Summary of Chromatographic Parameters Obtained for the NIST Column Selectivity Test Mixture<sup>a</sup>

column	mobile phase	$K_{\text{PhPh}}^b$	$K_{\text{BaP}}^c$	$K_{\text{TBN}}^d$	$\alpha_{\text{TBN/BaP}}$	$\alpha_{\text{PhPh/BaP}}$
[3,16]-ionene	85:15 AN:H <sub>2</sub> O <sup>e</sup>	0.34	0.34	0.34	1.00	1.00
	70:30 AN:H <sub>2</sub> O	0.48 <sup>f</sup>	0.48 <sup>f</sup>	0.69 <sup>f</sup>	1.43	1.00
	60:40 AN:H <sub>2</sub> O	0.90	1.17	1.54	1.32	0.77
	50:50 AN:H <sub>2</sub> O	2.38	2.91	4.26	1.46	0.82
[3,22]-ionene	70:30 AN:H <sub>2</sub> O <sup>e</sup>	0.88	1.18	1.50	1.27	0.75
	60:40 AN:H <sub>2</sub> O	1.32	1.86	2.59	1.39	0.71
	40:60 AN:H <sub>2</sub> O	4.03	4.77	7.68	1.64	0.85

<sup>a</sup> Chromatographic conditions: chromatographic system 1; flow rate, 1.00 mL/min; 25.0 °C; column back-pressure, 800–1490 psi; UV detection at 254 nm. <sup>b</sup> PhPh refers to phenanthro[3,4-*c*]phenanthrene. <sup>c</sup> BaP refers to benzo[*a*]pyrene. <sup>d</sup> TBN refers to tetrabenzonaphthalene. <sup>e</sup> Mobile phase composed of indicated percentages (v/v) of acetonitrile:water. <sup>f</sup> PhPh and BaP coeluted and were only partially resolved from the TBN peak.

the [3,16]-ionene phase, or slightly increased, in the case of the [3,22]-ionene (Table 4). This suggests that the intermediate character and behavior of these ionene phases is not appreciably altered over this mobile phase composition range, based on this classification scheme.

Differences in the retention factors of triphenylene (trip) and *o*-terphenyl (*o*-ter) have also been employed to further assess the shape selectivity of bonded stationary phases.<sup>45,48–50</sup> For recent perspectives on such tests, see refs 43 and 44. Triphenylene contains a fused-ring system in which the three phenyl rings are coplanar, whereas in *o*-terphenyl, the three phenyl groups are noncoplanar. The selectivity factor, alpha (planar/nonplanar),  $\alpha_{\text{trip}/o\text{-ter}}$ , is typically in the range of 1.0–1.7 for these two solutes on a monomeric-like C-18 phase and in the range of 2.0–2.7 for polymeric C-18 supports. Generally, a value of  $\alpha_{\text{trip}/o\text{-ter}} > 2.0$  is considered to be indicative of significant solute shape recognition ability. This selectivity factor was found to be between 4.3 and 6.3 (Table 5), depending upon the mobile phase composition, for the [3,16]-ionene column. This selectivity on the ionene column is much greater than that observed with ordinary C-18 phases. Thus, on the basis of this test mix criterion, the ionene stationary phases appear to possess a high degree of shape selectivity for small- to medium-sized aromatic analytes.

In comparison to other specialty stationary phases, the ionenes separate these two solutes to about the same degree as that reported for some pyrene-, C<sub>60</sub> fullerene-, or tetraphenylporphyrin-modified silica or comb-shaped octadecylacrylate polymeric and liquid-crystal-bonded stationary phases (for which  $\alpha_{\text{trip}/o\text{-ter}}$  ranged from 2.7, 3.61, 3–4, and 3.8–4.9, respectively).<sup>29,45,51–53</sup> However, the selectivity achieved on the ionene-bonded phases is not as large as that reported on an aminopropyl silica gel material that had been modified with copper phthalocyanine ( $\alpha = 189$ ),<sup>51</sup> native

Table 5. Summary of Chromatographic Parameters Obtained for the Column Shape Selectivity Test Mixture<sup>a</sup>

column	mobile phase	$K_{\text{trip}}^b$	$K_{o\text{-ter}}^c$	$\alpha_{\text{trip}/o\text{-ter}}$
[3,16]-ionene	60:40 (v/v) MeOH:H <sub>2</sub> O	4.96	1.26	3.94
	50:50 (v/v) MeOH:H <sub>2</sub> O	15.1	3.14	4.81
	40:60 (v/v) MeOH:H <sub>2</sub> O	50.9	8.1	6.3
	25:75 (v/v) MeOH:H <sub>2</sub> O	d	30.0	
[3,22]-ionene	100% MeOH <sup>e</sup>	0.29	0.14	2.07

<sup>a</sup> Conditions: chromatographic system 2; mobile phase flow rate, 1.00 mL/min; UV detection at 254 nm; 21.4 °C. <sup>b</sup> trip refers to triphenylene. <sup>c</sup> *o*-ter refers to *o*-terphenyl. <sup>d</sup> Elution time > 60 min. <sup>e</sup> Same conditions as noted in footnote a except that chromatographic system 3 was employed, temperature of 24.0 °C.

protoporphyrin IX ( $\alpha = 39.2$ ),<sup>54</sup> or on metalloprophyrin ( $\alpha$  in the range of 10.6–29.4, depending upon the metal ion).<sup>55</sup> It is interesting to note that the value of  $\alpha_{\text{trip}/o\text{-ter}}$  and, thus, planarity recognition ability on polymeric ODS or liquid-crystalline-bonded phases are largest when pure methanol is employed as the mobile phase and decrease with increasing water concentration in the mobile phase.<sup>45</sup> The behavior seen on the ionene-bonded phase is the opposite of that trend, because the selectivity factor increases with increasing amounts of water in the mobile phase (Table 5).

**The ASTM Selectivity Test.** Last, the ionene columns were subjected to the recommended ASTM test mixture.<sup>15</sup> The ability of the ionene-bonded phases to function as a reversed-phase-type support is demonstrated by the baseline separation of the components of this test mixture (Figure 1). Table 6 summarizes the retention factors obtained for the solutes on both the [3,16]- and [3,22]-ionene phases as a function of added methanol in the mobile phase. As shown by these data, the ionene-bonded phases behave as reversed-phase materials, because an increase in the amount of organic modifier, methanol, in the mobile phase resulted in decreased retention for all of the test solutes on both of the ionene stationary phases (Table 6). In addition, for identical mobile phase compositions, the retention factors obtained on the [3,22]-ionene phase were usually greater than those observed on the [3,16] phase, except at 50:50 (v/v) methanol:water, when essentially the same retention is observed. This probably reflects

(48) Jinno, K.; Wu, J.; Ichikawa, M.; Takata, I. *Chromatographia* **1995**, *37*, 627–634.

(49) Jinno, K.; Okumura, C.; Taniguchi, M.; Chen, Y. L. *Chromatographia* **1997**, *44*, 613–618.

(50) Jinno, K.; Yamamoto, K.; Kuwamoto, T.; Nagashima, H.; Ueda, T.; Tajima, S.; Kimura, E.; Itoh, K.; Fetzter, J. C.; Biggs, W. R. *Chromatographia* **1992**, *34*, 381–385.

(51) Mifune, M.; Mori, Y.; Onoda, M.; Iwado, A.; Motohashi, N.; Haginaka, J.; Saito, Y. *Anal. Sci.* **1998**, *14*, 1127–1131.

(52) Jinno, K.; Tanabe, K.; Saito, Y.; Nagashima, H. *Analyst* **1997**, *122*, 787–791.

(53) Nagaoka, S.; Tanaka, H.; Sakaki, S.; Hirayama, C. *J. Liq. Chromatogr. Relat. Technol.* **1996**, *19*, 2967–2984.

(54) Xiao, J.; Kibbey, C. E.; Coutant, D. E.; Martin, G. B.; Meyerhoff, M. E. *J. Liq. Chromatogr. Relat. Technol.* **1996**, *19*, 2901–2932.

(55) Chen, S.; Meyerhoff, M. *Anal. Chem.* **1998**, *70*, 2523–2529.

Table 6. Summary of the Retention Data Obtained for Some ASTM Test Mix Components on the Ionene-Bonded Phases for Mobile Phases Containing Different Mixtures of Water and Methanol<sup>a</sup>

mobile phase composition	retention factor of indicated components <sup>b</sup>				
	benzyl-alcohol	benzene	acetophenone	methyl-benzoate	dimethyl-terephthalate
05:95 (v/v) MeOH:H <sub>2</sub> O	0.86 <sup>b</sup> [1.51] <sup>b</sup>	1.28 [1.66]	1.70 [2.16]	2.59 [3.52]	5.99 [8.22]
10:90 (v/v) MeOH:H <sub>2</sub> O	0.76 [1.10]	1.09 [1.57]	1.37 [1.89]	2.16 [3.05]	4.93 [6.33]
15:85 (v/v) MeOH:H <sub>2</sub> O	0.68 [0.85]	1.03 [1.41]	1.15 [1.05]	1.93 [2.50]	4.17 [5.09]
20:80 (v/v) MeOH:H <sub>2</sub> O	0.62 [0.73]	0.91 [1.24]	1.04 [1.12]	1.67 [2.05]	3.65 [3.87]
25:75 (v/v) MeOH:H <sub>2</sub> O	0.55 [0.64]	0.90 [1.13]	0.90 [0.93]	1.45 [1.73]	2.75 [3.04]
30:70 (v/v) MeOH:H <sub>2</sub> O	0.51 [0.54]	0.79 [0.99]	0.79 [0.74]	1.28 [1.56]	2.26 [2.22]
40:60 (v/v) MeOH:H <sub>2</sub> O	0.38 [0.39]	0.51 [0.73]	0.51 [0.47]	0.80 [0.83]	1.24 [1.21]
50:50 (v/v) MeOH:H <sub>2</sub> O	0.32 [0.28]	0.32 [0.28]	0.32 [0.47]	0.45 [0.47]	0.63 [0.63]

<sup>a</sup> Chromatographic system 1; flow rate, 1.00 mL/min; UV detection at 254 nm; 25.0 °C. <sup>b</sup> The upper number refers to the retention factors determined on the [3,16]-ionene column, and the lower one [in brackets] on the [3,22]-ionene column.

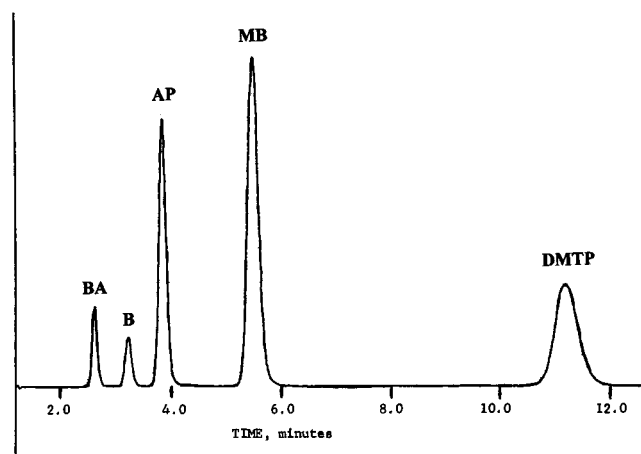


Figure 1. Chromatogram showing the separation of the ASTM test mix components, benzyl alcohol (BA), benzene (B), acetophenone (AP), methylbenzoate (MB), and dimethylterephthalate (DMTP) on the [3,22]-ionene-bonded phase with 95:05 (v/v) water:methanol mobile phase; 21.0 °C; flow rate 1.00 mL/min; detection at 254 nm; chromatographic system 1.

the fact that the former ionene is more hydrophobic and can form more compact, globular micelle-like aggregate structures than the latter, particularly in mobile phases containing a very high water content.<sup>7</sup>

It should be noted that it was not possible to elute all of the aldehyde or ketone components, such as benzaldehyde, from these ionene-bonded phases. This is probably due to the fact that some carbonyl-containing species can react with the residual free amine present in the aminopropyl moiety to form a Schiff's base-type condensation product. Such complications due to the reaction of aldehyde or ketone components with primary amine groups present on the chromatographic stationary phase have been reported for commercial *n*-propylamine-bonded phases as well as procedures identified for the reactivation of such amino columns.<sup>56</sup>

The relative elution order of the ASTM components observed on the ionene phases changes, depending on the amount of methanol modifier that is present. If eluted with 40:60 (v/v) methanol:water, the elution order on the ionene phases is benzyl alcohol < acetophenone < benzene < methylbenzoate < dimethylterephthalate, whereas with a 5:95 or 10:90 (v/v) methanol:water mobile phase, it is benzyl alcohol < benzene < acetophenone < methylbenzoate < dimethylterephthalate (Table 6). This order is slightly different from that observed on a conventional C-18 phase; that is, benzyl alcohol < acetophenone < methylbenzoate < benzene < dimethyl terephthalate (using a 40:60 (v/v) methanol:water or 30:70 (v/v) acetonitrile:water mobile phase). Thus, benzene elutes after methylbenzoate but before dimethylterephthalate on the C-18 phase, whereas it elutes either before or after acetophenone, depending upon the organic modifier content of the mobile phase on the ionene phases. The changes in elution order probably reflect the changing nature of the intrapolymeric ionene aggregate structure as a function of the methanol:water mobile phase composition. Previously, it has been noted that polyionenes can have different conformations, depending on the specific solution conditions (solvent, ionic strength).<sup>57</sup> Based upon the binding constants of these solutes to a cationic surfactant micelle such as hexadecyltrimethylammonium bromide (CTAB) or dodecyltrimethylammonium bromide (DTAB) in aqueous solution,<sup>58</sup> the predicted elution order would be: benzyl alcohol < acetophenone < benzene < methylbenzoate < dimethylterephthalate.

**Homologous Alkylbenzene Series Study.** A homologous series of *n*-alkyl benzenes was separated on the [3,22]-ionene column using as mobile phase solutions of different methanol–

(56) Karlesky, D.; Shelly, D. C.; Warner, I. M. *Anal. Chem.* **1981**, *53*, 2146–2147.

(57) Suzuki, Y.; Tazuke, S. *Macromolecules* **1980**, *13*, 25–35.

(58) Quina, F. H.; Alonso, E. O.; Farah, J. P. S. *J. Phys. Chem.* **1995**, *99*, 11708–11714.



water mixtures. The retention factor for such series is linearly related to the carbon number, according to eq 4:

$$\ln K_n = \ln k_0' + n \ln \alpha \quad (4)$$

where  $K_n$  is the retention factor of the  $n^{\text{th}}$  homolog;  $\alpha$  is the selectivity, defined as  $K_{n+1}/K_n$ ;  $n$  is the number of methylene units; and  $k_0'$  is the retention factor of the functional group of the particular series.<sup>59</sup> As previously noted in the literature,<sup>60,61</sup> when  $\log K$  values are plotted versus size ( $n_c$ , i.e., the number of methylene repeat units) for a homologous series using different organic modifier–water mobile phase mixtures, a family of curves is obtained. As seen in Figure 2A, such plots obtained on the [3,22]-ionene phase are linear, and the extrapolated straight lines converge at a common intersection point (P), whose  $y$ -axis convergence point ( $\log k^*$ ) is approximately equal to  $-1.0$  and whose  $x$ -axis ( $n_c^*$ ) point is ca.  $-3.25$  for this ionene-bonded phase. By comparison, such a plot for the separation of these alkylbenzenes on a Hypersil ODS column using aqueous methanolic mobile phases yielded an intersection point of  $\log k^* = -1.5$  and  $n_c^* = \text{ca. } -6.0$ .<sup>60</sup> The slope of each linear plot is equal to the logarithm of the hydrophobic methylene selectivity ( $\alpha$ ), which is proportional to the free energy of transfer of the methylene group between the mobile and stationary phases. This quantity is also useful for the estimation of the hydrophobicity of chemically bonded stationary phases, because its value increases with an increasing contribution of hydrophobic interactions in the stationary phase under otherwise identical conditions.<sup>62</sup> For the alkylbenzene series chromatographed on the [3,22]-ionene phase with aqueous methanolic mobile phases, the slopes of the plots (Figure 2A) decrease with decreasing amounts of water in the mobile phase. In addition, the slopes of the plots for a particular mobile phase composition for the [3,22]-ionene phase are less than that estimated from data in the literature reported for C-18 or C-8 stationary phase materials. For instance, if a 55:45 or 65:35 (v/v) methanol:water mobile phase was employed, the slopes of such plots on the [3,22]-ionene column were 0.17 or 0.12, respectively. This compares to an estimated slope of 0.57 for a 60:40 (v/v) methanol:water mobile phase on the ODS phase<sup>60</sup> or 0.37 on a Silasorb C-8 phase with a 60:40 (v/v) acetonitrile:water mobile phase,<sup>63</sup> which were the largest such values found in a cursory literature search. Included in Figure 2B are values for a variety of other C-18 phases (Hypersil C-18, Supelcosil C-18 LC PAH, Waters Resolve C-18, LiChrospher Si-C-18, and ASTEC C-18),<sup>59,64,65,69</sup> as well as some other stationary phase materials, such as porous glassy carbon (PGC) or PGC or zirconia (LTGC–Zr), that had been coated with low-temperature glassy carbon (LT-

GC),<sup>66</sup> restricted-access polymeric polyglycerol monomethacrylate-co-ethylene dimethacrylate (PGMADM),<sup>67</sup> a mixed phase containing aminopropyl and two alkyl (pentyl) amide moieties (SG-AP<sub>2</sub>),<sup>62</sup> and Unisphere Al–C-18.<sup>69</sup> Because the slope of plots of  $\log K$  vs  $n_c$  can be utilized as a rough measure of stationary phase hydrophobicity,<sup>63,68</sup> the data indicate that the ionene-bonded phase is less hydrophobic, as compared to conventional C-18 or C-8 stationary phases, particularly when it is used in conjunction with mobile phases containing a high proportion of organic modifier. However, they are more hydrophobic when compared to many of these other mentioned stationary phase materials.

The change in the standard free energy of transfer,  $-\Delta(\Delta G^\circ)$ , for each additional methylene group in a homologous series of solutes can be calculated from eq 5.<sup>69</sup>

$$\ln \alpha_{\text{CH}_2} = -\Delta(\Delta G^\circ)/RT \quad (5)$$

For the  $n$ -alkylbenzene series, the free energy of transfer of a methylene moiety from a 5:95 methanol:water mobile phase to the [3,22]-ionene stationary phase was estimated to be  $-2.1$  kJ/mol. This compares to a value of  $-2.4$  kJ/mol reported for an ODS (C-18) stationary phase with a 100% aqueous mobile phase.<sup>70,71</sup> A value of  $-1.9$  kJ/mol has been reported for the free energy of transfer per methylene group of the phenylalkyl moiety (of cationic surfactants) from bulk water to the micellar interface of cationic surfactant aggregates,<sup>72</sup> and a value of  $-2.2$  kJ/mol was recently determined for the transfer of a methylene group of  $n$ -alkylbenzenes from water to the micelles formed from the anionic surfactant sodium dodecyl sulfate.<sup>73</sup> The free energy of transfer per methylene group can also be estimated from the solute volume term ( $V_x$ ) in linear solvation free energy relationships for solute binding to micelles.<sup>58</sup> The  $\Delta(\Delta G^\circ)$  for transfer of the  $-\text{CH}_2-$  moiety from water to the cationic CTAB micelle estimated in this manner is ca.  $-2.7$  kJ/mol.<sup>58,74</sup>

**Chromatographic Efficiency.** As judged from criteria reported in the literature,<sup>75–78</sup> the chromatographic efficiency observed for the ionene-bonded phases is only slightly lower than that seen on commercial RP stationary phase materials. Figure 3 shows the plot of reduced plate height,  $h$ , vs mobile phase linear velocity,  $\mu$ , for ethylbenzene ( $K = 3.3$ ) eluted with the 5:95 (v/v) methanol:water mobile phase and for benzene ( $K = 0.84$ ) eluted with 25:75 (v/v) methanol:water as mobile phase. The minimum reduced plate height values ranged from  $\sim 4.1$  to 5.4 at an optimum

- (59) Grushka, E.; Colin, H.; Guiochon, G. *J. Chromatogr.* **1982**, *248*, 325–339.  
 (60) Colin, H.; Krstulovic, A. M.; Gonnord, M. F.; Guiochon, G.; Yun, Z.; Jandera, P. *Chromatographia* **1983**, *17*, 9–15.  
 (61) Tchaplá, A.; Colin, H.; Guiochon, G. *Anal. Chem.* **1984**, *56*, 621–625.  
 (62) Buszewski, B.; Gadzala-Kopiuch, R. M.; Jaroniec, M. *J. Liq. Chromatogr. Relat. Technol.* **1997**, *20*, 2313–2325.  
 (63) Bhagwat, V.; Berezniński, Y.; Buszewski, B.; Jaroniec, M. *J. Liq. Chromatogr. Relat. Technol.* **1998**, *21*, 923–939.  
 (64) Heron, S.; Tchaplá, A. *Chromatographia* **1993**, *36*, 11–18.  
 (65) Borgerding, M. F.; Quina, F. H.; Hinz, W. L.; Bowermaster, J.; McNair, H. M. *Anal. Chem.* **1988**, *60*, 2520–2527.  
 (66) Rittenhouse, C. T.; Olesik, S. V. *J. Liq. Chromatogr. Relat. Technol.* **1996**, *19*, 2997–3022.

- (67) Hosoya, K.; Kishii, Y.; Kimata, K.; Araki, T.; Tanaka, N. *Chromatographia* **1994**, *38*, 177–182.  
 (68) Bontrop, D.; Kohr, J.; Engelhardt, H. *Chromatographia* **1991**, *32*, 171–178.  
 (69) Arenas, R. V.; Foley, J. P. *Analyst* **1994**, *119*, 1303–1314.  
 (70) Hu, Y.; Carr, P. W. *Anal. Chem.* **1998**, *70*, 1934–1942.  
 (71) Carr, P. W.; Tan, L. C.; Park, J. H. *J. Chromatogr.* **1996**, *724*, 1–12.  
 (72) Okano, L. T.; El Seoud, O. A.; Halstead, T. K. *Colloid Polymer Sci.* **1997**, *275*, 138–145.  
 (73) Vitha, M. F.; Dallas, A. J.; Carr, P. W. *J. Phys. Chem.* **1996**, *100*, 5050–5062.  
 (74) Rodrigues, M. A.; Alonso, E. O.; Chang, Y. W.; Farah, Y. W.; Quina, F. H. *Langmuir* **1999**, *15*, 6770–6774.  
 (75) Snyder, L. R.; Glajch, J. L.; Kirkland, J. J. *Practical HPLC Method Development*, 2nd ed.; John Wiley & Sons: New York, 1997, Chapter 3, p 68.  
 (76) Walters, M. J. *J. Assoc. Off. Anal. Chem.* **1987**, *70*, 465–469.  
 (77) Engelhardt, H.; Jungheim, M. *Chromatographia* **1990**, *29*, 59–68.  
 (78) Engelhardt, H.; Arangio, M.; Lobert, T. *LC-GC* **1997**, *15*, 856–866.

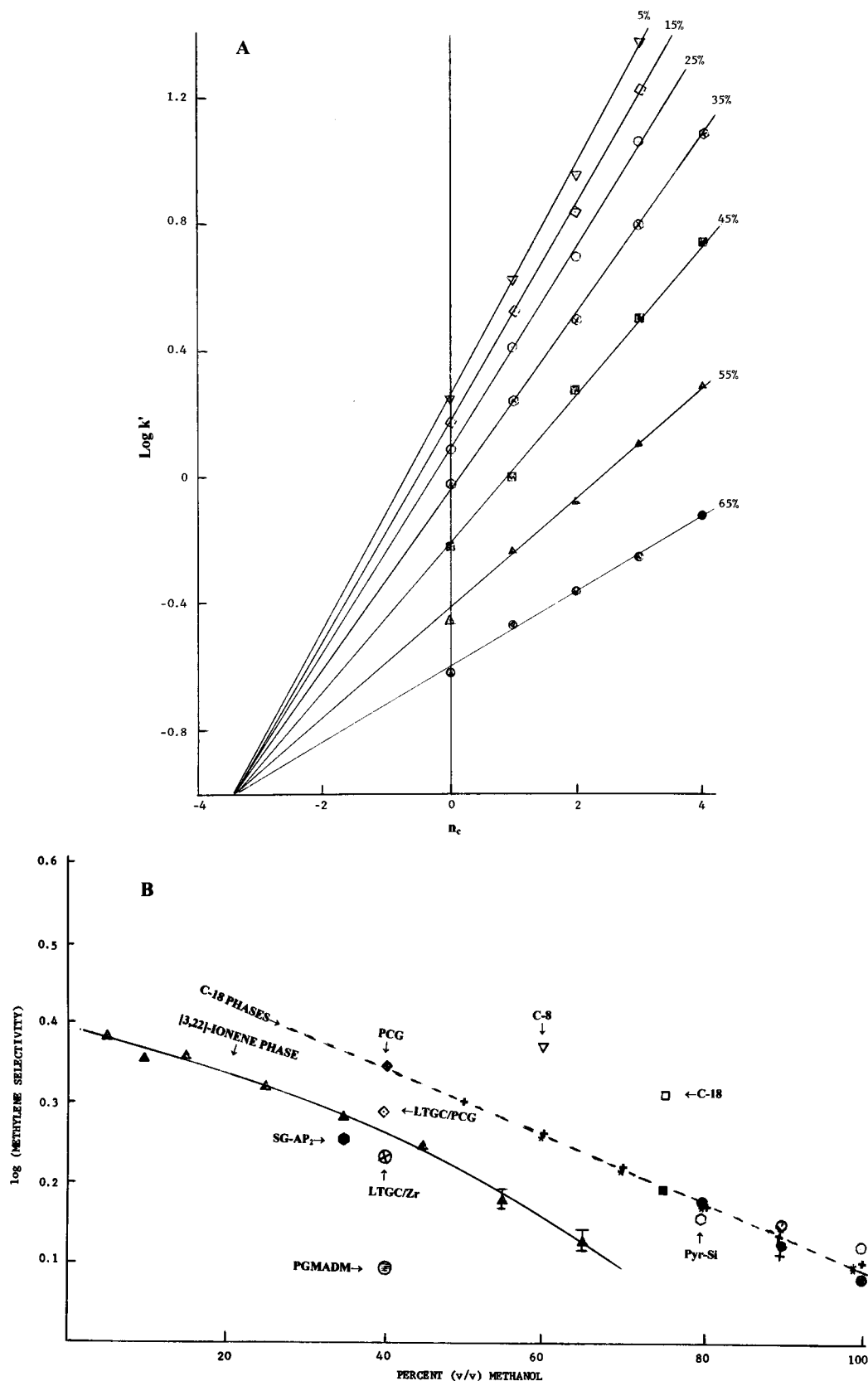


Figure 2. A.  $\log k'$  vs carbon number ( $C_n$ ) in the alkyl portion of a homologous series of  $n$ -alkyl benzenes separated on the [3,22]-ionene stationary phase. The curves correspond to mobile phases containing different percentages (v/v) of the organic modifier, methanol, in water; mobile phase flow rate 1.00 mL/min; 24.5 °C; detection at 254 nm. B. Plot of  $\log \alpha_{CH_2}$  (methylene selectivity) versus percent (v/v) methanol in the methanol:water mobile phase for  $n$ -alkylbenzenes for the [3,22]-ionene column at 24.5 °C. The dashed line is the corresponding plot obtained for various ODS stationary phases [based on literature data for Hypersil C-18, at 29.2–32 °C<sup>59</sup> (●),  $\mu$ Bondapak C-18<sup>61</sup> (×), Supelcosil C-18<sup>64</sup> (○), Waters Resolve C-18<sup>65</sup> (■), and LiChrospher Si-C-18 (+)<sup>69</sup>]. Also included are data obtained in this study on an ASTEC C-18 column (□) and literature data for C-8<sup>63</sup> (▽); PCG, LTGC/PCG, and LTGC/Zr;<sup>66</sup> SG-AP<sub>2</sub>,<sup>62</sup> PGMADM,<sup>67</sup> and Unisphere Al-C-18 (\*)<sup>69</sup> phases.

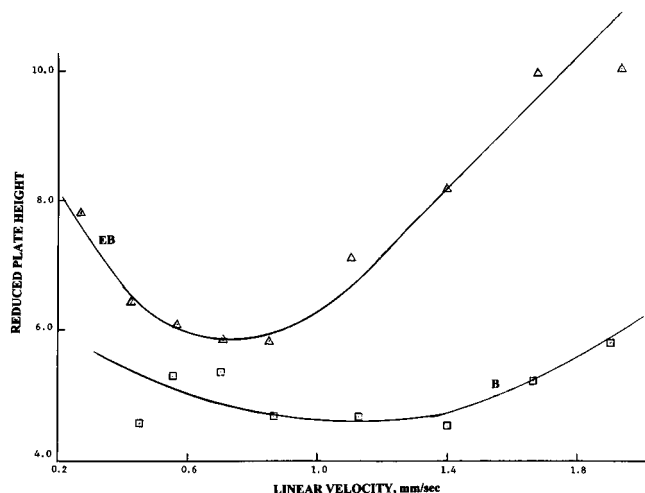


Figure 3. Effect of linear velocity on the reduced plate heights for a 10.0 cm  $\times$  4.6 mm column packed with the [3,16]-ionene-bonded phase at 22.0  $^{\circ}$ C for ethylbenzene ( $\Delta$ ,  $K' = 3.3$ ) eluted with a 5:95 (v/v) methanol:water mobile phase and benzene ( $\square$ ,  $K' = 0.84$ ) eluted with a 25:75 (v/v) methanol:water mobile phase.

linear velocity of  $\sim 0.8$  mm/sec. These values are slightly higher than "the minimum reduced plate height,  $h = 2$  to 4, that should be obtained with a well packed, high efficiency reversed phase packing material when operated at optimum linear velocities between 1 and 2 mm/sec for 5  $\mu$ m particles".<sup>77,78</sup> The peak asymmetry factors,  $A_{0.1}$ , of the benzene ( $K' = 1.09$ ) and ethylbenzene ( $K' = 3.31$ ) peaks ranged from 1.1 to 1.8 and 1.1 to 1.6, respectively, if eluted with 5:95 (v/v) methanol:water as mobile phase from the [3,16]-ionene phase. This asymmetry factor ranged from 0.9 to 1.1 for the benzene ( $K' = 0.84$ ) peak when eluted with a 25:75 (v/v) methanol:water mobile phase.

**Applications. Shape Recognition of Isomers.** In view of the apparent shape recognition ability of the ionene phases, these columns were first examined with respect to separation of components that have only very small structural differences between them. The chromatographic baseline separation of some positional isomers of disubstituted (ortho-, meta-, and para-cyanophenols and -phenylphenols) and trisubstituted benzenes (dinitroanilines) on the bonded micelle-mimetic ionene-bonded phases can be easily achieved under isocratic conditions using aqueous methanolic mobile phases, as illustrated by the data presented in Tables 7 and 8. Figure 4 shows the baseline separation of *o*-, *m*-, and *p*-phenylphenol on the [3,16]-ionene column under isocratic conditions with a 65:35 (v/v) methanol:water mobile phase. In contrast, the separation of these three phenylphenol isomers on a C-18 column was not possible under identical mobile phase conditions [i.e., 65:35 (v/v) methanol:water with or without added buffer (pH 6.3)]. Additionally, on an unmodified aminopropyl silica column with 75:25 (v/v) water:methanol as mobile phase, only the ortho isomer could be separated from the para and meta isomers, which coeluted<sup>79</sup> [with 65:35 (v/v) methanol:water as mobile phase, all three of the isomers coeluted]. This preliminary data suggests that the bonded ionene stationary phases have the ability to separate positional and geometric isomers quite well.

Table 7. Retention Factors of Isomeric Phenylphenols Separated on the Ionene Columns as a Function of Mobile Phase Composition

mobile phase composition	retention factor of indicated isomer <sup>a</sup>		
	<i>o</i> -phenyl-phenol	<i>m</i> -phenyl-phenol	<i>p</i> -phenyl-phenol
80:20 (v/v) MeOH:H <sub>2</sub> O <sup>b</sup>	0.39 [0.48]	0.65 [0.84]	0.84 [1.12]
70:30 (v/v) MeOH:H <sub>2</sub> O <sup>b</sup>	0.65 [0.99]	1.08 [1.73]	1.41 [2.47]
65:35 (v/v) MeOH:H <sub>2</sub> O <sup>b</sup>	0.81 [1.48]	1.42 [2.89]	1.86 [4.67]
60:40 (v/v) MeOH:H <sub>2</sub> O <sup>b</sup>	1.20 [2.39]	2.15 [4.97]	2.79 [6.77]
55:45 (v/v) MeOH:H <sub>2</sub> O <sup>b</sup>	1.67 [4.15]	3.12 [8.30]	4.05 [11.6]
50:50 (v/v) MeOH:H <sub>2</sub> O <sup>c</sup>	2.21	4.26	5.34
45:55 (v/v) MeOH:H <sub>2</sub> O <sup>c</sup>	5.02	10.3	12.9
35:65 (v/v) MeOH:H <sub>2</sub> O <sup>c</sup>	10.2	25.3	32.0
25:75 (v/v) MeOH:H <sub>2</sub> O <sup>c</sup>	20.1	58.6	72.5

<sup>a</sup> The upper number refers to the retention factor for the indicated isomer obtained on the [3,16]-ionene column, and the lower number [in brackets] to that obtained on the [3,22]-ionene-bonded-phase column. <sup>b</sup> Chromatographic system 1; flow rate, 1.00 mL/min; UV detection at 254 nm; 25.0  $^{\circ}$ C. <sup>c</sup> Chromatographic system 3; 21.0  $^{\circ}$ C; other conditions as noted in footnote *b*.

Table 7 summarizes the pertinent chromatographic parameters for the reversed-phase separation of the three phenylphenol positional isomers on both the [3,16]- and [3,22]-ionene columns as a function of the amount of methanol organic modifier in the mobile phase. When compared to the [3,16]-phase, the retention of these isomers was always greater on the [3,22]-ionene phase. This is undoubtedly due to its greater hydrophobicity and its ability to form more globular, more micelle-like aggregate structures. The selectivity factors,  $\alpha_{o/m}$  and  $\alpha_{o/p}$  increase with increases in the water content of the mobile phase, but  $\alpha_{m/p}$  remains relatively constant (Table 7). As expected, retention on both ionene phases decreased as the percent of methanol in the mobile phase was increased. Interestingly however, plots (not shown) of  $\log K'$  vs % methanol in the mobile phase for the isomeric phenylphenols are not linear and appear to be curved, with greater than expected retention observed for these analytes in the high-water region. Similar but more pronounced curvature has been reported in plots of  $\log K'$  vs % organic solvent for the separation of polycyclic aromatic hydrocarbons on a bonded liquid-crystalline stationary phase, where the effect was attributed to "an increase in ordering as the bonded moiety tries to minimize its contact with the highly polar mobile phase".<sup>80,81</sup> Such behavior is in contrast to the linearity that is typically observed in these plots when conventional C-8 or C-18 stationary phases are operated in the reversed-phase mode with hydroorganic mobile phases; however, even with such traditional reversed phases, "exceptions to the apparent linear behavior of  $\log K'$  with volume fraction of methanol [reportedly] can occur due to a flattening of the curve

(79) Braun, J. M.; Hinze, W. L. *Anal. Lett.* **2000**, 33, 2983–2997.

(80) Pesek, J. J.; Lu, Y.; Siouffi, A. M.; Grandperrin, F. *Chromatographia* **1991**, 31, 147–152.

(81) Pesek, J. J.; Williamsen, E. J. *Trends Anal. Chem.* **1992**, 11, 259–266.

Table 8. Summary of Some Separations Achieved on the [3,16]-Ionene-Bonded Stationary Phase<sup>a</sup>

components	retention factor	selectivity factor	mobile phase
<i>m</i> -cyanophenol	1.30	2.8	65:35 (v/v) MeOH:H <sub>2</sub> O
<i>p</i> -cyanophenol	3.70	3.2	
<i>o</i> -cyanophenol	11.9		
<i>m</i> -cyanophenol	2.28	3.0	50:50 (v/v) MeOH:H <sub>2</sub> O
<i>p</i> -cyanophenol	6.79	3.6	
<i>o</i> -cyanophenol	24.3		
2-nitroso-1-naphthol	1.57		75:25 (v/v) MeOH:H <sub>2</sub> O
1-nitroso-2-naphthol	8.88	5.6	
2-nitroso-1-naphthol	6.61		50:50 (v/v) MeOH:H <sub>2</sub> O
1-nitroso-2-naphthol	25.5	4.9	
<i>trans</i> -stilbene	1.47	1.4	50:50 (v/v) MeOH:H <sub>2</sub> O
<i>cis</i> -stilbene	2.11		
2,6-dinitroaniline	1.30		25:75 (v/v) MeOH:H <sub>2</sub> O
3,5-dinitroaniline	2.30	1.7	
2,4-dinitroaniline	3.82	1.6	
benzene	0.91 <sup>b</sup>		35:65 (v/v) MeOH:H <sub>2</sub> O <sup>b</sup>
toluene	1.78	1.9 <sup>b</sup>	
ethylbenzene	3.09	1.7	
<i>n</i> -propylbenzene	6.46	2.1	
<i>n</i> -butylbenzene	12.0	1.8	
benzene	0.34 <sup>c</sup> [0.26] <sup>d</sup>		55:45 (v/v) MeOH:H <sub>2</sub> O <sup>c</sup>
naphthalene	0.87 [0.73] <sup>d</sup>	2.6 [2.8] <sup>d</sup>	
anthracene	2.57 [1.43] <sup>d</sup>	3.0 [2.0] <sup>d</sup>	
naphthacene	8.51 [3.42] <sup>d</sup>	3.3 [2.4] <sup>d</sup>	
benzene	0.67 <sup>c</sup> [0.49] <sup>d</sup>		25:75 (v/v) MeOH:H <sub>2</sub> O <sup>c</sup>
naphthalene	4.21 [2.34] <sup>d</sup>	6.2 [4.8] <sup>d</sup>	
anthracene	37.2 [15.4] <sup>d</sup>	8.8 [6.5] <sup>d</sup>	

<sup>a</sup> Chromatographic system 3; flow rate, 1.00 mL/min; UV detection at 210, 254, or 274 nm; 21.0–22.0 °C. <sup>b</sup> Alkylbenzene series separation performed on the [3,22]-ionene-bonded phase; chromatographic system 1; mobile phase flow rate, 1.00 mL/min; UV detection at 254 nm; 24.5 °C. <sup>c</sup> Chromatographic system 3; [3,16]-ionene-bonded-phase column; flow rate, 0.5 mL/min; detection wavelengths were 202, 220, 252, and 274 nm for benzene, naphthalene, anthracene, and naphthacene, respectively; temperature, 21.0 °C. <sup>d</sup> Conditions the same as noted in footnote c, except that the temperature of mobile phase and column was 50.0 °C.

at high methanol concentrations and a somewhat steeper curve observed at very low methanol concentrations.”<sup>82</sup>

**Separation of Aromatic and Polyaromatic Hydrocarbons.** A brief study was conducted in order to determine the effectiveness of the ionene phases for the separation of aromatic hydrocarbons. A homologous series of alkylbenzenes was baseline-resolved via use of the [3,22]-ionene column with 35:65 methanol:water as the mobile phase (Table 8). Again, plots of log *K'* vs % organic modifier in the mobile phase were nonlinear (Figure 5) for these analytes, with the point of discontinuity occurring when the mobile phase contained ~30% methanol; however, for these solutes, the retention was lower than that expected in the high-water region, which is in contrast to the behavior observed with the isomeric phenylphenols.

The last two entries in Table 8 present data for the separation of some polycyclic aromatic hydrocarbons at 21.0° C and 50.0 °C on the [3,16]-ionene column. In all cases, baseline separations are achieved. For mobile phase compositions spanning the range from 65:35 (v/v) MeOH:H<sub>2</sub>O to 25:75 (v/v) MeOH:H<sub>2</sub>O (not all data

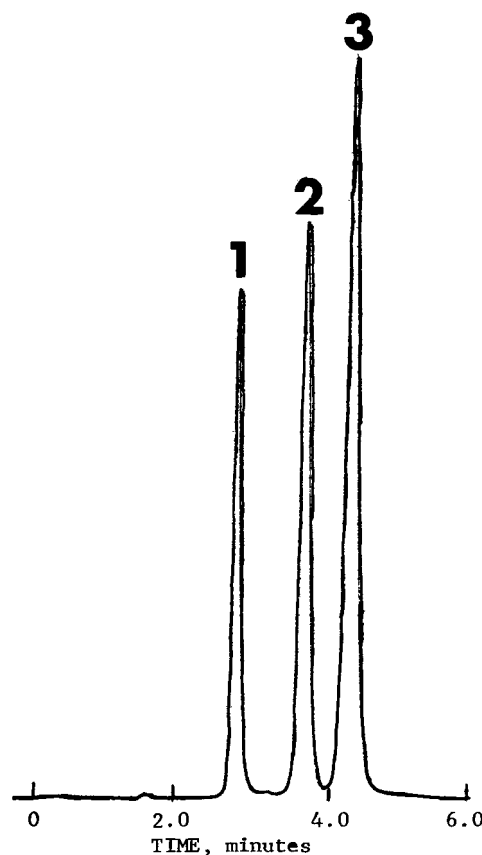


Figure 4. Separation of the positional isomers of phenylphenol on a column packed with [3,16]-ionene. Conditions: 65:35 (v/v) methanol: water mobile phase; flow rate, 1.00 mL/min; 23.0 °C; UV detection at 254 nm; injection volume, 1.0 µL; analytes, (1) *o*-phenylphenol, (2) *m*-phenylphenol, and (3) *p*-phenylphenol.

shown in the Table), an increase in column and mobile phase temperature from 21.0° C to 50.0° C resulted in an average decrease of ~33 to 46% in the retention factors of the four PAHs examined. The magnitude of this decrease in the retention factor on the ionene column was only somewhat larger than that observed on a conventional C-18 column [decreases in *K'* of from 25 to 38% (data not shown)].

**Ion-Exchange Capabilities.** Previously, nonamphiphilic ionenes (i.e., of the type that do not to form micelle-mimetic intrapolymeric aggregates) had been demonstrated to function successfully as anion exchange phases.<sup>11–13</sup> Employing aqueous mobile phases containing potassium hydrogenphthalate, such ionene phases successfully resolved mixtures of anions containing chloride, nitrite, nitrate, bromide, sulfate, acetate, bromate, and thiocyanate among others.<sup>11–13</sup> The [3,16]-ionene-bonded phase, when used in conjunction with an aqueous mobile phase containing 1.00 mM potassium hydrogenphthalate (pH 6.0) with indirect UV detection at 260 nm, allowed for the separation of a simple anion mixture. For example, for the separation of chloride, nitrite, and nitrate ions, the relative retention factors were 1.0, 3.3, and 5.2, respectively. This is the same elution order that had been reported by Pirogov for the separation of these anions on electrostatically coated [2,4]-, [4,6]-, [3,8]-, [2,8]-, [6,10]- and [6,8]-ionene phases.<sup>13</sup> This elution order is also the same as that reported for the separation of these anions on polyamine-bonded resin or phenylpropanolamine-silica-bonded materials.<sup>83,84</sup> This elution pattern

(82) Melander, W. R.; Horvath Cs. In *High-Performance Liquid Chromatography*; Horvath, Cs., Ed.; Academic Press: New York, 1980; Vol. 2, Chapter 3, pp 113–319.



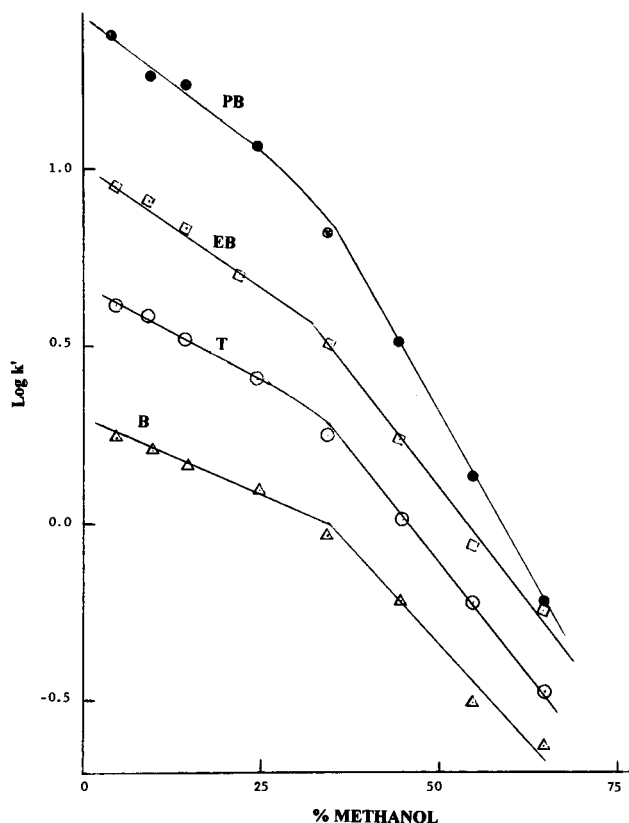


Figure 5. Log retention factor vs percent organic modifier in mobile phase (methanol:water) for benzene (B), toluene (T), ethylbenzene (EB) and *n*-propylbenzene (PB) chromatographed on the [3,22]-ionene-bonded phase (temperature, 24.5 °C; flow rate, 1.00 mL/min; UV detection at 254 nm).

seems to follow the normal Hofmeister lyotropic salt series for quaternary ammonium materials.<sup>85,86</sup>

## CONCLUSIONS

The results indicate that micelle-mimetic ionenes can be covalently attached onto the surface of silica supports to yield stationary phases that exhibit rather unique and interesting chromatographic properties. The immobilized ionenes appear to

interact with neutral solute molecules in the mobile phase primarily through hydrophobic interactions (in mobile phases of high water content, via incorporation of the solute into the micelle-like aggregate, or in methanol-rich mobile phases, via interaction with a more extended polyelectrolyte structure). The ionene-bonded phases are multimodal, because they also function as anion exchangers, showing the typical selectivity pattern of a quaternary ammonium ion exchange stationary phase. Thus, the mixed-mechanism ionene columns provide a flexible and versatile means for simultaneous separation of neutral, basic, and acidic compounds.

The new LC stationary phase material was found to exhibit chromatographic characteristics different from those of conventional C-18 reversed-phase materials in general and offers unique selectivity for the separation of some classes of analytes. In particular, the ionene-bonded phases appear to offer significant solute shape recognition ability, thus allowing for the efficient separation of isomeric components.

## ACKNOWLEDGMENT

We thank Dr. Lane C. Sander (National Institute of Standards and Technology, Gaithersburg, MD) for providing the NIST SRM-869 test mixture and Dr. Julianne M. Braun (Department of Chemistry, University of Georgia) for obtaining shape selectivity test mix data on the [3,22]-ionene column. Acknowledgment is made to Advanced Separation Technologies, Inc. (Whippany, NJ), Wake Forest University (Research & Publication Fund), the National Science Foundation (NSF Grant Int-9203011 to W.L.H.), the French Centre National de la Recherche Scientifique (UMR 5619 to A.B.) and Brazilian CNPq Grant (to F.H.Q.), and the donors of the Petroleum Research Fund, administered by the American Chemical Society (PRF Grant 20162-AC7 to W.L.H.) for their partial support of this research project. In addition, F.H.Q. gratefully acknowledges grant support from the Fundacao de Amparo a Pesquisa do Estado de São Paulo (FAPESP; Thematic Project 1994/3505-3) and PADCT-FINEP (Project No. 65-92-0063-00), I.U.M. acknowledges support in the form of a United States Government Fulbright Scholars Grant, and M.C. expresses his sincere appreciation to the Turkish Ministry of Education for the support of his stay at W.F.U. This article was written while a Wake Forest University Reynolds Research Leave supported the corresponding author.

Received for review August 22, 2000. Accepted January 28, 2001.

AC001003X

(83) Okada, T. *Anal. Chim. Acta* **1995**, *303*, 193–197.

(84) Wongyai, S. *Chromatographia* **1994**, *38*, 485–490.

(85) Barron, R. E.; Fritz, J. S. *J. Chromatogr.* **1984**, *284*, 13–25.

(86) Pfuller, U. *Mizellen, Vesikel, Mikroemulsionen: Tensidassoziate und ihre Anwendung in Analytik und Biochemie*; Verlag Volk und Gesundheit: Berlin, 1986; p 15.