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Liquid Chromatography/Mass Spectrometry Investigation of the Reversed-Phase Separation of Fullerenes and Their Derivatives[†]

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The separation of fullerenes and their derivatives on four commercially available reversed-phase columns was investigated using liquid chromatography/mass spectrometry with atmospheric pressure chemical ionization. Three octadecyl-silica stationary phases were incapable of adequately separating the fullerenes according to the number of carbons. However, baseline resolution of nearly all the fullerenes (up to at least C_{96}) was achieved with a 2-(1-pyrenyl)ethylsilica phase. The resolution of individual fullerene isomers, on the other hand, was best accomplished with a polymeric octadecylsilica bonded phase. A proposed mechanism for this unique ability combines the shape selectivity of polymeric phases, previously recognized for planar polycyclic aromatic hydrocarbons, and the different "molecular footprints" of the isomers. This retention mechanism can also be used to explain the increasingly lower retention times of successively substituted fullerenes; groups bonded to the fullerene surface appear to disrupt adsorptive interactions between the fullerene molecular footprints and the stationary phases.

INTRODUCTION

The development of improved methods for the production of large quantities of spherical carbon clusters, the fullerenes,[1-4] has led to an explosion of interest in the characterization of these molecules and their derivatives.[5-13] The major components present in soot produced by the vaporization of graphite, C_{60} and C_{70}, have been well-characterized by NMR, mass spectrometry (MS), and many other spectroscopic techniques because they can be conveniently isolated in relatively pure form by open column chromatography using either alumina or silica packing materials.[5,11-14] However, the fullerene-rich soots also contain small amounts (1–5%) of larger fullerenes, such as C_{76}, C_{78}, C_{84}, C_{90}, and C_{94}. These have been much more difficult to obtain pure in quantity, especially since most of these larger fullerenes are present in more than one isomeric form.[6-9,12,13,15] While the isolation and characterization of new fullerenes has captured the attention of many research groups, chemical derivatization of the fullerenes has been an equally intense area of research. Some of the organic reactions of C_{60} include the addition of nucleophiles,[16] dienes and 1,3-dipoles,[17] and radicals.[18,19] Since the fullerenes are polyfunctional molecules, a myriad of products can be formed after treatment with an excess of reagent.[20] The isolation of isomerically pure single compounds from such reactions using existing separation methods has been considered virtually impossible.[20]

Obviously, there is a need for the development of efficient and convenient chromatographic methods for the separation (and possibly large-scale purification) of fullerene isomers and derivatives. The separations of the larger fullerenes and C_{60} reaction mixtures tend to be very poor on either silica or alumina columns. Many of the current fullerene isolation methods employ a liquid chromatographic (LC) separation on bonded-phase columns as the final step in a series of

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separations.[12,13,21-23] The choice of the column used in this final separation will ultimately determine the purity of the collected fractions. Hawkins *et al.*[24] reasoned that a stationary phase containing π-acids might increase the retention and promote the discrimination of these π-basic aromatic clusters. While they found that Pirkle's phenylglycine-based column was useful for separating C_{60} and C_{70}, Welch and Pirkle[25] subsequently demonstrated that a novel tripodal π-acidic stationary phase designed for simultaneous multipoint interaction with fullerenes provided the greatest retention and separation factor for a C_{60}/C_{70} mixture. Similarly, Jinno *et al.*[26] described the use of "multilegged" phenyl group bonded silica phases, which form cavity-like spaces between methyl and phenyl groups, to separate C_{60} and C_{70} on the basis of interactions between the phenyl rings and methyl groups of the stationary phase and the solutes. The resulting separations were not much better than those achieved using an octadecylsilica (ODS) bonded phase.[26] For the separation of larger fullerenes, it was suggested that multilegged phases with appropriate cavity sizes to catch the fullerenes could be designed.[26] Recently, Kimata *et al.*[27] reported the use of a 2-(1-pyrenyl)ethylsilica stationary phase for the preparative separation of fullerenes using toluene as the mobile phase. Separation factors for C_{60} and C_{70} were comparable to those with other stationary phases that used much weaker solvents. It was also stated that this phase provided improved separations of late-eluting compounds, although these peaks were not identified.

Most of these[24-27] and other studies[14,22,28-32] have shown that mixtures of C_{60} and C_{70} can be readily separated on a variety of stationary phases, but the resolution of the larger fullerenes and their isomers has not been clearly demonstrated. A recent attempt to characterize the separation of higher fullerenes on reversed-phase ODS columns[23] relied on mass spectrometric and ^{13}C NMR identification of seven fractions collected above C_{84}. This method suffered from the need for a large number of injections and many days of labor to produce microgram quantities of isolated compounds. The acquisition of reliable spectroscopic data was hampered by the incomplete resolution of most of the large fullerenes.

We have recently demonstrated that extracts of fullerene soots produced by graphite arcs and benzene flames are extremely complex mixtures, containing numerous isomeric forms of large fullerenes as well as various fullerene derivatives.[6-9] In a continuation of this work, the present paper reports on an evaluation of four commercially available reversed-phase liquid chromatographic stationary phases for their ability to (i) separate fullerenes according to number of carbons (e.g., C_{76} from C_{78}, and so on), (ii) resolve individual isomeric forms of fullerenes (e.g., the isomers of C_{78}), and (iii) separate series of derivatized fullerenes. Two fullerene soot

extracts were utilized in this study: a high-temperature extract of a graphite-derived soot shown previously to contain fullerenes as high as C_{120}[9] and a flame-generated soot extract shown to contain a complex mixture of fullerene derivatives.[6-8] Compound identification was achieved by on-line coupling of liquid chromatography with atmospheric pressure chemical ionization (APCI) mass spectrometry.[6-9]

EXPERIMENTAL SECTION

Columns Studied. The following commercial reversed-phase liquid chromatography columns were used: (a) 5-μm Vydac 201TP52 C_{18}, 25 cm $\times$ 2.1 mm i.d. (Separations Group, Hesperia, CA); (b) 5-μm Hypersil-ODS, 25 cm $\times$ 2.1 mm i.d. (Shandon, Cheshire, UK); (c) 5-μm Zorbax Rx-C18, 15 cm $\times$ 2.1 mm i.d. (manufactured by Rockland Technologies, Inc., Newport, DE, under license from the Du Pont Co.); and (d) 5-μm Cosmosil PYE, 15 cm $\times$ 4.6 mm i.d. (Nacalai Tesque, Inc., Kyoto, Japan).

Materials. A sample of soot produced by resistive heating of graphite was purchased from the Texas Fullerene Corp. (TFC, Houston, TX) and extracted with boiling 1,2,4-trichlorobenzene (some C_{60} and C_{70} had been removed previously with a gentle toluene extraction).[9] A flame-generated material (soot and condensate) was extracted with toluene at room temperature using low-power ultrasonic agitation.[33] These solutions were the same as those characterized previously.[6-9,33,34] Spectroscopic grade toluene, benzene, dichloromethane, acetonitrile, and 1,2,4-trichlorobenzene were used as received from the suppliers without further purification.

LC with UV Detection. The mobile-phase gradient used with all columns was initially acetonitrile/dichloromethane (50:50), programmed linearly to 100% dichloromethane over 20 min, held for 25 min, and then programmed back to the initial composition over 5 min. The mobile-phase flow rate was 0.2 and 1.0 mL/min for 2.1- and 4.6-mm-i.d. columns, respectively. Injection volumes of 1 and 2 μL of concentrated solutions in dichloromethane were used for the 2.1- and 4.6-mm-i.d. columns, respectively. The small injection volumes were used to avoid band-broadening effects from the strong solvent. An HP1090M liquid chromatograph (Hewlett Packard Co., Palo Alto, CA), equipped with a ternary DR5 solvent delivery system, a variable-volume injector/autosampler, a built-in HP1040A diode array detector, and an HP7994A data system, was used in all LC/UVD analyses. The detector was configured for acquisition of absorbance at 368 $\pm$ 132 and 254 $\pm$ 2 nm.

LC with On-Line Mass Spectrometry. The LC conditions were identical to those used in the LC/UVD analyses except that 2- and 4-μL injection volumes were used for the 2.1- and 4.6-mm-i.d. columns, respectively. The LC/MS experiments were conducted using an API-III triple quadrupole mass spectrometer (SCIEX, Thornhill, ON, Canada), equipped with a heated pneumatic nebulizer interface and operated in the benzene charge-transfer chemical ionization mode as described previously.[6,7,9]

Full-scan mass spectra, in both positive and negative ion modes, were acquired over a range of m/z 650-1200 with a scan cycle time of 2 s. Subsequent selected-ion recording experiments on the TFC soot extract monitored 12 molecular ions (C_{60}-C_{96}) in positive ion mode with a dwell time of 100 ms per ion.

RESULTS AND DISCUSSION

Four very different stationary phases were selected for this study, *viz*: (a) Vydac 201TP, a polymeric octadecylsilane (ODS) phase bonded to 5-μm spherical silica (300-Å pore size, 100 m²/g surface area, 9% carbon loading); (b) Hypersil-ODS, a monomeric ODS phase bonded to 5-μm spherical silica (120-Å pore size, 170 m²/g surface area, 10% carbon loading); (c) Zorbax Rx-C18, a monomeric ODS phase bonded to 5-μm patented porous silica microspheres (80-Å pore size, 200 m²/g surface area, 12% carbon loading); and (d) Cosmosil PYE, a

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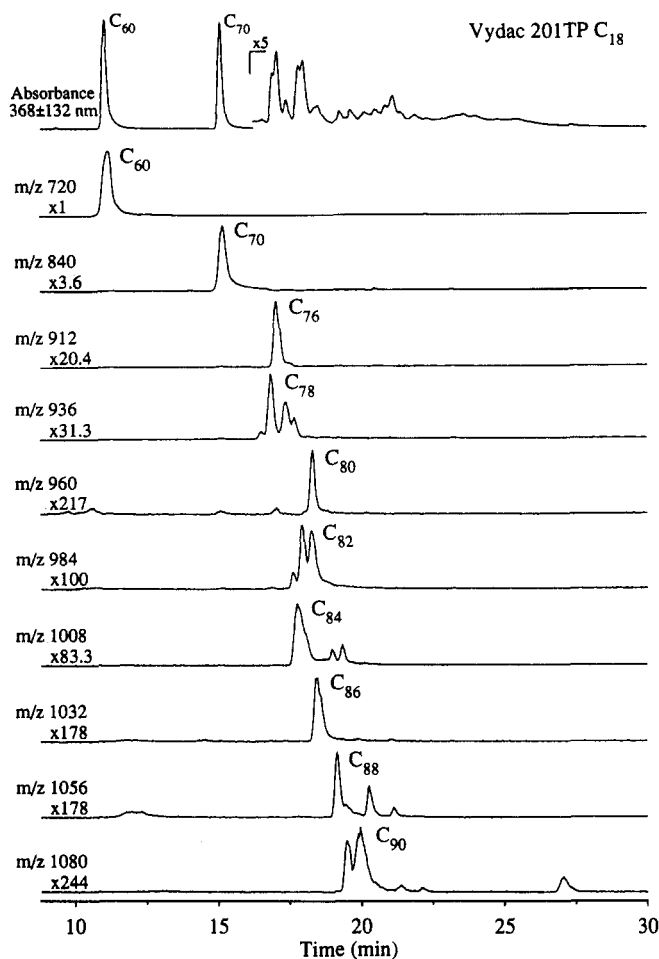
Figure 1. LC/UVD chromatogram (368 ± 132 nm, top trace) and LC/MS selected-ion chromatograms (monitoring molecular ions of the fullerenes C_{60}–C_{90}) from analyses of a trichlorobenzene extract of graphite soot using a Vydac 201TP column (polymeric octadecylsilica phase). Chromatographic conditions as described in the Experimental Section. Scaling factors are given for each normalized trace. Note: the peak shape on C_{60} is distorted slightly due to saturation of the LC/MS system.

Figure 2. LC/UVD chromatogram (368 ± 132 nm, top trace) and LC/MS selected-ion chromatograms (monitoring molecular ions of the fullerenes C_{60}–C_{90}) from the analyses of a trichlorobenzene extract of graphite soot using a Hypersil-ODS column (monomeric octadecylsilica phase). Chromatographic conditions as in Figure 1.

2-(1-pyrenyl)ethylsilane bonded to 5-μm spherical silica (110-Å pore size, 330 m^2/g surface area). Polymeric C_{18} phases are prepared by using trichlorosilane reagents with the addition of a small amount of water, while monomeric C_{18} phases use monochlorosilane reagents with the exclusion of water. Commercial columns packed with these stationary phases gave moderate to high efficiencies (plate counts from 9500 to 12 500) and good separations of C_{60} and C_{70} when used with an acetonitrile/dichloromethane mobile phase. The chromatograms for a trichlorobenzene extract of soot produced by resistive heating of graphite, using broad-band UV detection (UVD) and gradient elution, are given in the top traces of Figures 1–4 for each of the four columns. Of particular interest to this paper are the many small peaks (expanded scale is shown) corresponding to large (or giant) fullerenes. Little information on these compounds can be gained from the LC/UVD chromatograms (even through examination of UV spectra acquired by the diode array detector), and on the basis of these data alone, one might conclude that there is little difference among the columns.

We have shown previously that LC/MS using a heated pneumatic nebulizer interface is a powerful tool for the analysis of complex mixtures of fullerenes.[6,7,9] Soft ionization is achieved by atmospheric pressure chemical ionization in a plasma sustained by a corona discharge. Introduction of benzene to the APCI source ensures that the dominant ionization mechanism for the fullerenes in the positive ion
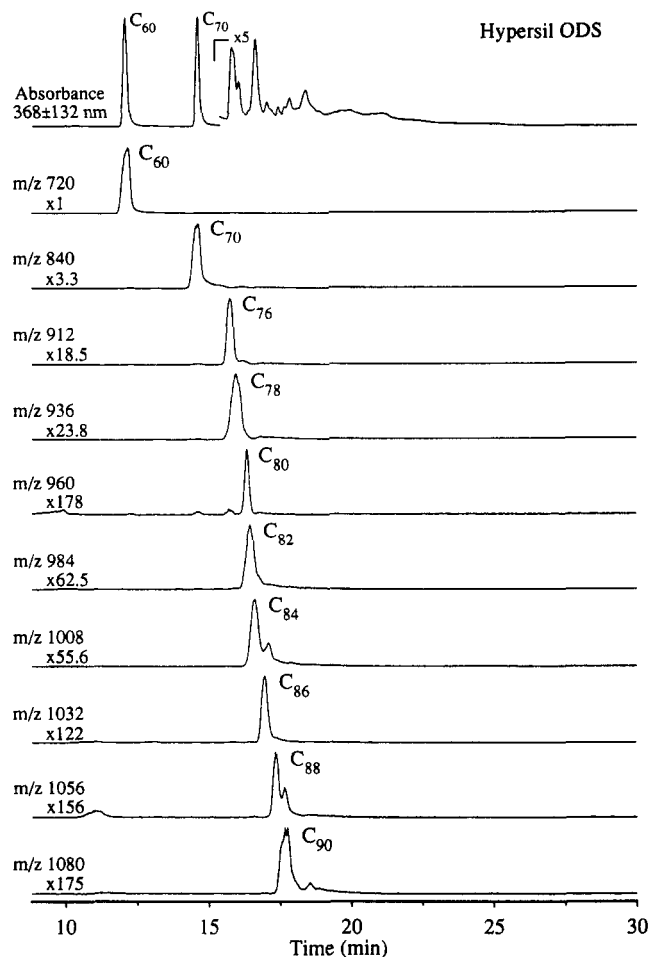
mode is charge transfer from $C_6H_6^{•+}$ ions which dominate the plasma.[6,7,9] In this study, the LC/MS technique has been used to characterize the stationary phases for the separation of fullerenes and their derivatives. Figures 1–4 show mass chromatograms produced from the LC/MS analyses using the four different columns. The mass chromatograms of the molecular ions allow the selective detection of C_{60}, C_{70}, and the fullerene series C_{76+2n} (n = 0–7). The high sensitivity of the technique allows the detection of many individual species in this obviously very complex mixture. We have also detected many fullerene derivatives, including hydrofullerenes ($C_{60}H_{2n}$), alkyl-substituted fullerenes, and fullerene oxides, in a flame-generated soot.[6,7]

In the sections below, we will discuss the influence of the different stationary phases on the separation of fullerenes according to carbon number, isomeric structure, and degree of substitution.

Separation of Fullerenes by Carbon Number. We have demonstrated recently that fullerenes as high as C_{120} are amenable to nonaqueous reversed-phase liquid chromatographic separations.[9] The LC/MS analysis of a high-temperature extract of fullerene soot using a polymeric Vydac 201TP C_{18} phase (Figure 1) revealed that, while there was a gradual increase in retention time with carbon number, there was still considerable overlap between fullerenes of differing mass. For example, C_{76} eluted in the center of four C_{78} isomers. Diederich et al.[12,13,21] relied on repeated chromatographic separations on a Vydac 201TP C_{18} column for the isolation of C_{76} and two isomers of C_{78} from a sample enriched in these higher fullerenes. A third isomer has been subsequently
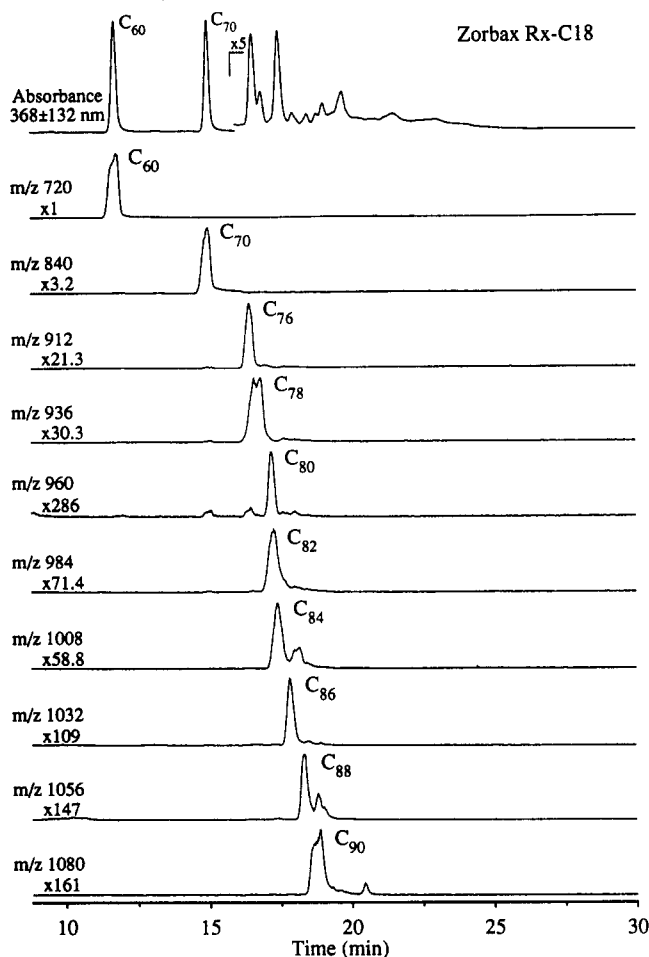
Figure 3. LC/UVD chromatogram (368 ± 132 nm, top trace) and LC/MS selected-ion chromatograms (monitoring molecular ions of the fullerenes C_{60}–C_{90}) from the analyses of a trichlorobenzene extract of graphite soot using a Zorbax Rx-C18 column (monomeric octadecylsilica phase bonded to patented porous silica microspheres). Chromatographic conditions as in Figure 1.

Figure 4. LC/UVD chromatogram (368 ± 132 nm, top trace) and LC/MS selected-ion chromatograms (monitoring molecular ions of the fullerenes C_{60}–C_{90}) from the analyses of a trichlorobenzene extract of graphite soot using a Cosmosil PYE column [2-(1-pyrenyl)ethylsilica phase]. Chromatographic conditions as in Figure 1. Note: the LC/MS relative responses of the large fullerenes ($>C_{70}$) are much greater than those observed in Figures 1–3 because of the absence of acetonitrile in the mobile phase after 20 min.

identified by the NMR analysis of crude C_{78} fractions.[15,35] The overlap of the fullerene masses shown in Figure 1 is associated with the unique ability of the polymeric C_{18} stationary phase of the Vydac column to separate fullerene isomers (*vide infra*).

The same analysis performed on a Hypersil ODS column (a monomeric C_{18} phase) produced dramatically different results (Figure 2). The fullerenes eluted in order of increasing mass and the individual isomers were no longer well resolved. The ability of the Hypersil monomeric phase to separate the fullerenes according to carbon number was only slightly better than that of the Vydac polymeric phase; there was still slight overlap (e.g., C_{76} and C_{78}) as well as coelution (e.g., C_{80} and C_{82}) of many fullerenes of different mass. In view of the dramatic difference between the monomeric and polymeric C_{18} phases with regard to separating fullerenes, a third novel C_{18} phase was also incorporated into this study. The patented porous silica microspheres used as a stationary phase support in Zorbax Rx columns is designed to produce columns with high efficiencies and reduce band tailing for basic compounds. The analysis of the graphite soot extract on a Zorbax Rx-C18 column (Figure 3) produced results that were very similar to those for the monomeric Hypersil ODS phase (Figure 2).

As an alternative to C_{18} reversed-phase columns, a 2-(1-pyrenyl)ethylsilica packing material (the Cosmosil PYE column) was evaluated for its ability to separate the fullerenes. This stationary phase has the added advantage of π-bond
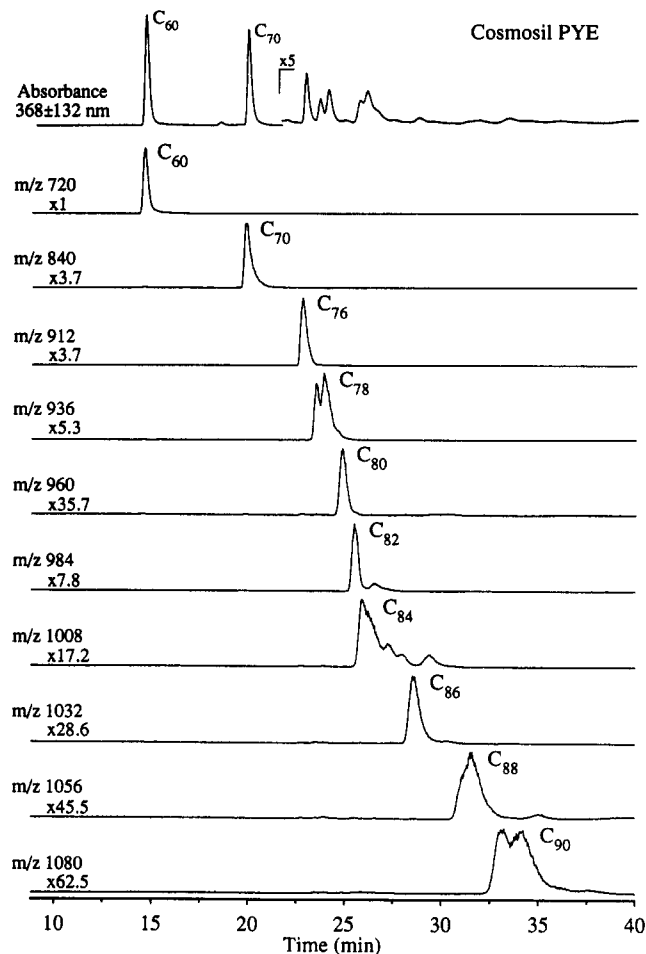
interactions with the fullerene analytes[27] (as opposed to only van der Waals interactions with the C_{18} materials). Figure 4 illustrates that nearly all of the fullerene masses could be baseline resolved (in particular, C_{76} from C_{78}) using the pyrenyl phase. There was only a slight improvement in isomer resolution for certain fullerenes (e.g., C_{78} and C_{84}) over the monomeric C_{18} phases (Figures 2 and 3).

A plot of the relative retention times versus carbon number for all of the fullerenes detected on the four columns (Figure 5), clearly shows that the pyrenyl column exhibited a significantly different behavior from that of the C_{18} columns. As the fullerenes increased in size (greater than C_{90}), the relative retention times on the three C_{18} phases began to level off, while the retention on the pyrenyl phase continued to increase almost exponentially. When chromatographic isolation of individual fullerenes by carbon number is required, the pyrenyl column would certainly be a better choice than the conventional C_{18} columns, especially for large fullerenes.

Separation of Fullerene Isomers. Unlike C_{60}, C_{70}, and C_{76}, where isolated-pentagon isomers are unique or small in number,[36,37] the large fullerenes have many more isomers satisfying the pentagon-isolation rule (it is generally accepted that structures with no adjacent pentagons are more stable

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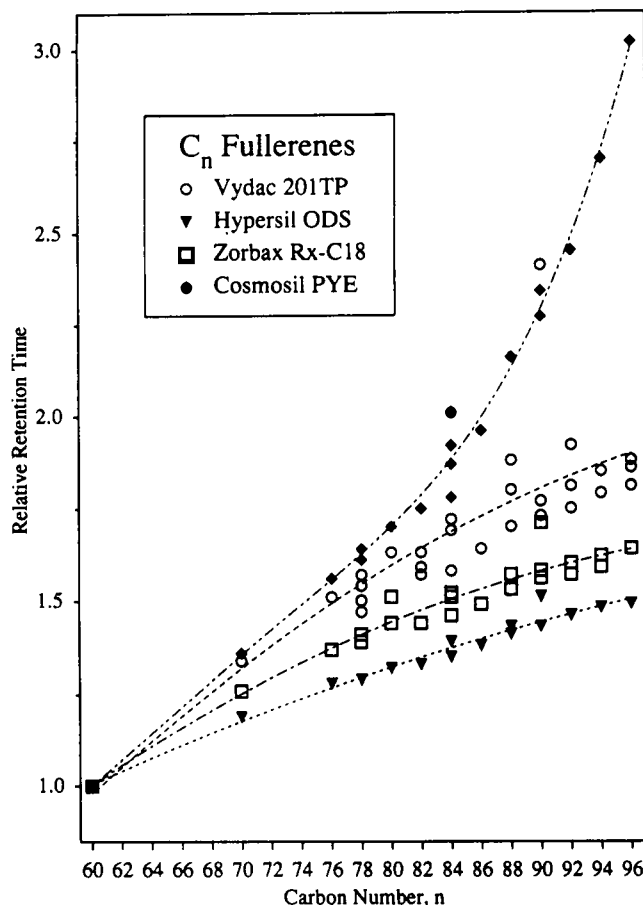
Figure 5. Plot of retention times relative to that of C_{60} versus carbon number of all the fullerenes detected in the LC/MS analyses of a trichlorobenzene extract of graphite soot shown in Figures 1–4. Chromatographic conditions as in Figure 1.

because this arrangement minimizes the strain energy). Therefore, the ability to chromatographically resolve isomers will become much more important when the isolation of larger fullerenes is desired.

The data shown in Figures 1–5 illustrate that the polymeric Vydac C_{18} stationary phase is superior in resolving fullerene isomers. It is of interest to inquire why this column is so much better at resolving these isomers than the other C_{18} columns. Previous studies have demonstrated that phase type (monomeric versus polymeric) greatly affects the separation of isomeric polycyclic aromatic hydrocarbons (PAH), with polymeric phases providing greater separation selectivity than the monomeric phases.[38–40] In general, for a set of isomeric PAH, retention on phases of both types increases with increasing length-to-breadth ratio of the molecule, although this effect is greatest for the polymeric phases. It has been proposed that this structure–retention relationship, and its similarities to PAH retention on alumina, is the result of an adsorption contribution in the retention mechanism.[38] Therefore, the more uniform and ordered polymeric C_{18} phases are pictured as more sensitive to PAH shape and planarity because of greater adsorptive interactions as a PAH molecule lines up along the C_{18} surface.[38]

A similar retention mechanism may be applied to the fullerenes by using the idea of "molecular footprints" introduced by Cox et al.[10] to discuss the chromatographic characteristics of C_{60} and C_{70}. These authors found that the

retention of the soccer-ball-shaped C_{60} on a dinitroanilino-propyl silica (DNAP) phase is very similar to that of triphenylene, a C_{18} planar PAH molecule, while the retention of the football-shaped C_{70} lies somewhere between the five-ring benzo[a]pyrene and the six-ring coronene PAH compounds. In other words, the retention of C_{60} and C_{70} closely resembles that of planar PAH molecules with correspondingly similar molecular footprints. Since isomeric fullerenes have different shapes, and therefore different footprints that resemble those of various PAH molecules, we believe that the shape selectivity of the polymeric phases toward PAH (described above) is probably responsible for the separation of fullerene isomers.

It should also be mentioned that a different PAH retention model has been proposed subsequently,[39] which more accurately describes the ability of the C_{18} phases to distinguish between nonplanar and planar PAH, as well as explaining the correlation between PAH retention and their length-to-breadth ratio. This so-called "slot model" envisages the bonded phase as consisting of a number of narrow slots into which the solute molecules can penetrate. Planar molecules may be able to fit more easily into such narrow slots and interact strongly with the C_{18} stationary phase, whereas nonplanar molecules cannot penetrate as far into the slots and thus interact less strongly with the stationary phase. Long narrow molecules (i.e., large length-to-breadth ratios) would fit into more of the available slots than would "square" molecules and so are retained longer than square molecules. However, in contrast to the small, relatively planar PAH, fullerenes have large spherical dimensions that are unlikely to penetrate into slots. Therefore, the discrimination of fullerene isomers by polymeric C_{18} phases is better explained by the earlier retention mechanism[10,38] (i.e., footprint adsorption to the C_{18} surface).

Separation of Simple Fullerene Derivatives. In addition to fullerenes, a flame-generated soot extract has been shown[7] to contain a number of simple fullerene derivatives including $C_{60}H_2$, $C_{60}H_4$, $C_{70}H_2$, and $C_{60}CH_4$. Although these relatively minor components are easily detected in both positive and negative ion modes, the LC/MS technique is much more sensitive in negative ion mode because of the efficient ionization of the derivatives by proton abstraction. Further detailed analysis of this soot extract in negative ion mode has now revealed the presence of many more minor derivatives that actually form a series of $C_{60}H_{2n}$ ($n = 1, 2, 3$, ...) and $C_{60}CH_{2n}$ ($n = 2, 3, 4$, ...) fullerenes. These minor components were used to probe the separation characteristics of the four columns with respect to a series of simple fullerene derivatives.

The relative retention times of the $C_{60}H_{2n}$ derivatives on the four columns used in this study are plotted against the numbers of hydrogen atoms, $2n$, in Figure 6. Chromatographically resolved isomeric forms of the hydrofullerenes were observed for only the $C_{60}H_8$ and $C_{60}H_{10}$ compounds, although very large numbers of isomers are theoretically possible for each species (e.g., $C_{60}H_2$ has 23 possible isomeric forms).[41,42] The separation behavior on all four columns is very similar, but the Zorbax Rx-C18 appears to be slightly better at separating these derivatives than the other columns.

The relative retention times of the second series of fullerene derivatives, the $C_{60}CH_{2n}$ species, are plotted in Figure 7. The actual structures of these derivatives are presently unknown, although they could be thought of either as a series of hydrogenated methyl-substituted fullerenes [i.e., $C_{60}(CH_3)H_n$, where $n = 1, 3, 5$, etc.] or as hydrogenated methylene-substituted fullerenes or fulleroids[16,17,43] [i.e., $C_{60}(CH_2)H_n$,

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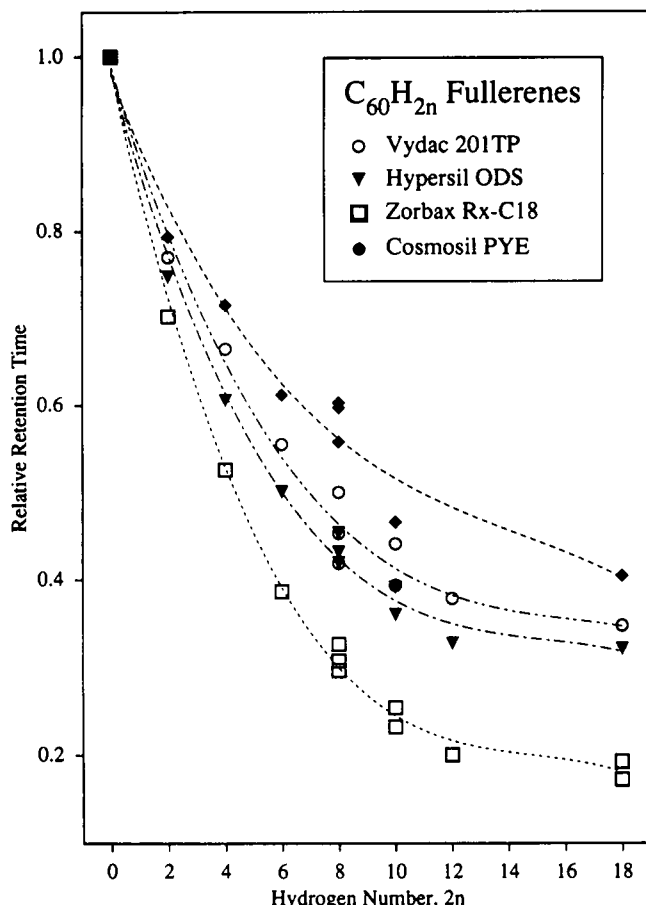
Figure 6. Plot of retention times relative to that of C_{60} versus hydrogen number of the hydrofullerenes detected in the LC/MS analyses of a flame-generated soot extract. Chromatographic conditions as in Figure 1.

Figure 7. Plot of retention times relative to that of $C_{60}CH_4$ versus hydrogen number of the $C_{60}CH_{2n}$ derivatives detected in the LC/MS analyses of a flame-generated soot extract. Chromatographic conditions as in Figure 1.

where $n = 2, 4, 6$, etc.]. However, the absence of any detectable amounts of $C_{60}CH_2$, the recently synthesized parent compound for the fulleroid series,[43] seems to support the methyl-substituted description. The retention behavior is very similar to that of the $C_{60}H_{2n}$ derivatives, and once again, the Zorbax Rx-C18 column is clearly better than the other three columns at separating these simple derivatives. No explanation of these observations can be proposed at this time.

It is interesting to note that all of the fullerene derivatives detected in the flame soot elute before the parent compound.[7] This was also observed previously for the separation of *tert*-butyl derivatives of C_{60} on a C_{18} column,[20] although the reasons for this behavior were not addressed. Since retention in reversed-phase chromatography is largely dependent on the solubility of the analyte in the mobile phase, the alkylated derivatives of the fullerenes might be more soluble in the mobile phase (acetonitrile/dichloromethane) and hence elute earlier. However, this is the exact opposite of what is observed for PAH; alkylation produces an increase in retention over that of the parent compound.[44] The isolation of these alkylated fullerene derivatives, and the determination of their solubilities relative to those of the respective parent compounds, will ultimately reveal whether solubility is responsible for the retention characteristics of the derivatives.

A more probable explanation of the early elution of the derivatives relies on the idea of fullerene molecular footprints. The addition of an alkyl group (or possibly any group) to the surface of the fullere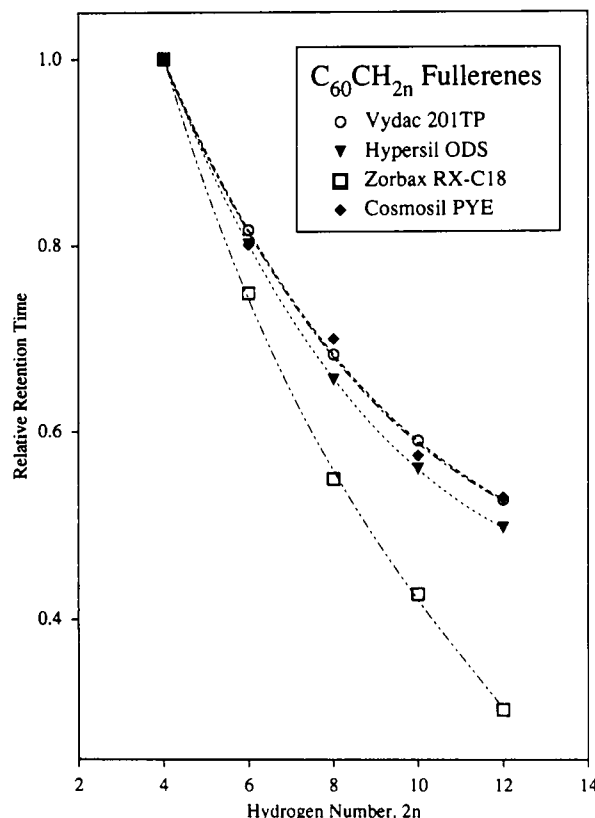ne would not only disrupt the shape of the footprint but also prevent intimate contact between the entire fullerene footprint and the stationary phase. The reduction of adsorptive interactions between the solute and the stationary phase would lead to a decrease in retention, and as more groups are added to the available surface, the effect becomes greater. This concept is paralleled by the alkylation of PAH in the "bay" region of the molecule, which results in the loss of planarity and a consequent decrease in retention relative to that of the parent compound.[44]

Thermally Metastable Derivatives. The original analysis of the flame-generated soot extract revealed the presence of several abundant components with fullerene-like characteristics.[33] According to subsequent LC/MS analyses using APCI, these additional components behaved in all respects as thermally metastable isomers of the stable C_{60} and C_{70} fullerenes.[6] Further analysis by charge-transfer ion spray LC/MS, however, showed that these constituents actually yield mass spectra consistent with their identification as fullerene adducts with highly unsaturated moieties.[8] The most abundant of these compounds produced mass spectra containing only molecular ions at m/z [M + 94], where M = 720 for C_{60} or 840 for C_{70}, while less abundant species with masses [M + 66] were also detected in the extract. Additional compounds, with masses [M + 80], were discovered in a fraction enriched with these species, but were undetected in the original soot extract. The three metastable components are homologues, with the general formula $C_xC_nH_{2n-4}$, with $n = 5$, 6, or 7, and $x = 60$ or 70. LC/MS/MS experiments revealed that the alkyl moieties exist as single substituent groups (collision-induced dissociation readily yields ions corresponding to the parent fullerenes and no intermediate ions corresponding to successive losses of other alkyl groups).[8] On the basis of this evidence, Rotello *et al.*[34] recently synthesized the C_{60}-cyclopentadiene adduct and found that it had spectra similar to that of the $C_{60}C_5H_6$ compound. We have now confirmed the identity of this compound by matching the

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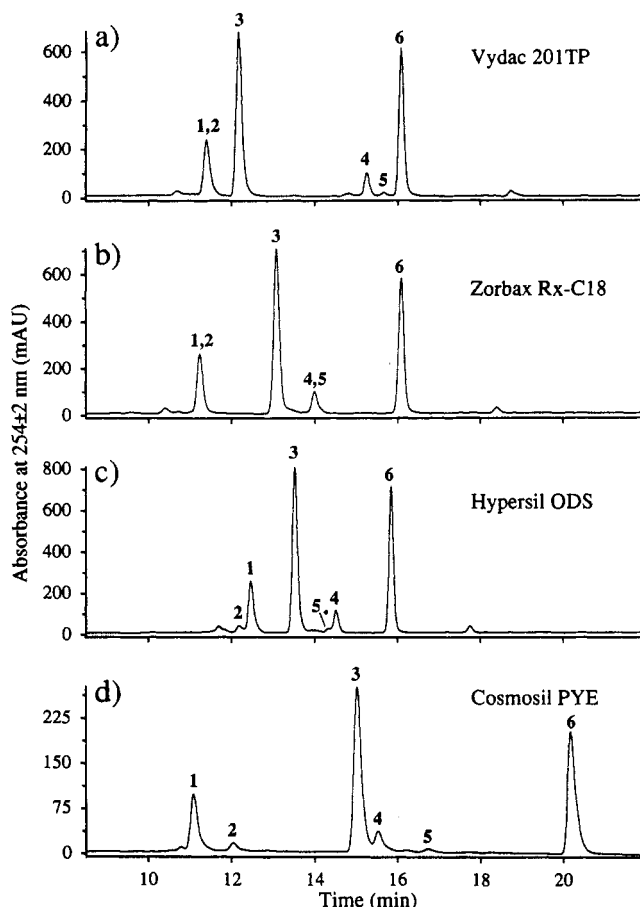
Figure 8. LC/UVD chromatograms (368 ± 132 nm) of a flame-generated soot extract using (a) a Vydac 201TP column, (b) a Zorbax Rx-C18 column, (c) a Hypersil-ODS column, and (d) a Cosmosil PYE column. Peak identities: (1) $C_{60}C_7H_{10}$; (2) $C_{60}C_5H_6$; (3) C_{60}; (4) $C_{70}C_7H_{10}$; (5) $C_{70}C_5H_6$; (6) C_{70}. Chromatographic conditions as in Figure 1.

retention times of the synthesized product on the four columns with those of the metastable component in the flame soot extract. We hypothesize that the other metastable components are methyl- and dimethylcyclopentadiene adducts of C_{60} and C_{70}.

The LC/UVD chromatograms obtained from the analysis of the flame soot extract on all four columns are shown in Figure 8. All of the major components, and most of the smaller ones, have been identified previously by LC/MS analyses.[6–9] The peaks labeled 3 and 6 correspond to C_{60} and C_{70}, respectively. Peaks 1 and 2 correspond to the metastable components $C_{60}C_7H_{10}$ and $C_{60}C_5H_6$, while peaks 4 and 5 correspond to the metastable $C_{70}C_7H_{10}$ and $C_{70}C_5H_6$, respectively. The Vydac column (Figure 8a) is able to resolve the C_{70} derivatives, peaks 4 and 5, but not the C_{60} derivatives, peaks 1 and 2. At first, these results were rather surprising in view of the ability of the polymeric phase to resolve fullerene isomers and the derivatives discussed earlier. However, in contrast to the series of simple derivatives, where successive hydrogenation occurs over the entire surface of the fullerene, the homology of these cyclopentadiene adducts is in a single attached group. Therefore, the molecular footprints of components 1 and 2 (or 4 and 5) would essentially be the same. The separation of the metastable homologues would thus have to rely on a difference in solubility or additional interactions between the cyclopentadiene moiety and the stationary phase.

The other two C_{18} stationary phases studied are also very poor at resolving these compounds. The Zorbax Rx-C18 cannot resolve either of the C_{60} or C_{70} metastable derivatives (Figure 8b), while the Hypersil ODS column can only partially resolve both sets of derivatives (Figure 8c). The order of elution of the metastable compounds on the monomeric Hypersil phase is the opposite of that on the polymeric Vydac phase (compare peaks 4 and 5, Figure 8a,c). This may be related to the interactions between the cyclopentadiene moieties of the metastable components and the two different C_{18} phases. The chromatogram in Figure 8d illustrates that the pyrenyl column could easily separate the metastable components. Once again, this is probably a result of additional π-bond interactions between the unsaturated alkyl moieties and the stationary phase.

CONCLUSIONS

The present work has shown that three different commercially available reversed-phase C_{18} columns are incapable of adequately separating the fullerenes, present in a high-temperature soot extract, according to carbon number. However, baseline resolution of nearly all of the fullerenes (up to at least C_{96}) according to carbon number may be achieved with a reversed-phase pyrenyl column. This is probably a result of increased π-bond interactions between the stationary phase and the fullerenes.

The resolution of individual fullerene isomers is best accomplished with a polymeric C_{18} phase, such as the Vydac 201TP. All of the other stationary phases, monomeric C_{18} and pyrenyl, are very poor in this respect. A proposed mechanism for this unique ability combines the shape selectivity (with respect to planar PAH molecules) of polymeric phases and the different molecular footprints (which resemble PAH) of the individual isomers. A possible separation scheme based on these results, to isolate individual fullerene isomers, would utilize the pyrenyl column to first separate the fullerenes according to carbon number (e.g., C_{78}, C_{80}, C_{82}, etc.), followed by the separation of individual isomers with the Vydac column.

Finally, the chromatographic separation of a series of hydrogenated derivatives of C_{60} and $C_{60}CH_4$ is easily achieved using any of the four columns used in this study, but the Zorbax Rx-C18 column performs slightly better than the others. The increasingly shorter retention times of the successively hydrogenated derivatives is probably related to the disruption of the adsorptive interactions between the fullerene molecular footprints and the stationary phase. The separation of the homologous series of thermally metastable cyclopentadiene derivatives is much more difficult since each of the alkyl moieties exist as one intact group and their molecular footprints are therefore very similar. The final proof of this proposed mechanism will rely on the determination of the solubilities of the derivatives relative to those of the parent compounds.