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Progress in the design of selectors for buckminsterfullerene

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ABSTRACT

The chromatographic retentions of buckminsterfullerene (C_{60}), the related C_{70} carbon cluster, and several polycyclic aromatic hydrocarbons are evaluated using ten high-performance liquid chromatography stationary phases, including several stationary phases designed specifically for recognition of the fullerenes. All of the stationary phases examined provide some degree of retention and selectivity in the separation of C_{60} and C_{70}. A novel tripodal π-acidic stationary phase designed for simultaneous multipoint interaction with buckminsterfullerene provides the greatest retention and the greatest separation factor for the C_{60}–C_{70} mixture.

INTRODUCTION

Improved methods for the preparative synthesis [1–3] of the spherical carbon cluster, buckminsterfullerene (C_{60}), and related "fullerenes" have precipitated an explosion of interest in the investigation of these molecules and their derivative (for a recent review, see ref. 4). Current methods for the preparation of buckminsterfullerene yield a complex mixture consisting primarily of C_{60} and C_{70}. None of the methods for the chromatographic purification of buckminsterfullerene which have been reported to data allow for convenient purification of gram quantities of material [1–8]. We recently reported that an unusual effect of temperature on the chromatographic behavior of C_{60} and C_{70} on a π-acidic 3,5-(dinitrobenzoyl)phenylglycine-derived stationary phase can be exploited for improved preparative resolution of these compounds [7]. Nevertheless, only about 100 mg of the crude C_{60}–C_{70} mixture can be resolved per run on a 4 ft. × 2 in. I.D. preparative column containing this stationary phase, a column which can normally separate 20 g per run of a soluble mixture having a similar separation factor.

The difficulty in the preparative chromatographic purification of buckminsterfullerene lies in its relative isolubility. Several hundred ml of benzene or toluene ("good" solvents for the crude fullerene mixture) would be required for injection of 1 g of this material. Furthermore, use of benzene or toluene as a mobile phase with a column containing the aforementioned π-acidic stationary phase results in virtually no retention of the analytes. Use of hexane as a mobile phase gives suitable retention, however, several *liters* of hexane would be required to dissolve 1 g of the fullerene mixture. In the previously cited study, we settled upon a method using hexane as a mobile phase with injection of the fullerene mixture in benzene.

An efficient and convenient method for large scale purification of buckminsterfullerene may be beyong the scope of liquid chromatography, even though the development of improved solvent systems and improved stationary phases can be expected. Nevertheless, investigation of the ever growing family of fullerenes and fullerene derivatives, many of which exist as closely related geometrical isomers, will require improved analytical tools. In this

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study, the chromatographic behavior of C_{60}, C_{70}, and eight polycyclic aromatic hydrocarbons (PAHs, Fig. 1) was investigated using the ten high-performance liquid chromatography (HPLC) stationary phases illustrated in Fig. 2.

The chromatographic separation of π-electron-rich polycyclic aromatic hydrocarbons on π-electron deficient stationary phases has been known for quite some time [9,10]. Thus, the report by Hawkins *et al.* [5] of the ability of the π-acidic stationary phase **I** to separate C_{60} and C_{70} was not without precedent. The chirality of phase **I** is irrelevant to the separation of achiral analytes such as C_{60} and C_{70}. In these laboratories, chiral stationary phases such as phase **I** are often used to separate non-enantiomeric analytes such as diastereomers, positional isomers, etc.

Commercially available columns containing phase **I** and the closely related phase **II** were used in this study. In addition, commercially available columns containing π-basic phases **III** and **IV** were examined to assess the ability of π-basic stationary phases to afford retention for the fullerenes. The π-acidic stationary phase **V–X** were prepared for this study.

Phase **V**, an achiral glycine analog of phases **I** and **II**, was prepared as illustrated in Fig. 3. Acylation of glycine, **11**, with 3,5-dinitrobenzoyl chloride affords acid **12**. Coupling with 4-aminobutyldimethylmethoxysilane affords silane **13** which was immobilized upon silica gel to give phase **V**.

Phase **VI**, which contains an isolated 3,5-dinitrobenzamide system, was prepared as illustrated in Fig. 4. Acylation of 4-aminobutyldimethylmethoxysilane, **14**, with 3,5-dinitrobenzoyl chloride provides silane **15** which was bonded to silica gel to afford phase **VI**.

Phase **VII**, which contains the 2,4-dinitroaniline system, was prepared as illustrated in Fig. 5. Reaction of allyl amine, **16**, with 2,4-dinitrofluorobenzene provides olefin **17** which was hydrosilylated to afford silane **18**. Immobilization of silane **18** on sil-

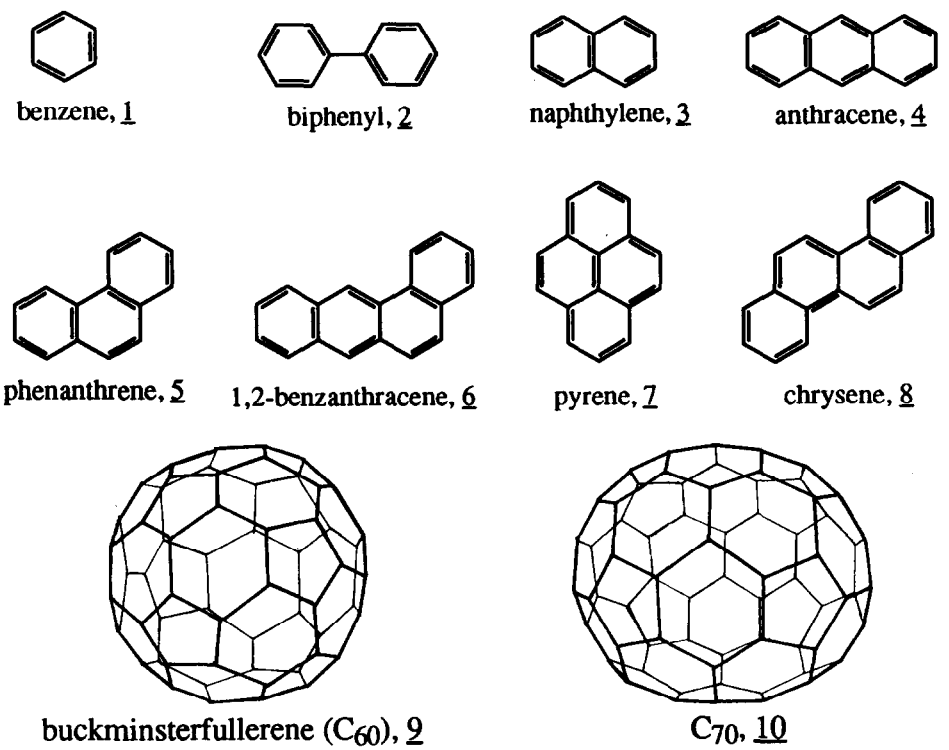

Fig. 1. Polycyclic aromatic hydrocarbons and fullerenes used in the study.

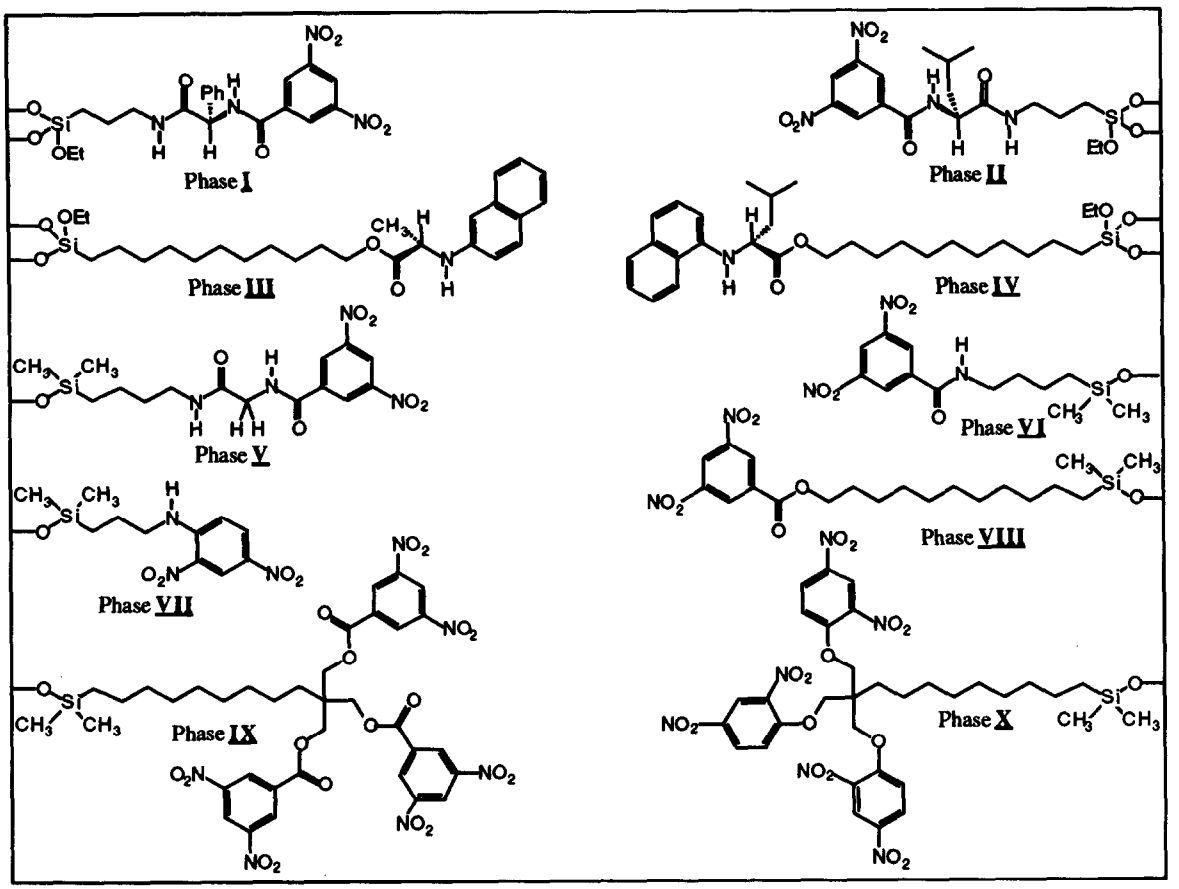

Fig. 2. Stationary phases used in the study. Et = Ethyl; Ph = phenyl.

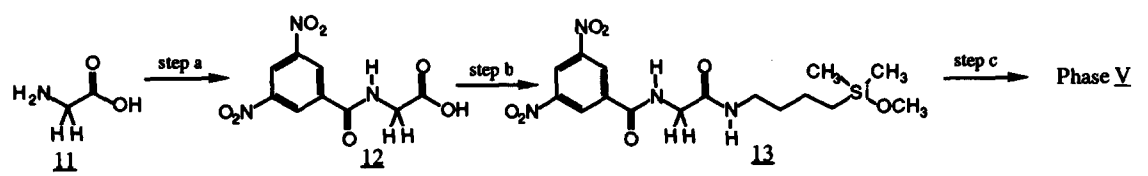

Fig. 3. Synthetic route for preparation of phase V. Step a: 3,5-dinitrobenzoyl chloride–triethylamine, tetrahydrofuran. Step b: 1-ethoxycarbonyl-2-ethoxy-1,2-dihydroquinoline (EEDQ)–aminobutyldimethylmethoxysilane, tetrahydrofuran. Step c: 5 μm/100 Å silica gel, 130°C, 1 Torr, 18 h.

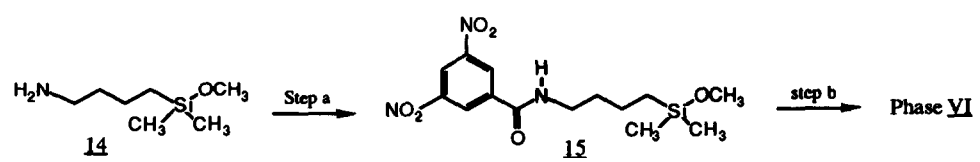

Fig. 4. Synthetic route for preparation of phase VI. Step a: 3,5-dinitrobenzoyl chloride–triethylamine, dichloromethane. Step b: 5 μm/100 Å silica gel, 130°C, 1 Torr, 18 h.

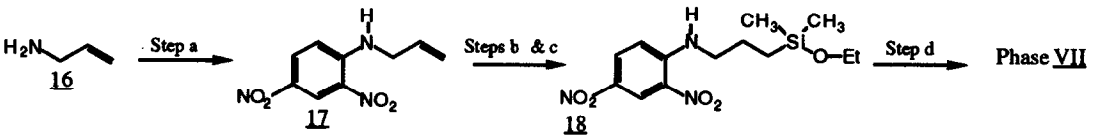

Fig. 5. Synthetic route for preparation of phase **VII**. Step a: 2,4-dinitrofluorobenzene–triethylamine, dichlormethane. Step b: dimethylchlorosilane, chloroplatinic acid (catalyst), dichloromethane. Step c: ethanol, triethylamine, diethyl ether. Step d: 5 μm/100 Å silica gel, 130°C, 1 Torr, 18 h.

ica gel affords phase **VI**. Cox *et al.* [6] reported the use of a "dinitroaniline stationary phase" for the separation of the C_{60}–C_{70} mixture. Although the structure of this stationary phase was not reported, it is probably similar, if not identical, to phase **VII**.

Phase **VIII**, which contains a 3,5-dinitrobenzoate ester, was prepared as illustrated in Fig. 6. Acylation of ω-undecenylenyl alcohol, **19**, with 3,5-dinitrobenzoyl chloride provides ester **20** which was hydrosilylated to provide silane **21**. Bonding of silane **21** to silica gel affords phase **VIII**.

Selector preorganization is known to have a dramatic effect upon the binding guest molecules [11]. We reasoned that functionalization of triols such as **23**, readily prepared using a variation of the pentaerythritol synthesis [12,13], could afford selectors containing a concave disposition of π-acidic aromatic rings complementary to the convex surface of C_{60}. Using this approach, phases **IX** (Fig. 7) and **X** (Fig. 8) were prepared. While the selectors in these stationary phases admittedly possess some degree of conformational "floppiness" and are thus not ideally preorganized, examination of Corey–Pauling–Koltun (CPK) space-filling molecular models indicates that they should be capable of some degree of simultaneous multipoint interaction with the fullerene analytes.

The tripodal 3,5-dinitrobenzoate ester phase, **IX**, was prepared as illustrated in Fig. 7. Reaction of ω-undecenylenyl aldehyde, **22**, with formaldehyde under basic conditions provides triol **23**, which was acylated with 3,5-dinitrobenzoyl chloride to afford triester **24**. Hydrosilylation of **24**, followed by immobilization on silica gel, affords phase **IX**.

The tripodal 2,4-dinotrophenyl ether phase, **X**, was prepared as illustrated in Fig. 8. Reaction of triol **23** with 2,4-dinitrofluorobenzene provides triether **26** which was hydrosilylated to afford silane **27**. Bonding of silane **27** to silica gel affords phase **X**.

MATERIALS AND METHODS

Apparatus

Chromatographic analysis was performed using

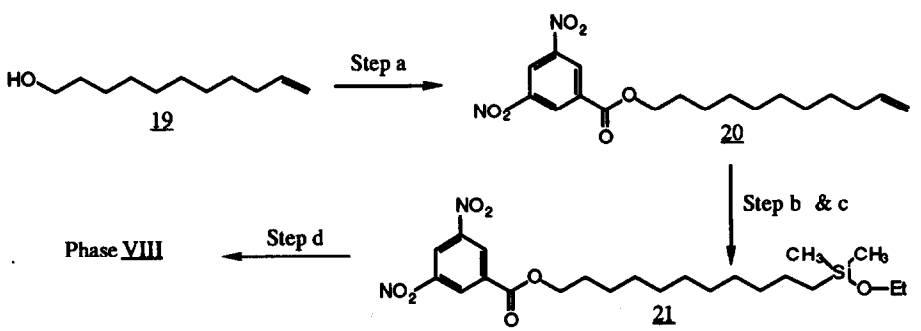

Fig. 6. Synthetic route for preparation of phaxe **VII**. Step a: 3,5-dinitrobenzoyl chloride–triethylamine, tetrahydrofuran. Step b: dimethylchlorosilane, chloroplatinic acid (catalyst), dichloromethane. Step c: ethanol, triethylamine, diethyl ether. Step d: 5 μm/100 Å silica gel, 120°C, 1 Torr, 24 h.

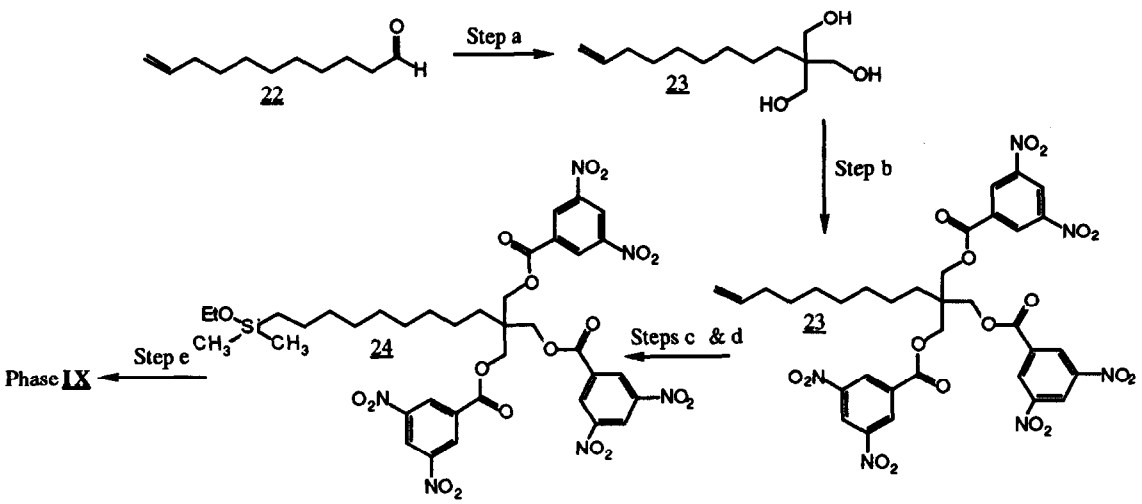

Fig. 7. Synthetic route for preparation of phase **IX**. Step a: formaldehyde–KOH, ethanol–water. Step b: 3,5-dinitrobenzoyl chloride–triethylamine, dichloromethane. Step c: dimethylchlorosilane, chloroplatinic acid (catalyst), dichloromethane. Step d: ethanol, triethylamine, diethyl ether. Step e: 5 μm/100 Å silica gel, 120°C, 1 Torr, 24 h.

a Beckman-Altex 100-A pump, a Rheodyne Model 7125 injector with a 20-μl sample loop, a Linear UVIS 200 variable-wavelength absorbance monitor set at 254 nm, and a Hewlett-Packard HP 3394-A integrating recorder. All ^{1}H NMR spectra were recorded on a Varian XL 200 FT NMR spectrometer. ^{1}H NMR chemical shifts are reported in ppm (δ) relative to tetramethylsilane.

Materials

Rexchrom 5 μm/100 Å silica gel and columns containing phases **I–IV** were obtained from Regis, Morton Grove, IL, USA. Phases **V–X** were prepared as outlined in the *Synthesis* section below. Dimethylchlorosilane and 4-aminobutyldimethylmethoxysilane were obtained from Petrarch Systems, Bristol, PA, USA. PAHs (analytes **1–8**) were available from previous studies. A crude mixture containing C_{60} and C_{70} was obtained within the department from Drs. John Shapley and Scott Koefod.

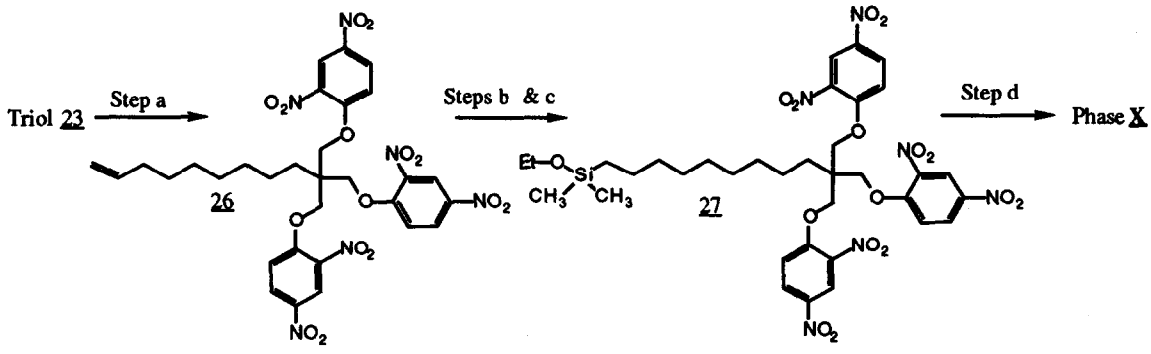

Fig. 8. Synthetic route for preparation of phase **X**. Step a: 2,4-dinitrofluorobenzene–triethylamine, dichloromethane. Step b: dimethylchlorosilane, chloroplatinic acid (catalyst), dichloromethane. Step c: ethanol, triethylamine, diethyl ether. Step d: 5 μm/100 Å silica gel, 120°C, 1 Torr, 24 h.

Methods

All chromatographic experiments were carried out at a nominal flow rate of 2.00 ml/min. Column void time was determined by injection of tri-*tert.*-butylbenzene [14].

Synthesis

Preparation of phase **V**

The synthetic route for the preparation of phase **V** is illustrated in Fig. 3.

Preparation of 3,5-(dinitrobenzamido)glycine, ***12.*** Glycine (**11**, 5.0 g) was suspended in 100 ml of dry tetrahydrofuran and cooled in an ice bath. 3,5-Dinitrobenzoyl chloride (16.9 g) and propylene oxide (7.0 ml) were added and the mixture was stirred under a nitrogen atmosphere and gradually allowed to warm to room temperature. After 10 h, the crude reaction mixture was evaporated to afford a brown oil. Addition of 100 ml of dichloromethane resulted in crystallization after several min. Filtration, followed by several washes with dichloromethane and drying under high vacuum, gave 13.9 g **12** (78% yield) as a pale yellow powder. ^{1}H NMR ([$^2H_6$]dimethyl sulfoxide) δ: 12.8 (s, 1H), 9.6 (t, 1H), 9.1 (s, 2H), 9.0 (s, 1H), 4.0 (d, 2H).

Preparation of organosilane ***13.*** To a cooled (ice bath) solution of 1.0 g of acid **12** in tetrahydrofuran was added 0.92 g 1-ethoxycarbonyl-2-ethoxycarbonyl-2-ethoxy-1,2-dihydroquinoline (EEDQ). This mixture was stirred 45 min. under a nitrogen atmosphere, 0.50 g of 4-aminobutyldmethylmethoxysilane was added, and the reaction mixture was allowed to gradually warm to room temperature. After 10 h, the crude reaction mixture was evaporated and purified by flash chromatography on silica to give 0.65 g (42% yield) of **13** as a slightly pink foam.

Preparation of phase ***V.*** Silica gel (5.0 g, Rexchrom, 5 μm/100 Å) was placed in a round-bottom flask fitted with a Dean–Star trap, condenser, and boiling stick. About 30 ml of benzene were added and the mixture was heated to reflux for several hours. Dimethylformamide (1 ml) was then added to the benzene slurry and the sample was evaporated to near dryness on a rotary evaporator. A dichloromethane solution of silane **13** (0.65 g) was then added and the resulting slurry was sonicated for several minutes before being evaporated to near dryness. The sample was again slurried in dichloromethane, sonicated, and evaporated to near dryness, this sequence being repeated several times to insure complete coverage of the silica gel. The nearly dry silica gel–silane mixture was then heated on an oil bath under reduced pressure (130°C, 1 Torr, 18 h). The silica gel was then slurried in ethanol, filtered through a fine sintered glass funnel, and washed repeatedly with ethanol and then methanol. Evaporation and analysis of the ethanol washes can be done at this point to monitor degradation of the silane during the course of the bonding reaction. The washed silica gel was then slurried in methanol and packed into a 25 cm × 4.6 mm I.D. stainless-steel HPLC column using an air-driven Haskell pump operating at about 9000 p.s.i. Stationary phase recovered from the column packer was dried thoroughly under high vacuum and submitted for elemental analysis (C 3.39%) which indicates a loading of $1.9 \cdot 10^{-4}$ mol of selector per gram of stationary phase. Residual silanols on the chromatographic support were then "endcapped" by passing a solution of 1 ml of hexamethyldisilazane dissolved in 50 ml of dichloromethane through the dichloromethane-equilibrated column at a flow rate of 1 ml/min [15]. The column was then sequentially eluted with dichloromethane, methanol and 20% 2-propanol in hexane.

Preparation of phase ***VI***

The synthetic route for the preparation of phase **VI** is illustrated in Fig. 4.

Preparation of organosilane ***15.*** Triethylamine (0.69 g) and 4-aminobutyldimethylmethoxysilane (1.00 g) were dissolved in 20 ml dichloromethane and cooled in an ice bath. 3,5-Dinitrobenzoyl chloride (1.43 g) dissolved in 10 ml of dichloromethane was then added dropwise over several minutes with stirring under a nitrogen atmosphere. The reaction mixture was allowed to warm to room temperature and stirred for an additional h. The crude reaction mixture was evaporated to dryness and purified by flash chromatography (silica gel, 5% acetonitrile in dichloromethane) to afford **15** as a clear oil (840 mg, 69,4% yield). ^{1}H NMR (C^2HCl_3) δ: 9.15 (s, 1H), 9.0 (s, 1H), 6.8 (bs, 1H), 3.55 (m, 2H), 4.3 (s, 3H), 1.75 (m, 2H), 1.5 (m, 2H), 0.65 (t, 2H), 0.15 (s, 6H).

Preparation of phase ***VI.*** The bonding of silane **15**

to silica and the subsequent packing of the resulting stationary phase into an HPLC column followed the procedure reported for the preparation of phase **V** except that a Kügelrohr distillation apparatus was used in the bonding reaction (130°C, 1 Torr, 18 h). Stationary phase recovered from the column packer was submitted for elemental analysis (C 4.69%) indicated a loading of $3.0 \cdot 10^{-4}$ mol of selector per gram of stationary phase.

Preparation of phase ***VII***

The synthetic route for the preparation of phase **VII** is illustrated in Fig. 5.

Preparation of olefin ***17***. To a cooled, stirred solution of allylamine (**16**, 0.46 g) and triethylamine (0.90 g) in dichloromethane was added 2,4-dinitrofluorobenzene (1.5 g). After warming to room temperature, the reaction mixture was stirred for 3 h, then evaporated to dryness and purified by flash chromatography (silica, dochloromethane) to afford olefin **17** as a crystalline solid (1.09 g, 61% yield). ^{1}H NMR (C^2HCl_3) δ: 9.25 (d, 1H), 8.8 (bs, 1H), 8.3 (dd, 1H), 7.0 (d, 1H), 6.0 (m, 1H), 5.8 (dd, 2H), 4.1 (t, 2H).

Preparation of Organosilane ***18***. Olefin **17** (1.09 g) was dissolved in 10 ml of dichloromethane and 10 ml of dimethylchlorosilane. Chloroplatinic acid (10 mg) dissolved in a minimum amount of 2-propanol was then added and the mixture was heated at reflux. Progress of the reaction was monitored by disappearance of starting material in quenched reaction aliquots (the quenching solution was composed of 5 ml of absolute ethanol, 5 ml of triethylamine, and 5 ml of diethyl ether). The assay procedure consists of removing several drops of reaction mixture, evaporating to dryness under high vacuum to remove excess dimethylchlorosilane, and addition of several drops of quenching solution. The mixture was then heated for several min on an oil bath, diluted with dichloromethane, and examined by thin-layer chromatography (TLC). After about 3 h, TLC analysis of quenched reaction aliquots indicated complete consumption of starting material. The crude reaction mixture was evaporated to dryness, with several additions and evaporations of dichloromethane to insure complete evaporation of residual dimethylchlorsilane, then quenched by addition of the quenching solution with stirring for 30 min. The quenched solution was filtered to remove precipitated trithylamine hydrochloride, then evapored and purified by flash chromatography on silica using 2% ethanol in dichloromethane to afford 0.80 g **18** (50% yield). ^{1}H NMR (C^2HCl_3) δ: 9.2 (s, 1H), 8.6 (bs, 1H), 8.3 (d, 1H), 7.0 (d, 1H), 3.6 (m, 2H), 3.4 (m, 2H), 1.8 (m, 2H), 1.2 (t, 3H), 0.7 (t, 2H), 0.1 (s, 6H).

Preparation of phase ***VII***. Silane **18** was bonded to silica and the resulting stationary phase was packed into an HPLC column as described for phase **V**, except that a Kügelrohr distillation apparatus was used in the bonding reaction (115°C, 1 Torr, 24 h). Packing material recovered from the column packer was submitted for elemental analysis, the result (C, 3.28%) indicates a loading of $2.5 \cdot 10^{-4}$ mol of selector per gram of stationary phase.

Preparation of phase ***VIII***

The synthetic route for the prepartion of phase **VIII** is illustrated in Fig. 6.

Preparation of olefin ***20***. To a cooled solution of ω-undecenylenyl alcohol (**19**, 1.0 g) and triethylamine (0.65 g) in 10 ml of dry tetrahydrofuran were added 3,5-dinitrobenzoyl chloride (1.36 g) with stirring. The reaction mixture was allowed to warm to room temperature and stirred for an additional h. The heterogeneous solution was diluted with diethyl ether and extracted three times with a 1.0 *M* HCl solution. The organic layer was washed with water, then brine, then dried over anhydrous magnesium sulfate. Filtration and evaporation yielded **20** (2.00 g, 93% yield) as a pale yellow solid. ^{1}H NMR (C^2HCl_3) δ: 9.25 (d, 1H), 9.2 (d, 2H), 5.8 (m, 1H), 4.95 (m, 2H), 4.4 (t, 2H), 2.05 (m, 2H), 1.85 (m, 2H), 0.9 (m, 12H).

Preparation of organosilane ***21***. Olefin **20** was converted into ethoxysilane **21** using the hydrosilylation procedure reported for preparation of organosilane **18**. Crude **21** was purified by flash chromatography on silica using dichloromethane to afford 1.04 g **21** (80.6% yield) as a yellow oil. ^{1}H NMR (C^2HCl_3) δ: 9.25 (d, 1H), 9.15 (d, 2H), 4.45 (t, 2H), 3.65 (q, 2H), 1.85 (m, 2H), 1.3 (m, 16H), 0.6 (t, 2H), 0.1 (s, 6H).

Preparation of phase ***VIII***. Silane **21** was bonded to silica and the resulting stationary phase was packed into an HPLC column as described for phase **V** (120°C, 1 Torr, 24 h). Stationary phase recovered from the column packer was submitted for

elemental analysis, the result (C, 3.94%) indicates a loading of $1.6 \cdot 10^{-4}$ mol of seector per gram of stationary phase.

Preparation of phase ***IX***

The synthetic route for the preparation of phase **IX** is illustrated in Fig. 7.

Preparation of triol ***23***. Undecylenic aldehyde (**22**, 50 g) and 200 g of 40% formaldehyde solution were dissolved in 500 ml of ethanol–water (1:1). Potassium hydroxide (16.30 g) dissolved in 150 ml of ethanol–water (1:1) was then added dropwise to the cold (0°C) stirred solution. The reaction was allowed to warm to room temperature, stirred for 4 h then heated to 60°C and stirred for an additional 2 h at which time TLC indicated complete consumption of starting material and formation of a new product. The crude reaction mixture was concentrated under vacuum to remove ethanol and then extracted several times with diethyl ether. The combined ether extracts were washed several times with water, washed with brine, dried over anhydrous magnesium sulfate, filtered and evaporated to dryness. Flash chromatography on silica gel using 10% methanol in dichloromethane gave triol **23** (28.2 g, 41.4% yield) as a white solid. ^{1}H NMR (C^2HCl_3) δ: 5.85 (m, 1H), 5.0 (m, 2H), 3.75 (d, 6H), 2.75 (t, 3H), 2.05 (m, 2H), 1.3 (m, 12H).

Preparation of triester ***24***. Triol **23** (1.0 g) and triethylamine (1.5 g) were dissolved in 50 ml of dry tetrahydrofuran and cooled in an ice bath. 3,5-Dinitrobenzoyl chloride (3.0 g) was then added and the solution was allowed to gradually warm to room temperature, then stirred overnight under a nitrogen atmosphere. Precipitated triethylammonium chloride was removed by filtration and the filtrate was washed several times with a 1 *M* HCl solution dried over anhydrous magnesium sulfate, filtered, and evaporated to dryness. Ether trituration of the resulting oil give triester **24** (1.64 g, 47% yield) as a white solid. ^{1}H NMR (C^2Cl_3, [$^2H_6$]dimethyl sulfoxide) δ: 9.2 (d, 3H), 9.1 (d, 6H), 5.75 (m, 1H), 4.9 (m, 2H), 4.65 (s, 6H), 2.0 (m, 2H), 1.8 (m, 2H), 1.4 (m, 10H).

Preparation of silane ***25***. Triester **24** (1.60 g) was converted into ethoxysilane **25** using the hydrosilylation procedure reported for preparation of organosilane **18**. Crude **25** was purified by flash chromatography (silica, 5% acetonitrile in dichloromethane) to afford **25** (1.03 g, 57.2% yield) as a yellowish solid. ^{1}H NMR (C^2HCl_3) δ: 9,25 (d, 3H), 9.15 (d, 6H), 4.6 (s, 6H), 3.6 (q, 2H), 1.8 (m, 2H), 1.6 (m, 2H), 1.3 (m, 12H), 0.5 (t, 2H), 0.05 (s, 6H).

Preparation of phase ***IX***. Silane **25** was bonded to silica and the resulting stationary phase was packed into an HPLC column as described for the preparation of phase **V** (120°C, 1 Torr, 24 h). Stationary phase recovered from the column packer was submitted for elemental analysis, the result (C, 6.30%) indicates a loading of $1.5 \cdot 10^{-4}$ mol of selector per gram of stationary phase.

Preparation of phase ***X***

The synthetic route for the preparation of phase **X** is illustrated in Fig. 8.

Preparation of triether ***26***. Triol **23** (1.0 g) and triethylamine (2.2 g) were dissolved in 50 ml of dichloromethane and cooled in an ice bath. 2,4-Dinitrofluorobenzene (2.7 g) was then added. After 30 min, the ice bath was removed and the reaction mixture was allowed to stir overnight at room temperature under a nitrogen atmosphere. The crude reaction mixture was evaporated and purified by flash chromatography on silica gel using dichloromethane to give **26** (1.39 g, 44% yield) as a pale yellow foam. ^{1}H NMR (C^2HCl_3) δ: 8.8 (d, 3H), 8.5 (dd, 3H), 7.35 (d, 3H), 5.8 (m, 1H), 4.95 (m. 2H), 4.5 (s, 6H), 2.05 (m, 2H), 1.85 (m, 2H), 1.4 (m, 12H).

Preparation of silane ***27***. Triether **26** (1.39 g) was converted into ethoxysilane **27** using the hydrosilylation procedure reported for preparation of organosilane **18**. Crude **27** was purified by flash chromatography on silica using 5% acetonitrile in dichloromethane as eluent to afford 1.0 g **27** (63% yield) ^{1}H NMR (C^2HCl_3) δ: 8.8 (d, 3H), 8,5 (dd, 3H), 7.35 (d, 3H), 4.45 (s, 6H), 3.65 (q, 2H), 1.85 (m, 2H), 1.25 (m, 14H), 1.2 (t, 3H), 0.55 (t, 2H), 0.1 (s, 6H).

Preparation of phase ***X***. Silane **27** was bonded to silica, and the resulting stationary phase was packed into an HPLC column as described for the preparation of phase **V** (130°C, 1 Torr, 24 h). Stationary phase recovered from the column packer was submitted for elemental analysis, the result (C, 6.29%) indicates a loading of $1.6 \cdot 10^{-4}$ mol of selector per gram of stationary phase.

RESULTS AND DISCUSSION

Evaluation of phases **I–X**

All of the stationary phases evaluated in the study provided some degree of retention and separation for the C_{60}–C_{70} mixture. Table I shows chromatographic data relevant to the separation of analytes **1–10** on phases **I–X**. Phase **X** affords the largest capacity factor (k') for buckminsterfullerene, **9**, and is the only phase to offer increased retention of this analyte relative to phase **I**. Phases **III–X** all afford improved separation factors (α') relative to phase **I** for separation of the C_{60}–C_{70} mixture. Interestingly, even the π-basic phases **III**, and **IV**, are quite effective in retaining and separating the fullerenes although these phases provide only marginal retention of the polycyclic aromatic hydrocarbons (analytes **1–8**).

The phenylglycine-derived phase, **I**, consistently affords greater retention than does the leucine-derived phase, **II**. In the plot of the the relative capacity factors observed with the two columns (Fig. 9), it can be seen that all of the data points fall approximately on a line having a slope of about 0.37. A linear plot indicates that each analyte in the series is being retained by essentially the same mechanistic process on each column, although the processes may differ between columns. When comparing two identical columns, a slope of unity would be expected. Two columns containing the same selector with different loadings (*i.e.* surface coverage) would be expected to give a straight line, the slope of which is indicative of the relative loadings of the two phases. When comparing two nonidentical columns, differences in selector structure and differences in loading will both contribute to the observed sloep.

The loadings of phases **I** and **II** are approximately the same, thus the differences in retention observed with the two phases must reflect differences in the selectors. We recently suggested that π-acidic stationary phases such as phase **I** (which contains a π-basic phenyl substituent at the stereogenic center) may be capable of undergoing simultaneous face to edge and face to face π–π interactions with π-basic analytes [16]. If this is indeed the explanation for the increased retention of the PAH analytes on phase **I** relative to phase **II**, then the fullerenes, which have no "edges', must derive a similar benefit from the presence of the phenyl group in phase **I**, since the data points for analytes **9** and **10** fall upon the line described by the PAH analytes.

Stationary phases **III** and **IV** provide substantial retention for the fullerene analytes but only moderate retention of the PAH analytes. Phases **III** and **IV** were included in the study to assess the ability of π-basic stationary phases to provide retention of the

TABLE I

CAPACITY FACTORS FOR ANALYTES **1–10** ON STATIONARY PHASES **I–X**

Conditions: mobile phase = 5% dichloromethane in hexane, flow-rate = 2.00 ml/min, ambient temperature. Void time determined using 1,3,5-tri-*tert.*-butylbenzene [14]. α = separation factor for C_{60} and C_{70}.

Analyte	Stationary phase									
	I	**II**	**III**	**IV**	**V**	**VI**	**VII**	**VIII**	**IX**	**X**
1	0.18	0.15	0.11	0.12	0.12	0.13	0.10	0.10	0.14	0.17
2	0.53	0.35	0.19	0.17	0.33	0.34	0.22	0.19	0.38	0.44
3	0.77	0.46	0.19	0.19	0.40	0.44	0.26	0.27	0.58	0.58
4	2.19	1.06	0.34	0.30	1.12	1.24	0.70	0.70	1.74	1.28
5	2.57	1.19	0.34	0.31	1.22	1.35	0.72	0.81	2.06	1.41
6	5.55	2.27	0.57	0.47	3.00	3.31	1.97	1.88	5.61	3.30
7	5.96	2.27	0.43	0.39	2.53	2.73	1.49	2.11	5.84	2.68
8	6.30	2.43	0.60	0.50	3.23	3.46	2.09	2.13	6.45	3.57
9	2.99	1.19	1.98	2.62	1.01	2.26	2.45	0.50	1.61	6.59
10	4.65	1.90	4.16	5.31	1.80	4.76	7.10	0.80	3.28	20.77
α	1.60	1.56	2.10	2.03	1.78	2.11	2.89	1.60	2.03	3.15

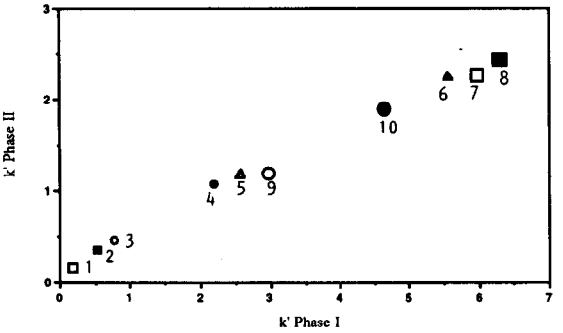

Fig. 9. Relative capacity factors for analytes **1–10** (as indicated) on phases **I** and **II**.

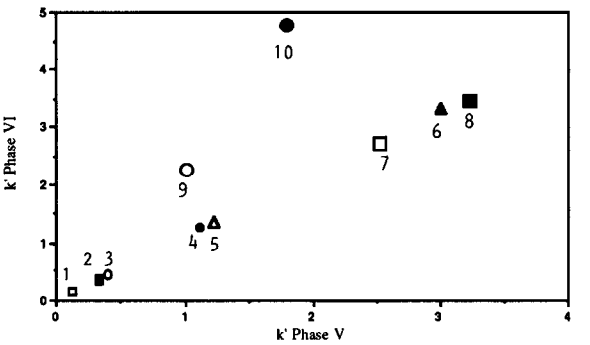

Fig. 11. Relative capacity factors for analytes **1–10** (as indicated) on phases **V** and **VI**.

fullerenes. In the plot of the the relative capacity factors observed with the two columns (Fig. 10), it can be seen that the data points for fullerene analytes **9** and **10** fall far from the line defined by the PAH analyte data points (slope = 0.07). This indicates that adsorption of the fullerenes occurs by a different mechanism than adsorption of the PAH analytes on phases **III** and **IV**. This unexpected finding suggests that the fullerene analytes may be polarized during adsorption so as to respond as either π-bases or π-acids. Alternatively, the aniline hydrogens of phases **III** and **IV** may hydrogen bond to the π-clouds of the fullerene analytes. Hydrogen bonds to π-clouds of aromatic systems are well precedented [17] and the convex surface of the fullerenes may render these analytes better able to undergo this interaction than the planar PAH molecules.

The glycine-derived phase **V** offers little advantage over the related π-acidic amino acid-derived phases, **I** and **II**. Capacity factors on phase **V** are similar those provided by phase **II** and smaller than those provided by phase **I**, again suggesting the involvement of the phenyl ring of phase **I** in interaction with the analytes.

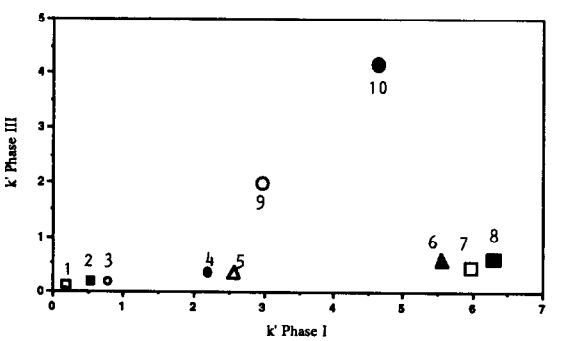

Fig. 10. Relative capacity factors for analytes **1–10** (as indicated) on phases **I** and **III**.

The isolated dinitrobenzamide system of phase **VI** retains the PAH analytes to about the same extent as do phases **II** and **V** but to a lesser extent than the phenylglycine-derived phase **I**. Fig. 11 shows the relative retentions of analytes **1–10** on phases **V** and **VI**. The data points for the PAH analytes **1–8** fall on a line having a slope of 1.08, the fullerene analytes lying off of this line owing to their selective adsorption by phase **VI**. Whatever the nature of this selective adsorption, it can be readily seen that the second amide group of the amino acid-derived π-acidic phases, **II** and **V**, makes little contribution to the retention of the fullerenes. Possibly, intramolecular hydrogen bonding between the 3,5-dinitrobenzamide hydrogen and the C-terminal carbonyl oxygen may interfere with the ability of phases **II** and **V** to hydrogen bond to the fullerenes.

The dinitroaniline-containing phase, **VII**, does not retain the PAH analytes as well as the dinitrobenzamide-containing phase, **VI**, but retains the fullerene analytes rather more strongly than does phase **VI**. Fig. 12 illustrates the relative capacity factors of analytes **1–10** on these two stationary phases. The data points for the PAH analytes fall on a line of slope 0.58 with the data points for the fullerene analytes lying considerably off of this line. The selective retention of the fullerene analytes (especially C_{70}) on phase **VII** relative to phase **VI** may

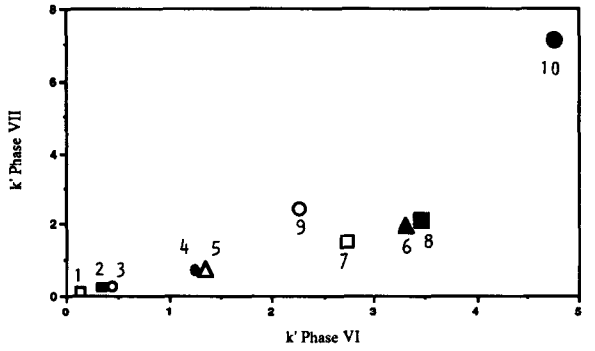

Fig. 12. Relative capacity factors for analytes **1–10** (as indicated) on phases **VI** and **VII**.

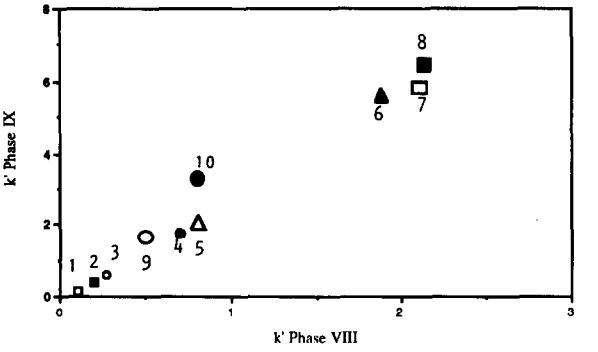

Fig. 14. Relative capacity factors for analytes **1–10** (as indicated) on phases **VIII** and **IX**.

be attributable to a difference in the hydrogen bonding ability of the aniline and amide hydrogens which may be oriented differently with respect to the π-acidic aromatic ring. The 3,5-dinitrobenzamide and 2,4-dinitroaniline moieties differ in π-acidity but this might not be expected to differentially affect the PAH and fullerene retentions.

As shown in Fig. 13, the dinitrobenzoate ester phase, **VIII**, shows reduced retention for the PAH analytes relative to the dinitrobenzamide phase, **VI**, the best line through these data points having a slope of 0.63. The data points for the fullerene analytes fall significantly below the line described by the PAH analyte data points, reflecting the poor retention of the fullerenes on the ester phase (which contains no hydrogen bond donors) relative to the amide phase (which does contain such a donor). This result may again indicate the importance of hydrogen bonding interactions in the retention of the fullerene analytes.

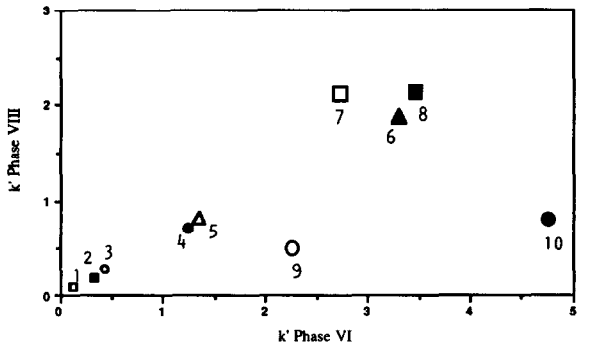

Fig. 13. Relative capacity factors for analytes **1–10** (as indicated) on phases **VI** and **VIII**.

The tripodal ester phase, **IX**, is similar to phase **VIII**, except that it may be capable of simultaneous multipoint interaction with the fullerene and PAH analytes. Interestingly, phase **IX** is the only one of the stationary phases examined which shows retentions for the PAH analytes which are comparable to those provided by phase **I**. Phases **VIII** and **IX** have similar loadings (about $1.5 \cdot 10^{-4}$ mol/g). However, since phase **IX** contains three π-acidic dinitrobenzoyl groups on every strand of selector, the "effective" loading of phase **IX** is roughly three times that of phase **VIII**. Fig. 14 depicts the relative capacity factors of analytes **1–10** on these two stationary phase.s The best line through the data points for the PAH analytes has a slope of 3.03, which would seem to indicate that each of the π-acidic rings of the tripodal phase **IX** are acting independently in retention of the PAH analytes. The data points for the fullerene analytes fall only slightly above the line defined by the PAH analytes, suggesting that simultaneous multipoint interaction of phase **IX** with the fullerene analytes is not a predominant retention mode.

Tripodal phase **X** afford excellent retention and separation for all of the analytes, showing the greatest retention for the fullerenes and the best separation factor for the C_{60}–C_{70} mixture. The relative capacity factors for analytes **1–10** on the two tripodal phases **IX** and **X** are illustrated in Fig. 15. The reason why phase **X** provides the degree of increased retention for the fullerenes relative to the

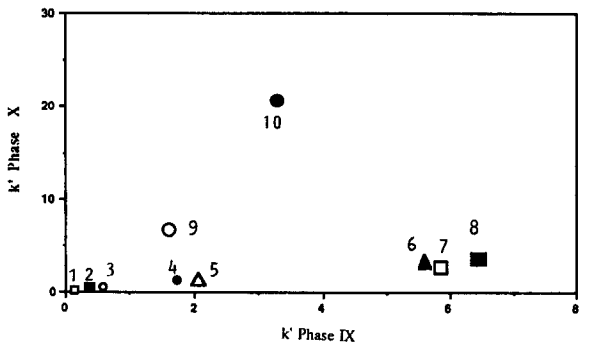

Fig. 15. Relative capacity factors for analytes **1–10** (as indicated) on phases **IX** and **X**.

tripodal ester phase **IX** is currently not well understood. A dinitrophenyl ether stationary phase analogous to phase **VIII** would be helpful in determining the contribution of the 2,4-dinitrophenyl group itself to this behavior. The π-acidic groups in phase **X** are positioned one atom closer to the branching point than those of phase **IX**, and this might conceivably impart some degree of conformational rigidity or a more favorable geometry for simultaneous multipoint interaction than in the case of phase **IX**.

Development of improved selectors

Although phase **X** provides superior retention and separation for the fullerene analytes, the current study suggests several possibilities for further improvements. The apparent ability of π-basic selector to afford significant retention for these compounds should be further investigated to determine whether it is π-π interaction or hydrogen bonding which retains the fullerenes on phases **III** and **IV**. The apparent importance of hydrogen bonding in fullerene retention and the complete absence of hydrogen bond donors in phase **X** (the best phase developed to date) suggests that phases such as the triamine-derived analogues of phases **IX** and **X** may prove even more useful for fullerene separation. In addition to having both π-acidic and hydrogen bonding groups, such phases may also adopt conformations in which intramolecular hydrogen bonding stabilizes the concave disposition of aromatic groups, thus providing a more preorganized selector for the fullerenes.

We recently described the use of the "immobilized guest method" in the development of improved selectors for enantioselective recognition of the non-steroidal anti-inflammatory drug, naproxen [18]. In this approach the "guest" is immobilized on silica gel to afford a stationary phase which is used to evaluate candidate "hosts". Such an approach offers the advantage of requiring only very small amounts of prospective selectors for analysis, in contrast to developing a new stationary phase for every selector to be evaluated. We initially reasoned that such an approach would be impossible for the fullerenes since they possess no functionality through which they can be conveniently immobilized. However, recent advances in the functionalization of buckminsterfullerene [19,20] suggest that this approach may, at some point, be feasible.

CONCLUSIONS

All of the stationary phases examined in the study provide some degree of retention and separation for the fullerenes, C_{60} and C_{70}. The π-acidic tripodal phase, **X**, designed for simultaneous multipoint interaction with the fullerenes, provides the highest degree of retention and the highest separation factor for C_{60} and C_{70}. This stationary phase may prove useful for preparative scale chromatographic purification of buckminsterfullerene as well as for chromatographic analysis and purification of the ever growing family of fullerenes and fullerene derivatives.

In one sense, this study raises a many questions as it answers. For example, it suggests that fullerene selectors affording greater retention and selectivity than does phase **X** may be developed. Selectors with dramaticaly increased retention and/or selectivity might allow for purification of multigram quantities of buckminsterfullerene, perhaps through batch adsorption techniques or other non-tranditional purification methods. In broader terms, polypodal selectors may prove useful in a host of other molecular recognition and nanotechnological applications.

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