Automated HPLC Separation of Endohedral Metallofullerene Sc@C_{2n} and Y@C_{2n} Fractions

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We describe an automated HPLC separation of the endohedral metallofullerenes such as Sc@C_{2n} and Y@C_{2n} from empty-cage fullerenes utilizing two polystyrene chromatographic columns (500 and 1000 Å) in series. Rapid separation of the metallofullerene fraction from the empty-cage fullerenes (e.g., C_{60}) under anaerobic conditions is achieved. For the isolated Sc@C_{2n} fraction, all even-carbon-membered species from Sc_2@C_{74} to Sc_2@C_{104} were identified by negative-ion chemical ionization mass spectrometry. In addition, Sc_3@C_{82} was a prominent component of this fraction. For the separated Y@C_{2n} sample, the mass spectral data indicate the presence of Y@C_{82} and all even-carbon-numbered diyttrium species from Y_2@C_{82} to Y_2@C_{104}.

Endohedral metallofullerenes in which metal atom(s) are encapsulated in an all-carbon fullerene framework (M@C_{2n}) have received considerable interest recently because of their unique structure and potential applications.[1,2-9] To date, however, extensive structural elucidation studies have been hampered by the difficulty in isolating macroscopic amounts of pure samples. The usual mode of preparation, electric arc heating of graphite/metal or graphite/metal oxide mixtures in a He gas atmosphere, generally results in low yields of the metallofullerene fraction (<1%). Furthermore, the soluble fullerene fraction is dominated by the more abundant empty-cage fullerenes (e.g., C_{60} and C_{70}). Thus, separation of the minor metallofullerene fraction represents a formidable challenge. Another important factor is the uncertainty of the stability of metallofullerenes under aerobic conditions.[9,12]

Furthermore, selective detection of the minor metallofullerene fraction is an essential prerequisite in order to establish the effectiveness of a given separation.

High-performance liquid chromatography (HPLC) utilizing polystyrene columns provides methodology capable of metallofullerene[9,10] and fullerene[11] separations under relatively mild conditions. In the present study, an automated HPLC system for relatively large-scale separation of the metallofullerene fractions is described. Specifically, both scandium and yttrium metallofullerene fractions (Sc@C_{2n} and Y@C_{2n}) have been separated from the empty-cage fullerenes including C_{60}, C_{70}, C_{76}, C_{84}, etc. In addition, electron paramagnetic resonance (EPR) has been employed as a selective detector in an off-line mode for detection of the paramagnetic metallofullerenes (e.g., Y@C_{82} and Sc_3@C_{82}). These "EPR-active" metallofullerenes serve as characteristic "markers" for the entire metallofullerene fraction. EPR analysis in conjunction with off-line negative-ion chemical ionization (CI) mass spectrometry provides a convenient method of monitoring the separation.

EXPERIMENTAL SECTION

The metallofullerene samples were prepared by electric arc vaporization of 6-mm-diameter graphite rods which were core-drilled and packed with either Sc_2O_3 or Y_2O_3 and powdered graphite. The atomic weight percent of the metal (Sc or Y) in these core-packed rods ranged from 3 to 5%. Arc-burning was conducted in a He atmosphere (~200 Torr).[4,8] The soot was extracted with CS_2 and then further treated in a soxhlet extractor with refluxing toluene as the solvent. After solvent removal in vacuo, the soluble extracts were washed with diethyl ether. The extractions and other operations were performed under a N_2 atmosphere (from liquid N_2 boil-off, 99.9995% pure).

A diagram of the automated HPLC apparatus is shown in Figure 1. The fraction collector was contained within a portable plastic N_2 atmosphere bag and all inlet solvents were kept under a N_2 atmosphere. The metallofullerene soluble extract was initially dissolved in a 80/20 (v/v) mixture of toluene/decalin (degassed in N_2) at a concentration of ~2-3 mg/mL. The stock solution was filtered twice through a small

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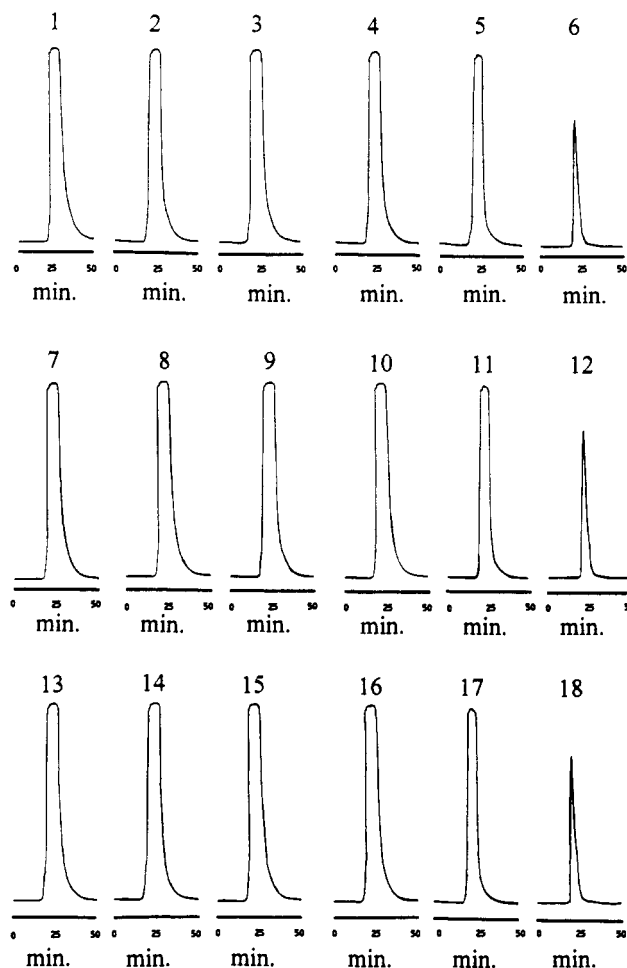‡ California Institute of Technology.
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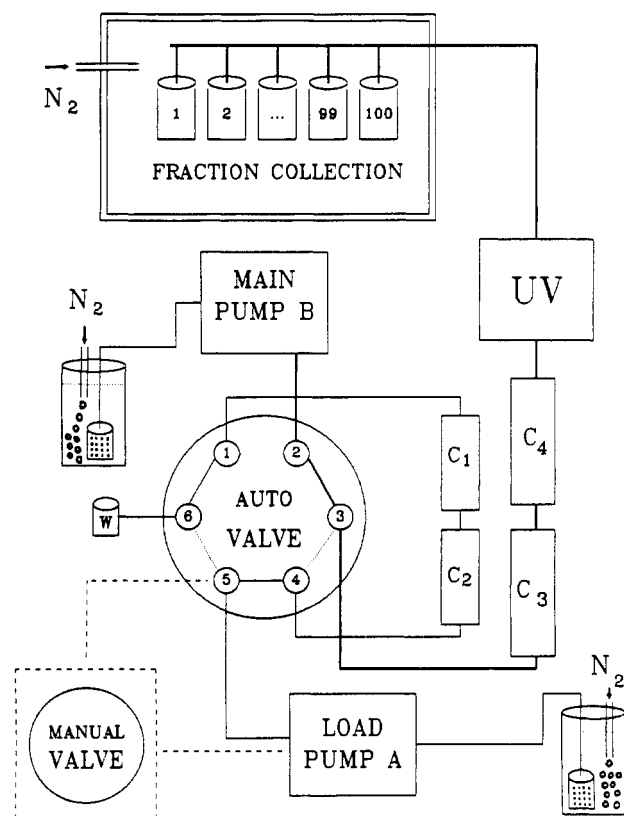
Figure 1. Automated HPLC apparatus: C_1, C_2 and C_3, C_4 are the load and separation polystyrene columns, respectively. The manual valve is only used in optional modes (see text).

Figure 2. Sequence of 18 automated Y@C_{2n} injections. The flow rate was 1 mL/min, solvents, toluene/decalin, 80/20 (v/v), and UV detection at 340 nm. The sequence represents six separate injections (~8 mg of Y@C_{2n}/injection) from the C_1 and C_2 load columns with the entire sequence repeated three times (C_1 and C_2 reloaded three times) as described in the text. The apparent reduced quantity injected on the last run of each sequence (sixth, twelfth, and eighteenth) reflects final voiding of the load columns (C_1 and C_2).

chromatographic column containing silica gel (~3 cm high, 2 cm wide) with a layer of clean sand and a glass wool plug at each end of the column. After filtration the solution was directly connected to the inlet of the loading pump A (Waters). A ChronTrol controller and timer (4–115-V outlets) initiate the start of load pump A. Pump A runs for 6 min (4 mL/min) to load the extract into the two load columns C_1 and C_2 (25 × 10 mm μStyragel columns). Then the ChronTrol controller simultaneously turns on the main pump B, activates the fraction collector (HAAKE Buchler, C-100), and externally activates the integrator recorder (Hitachi, D-2500). The integrator sends a control signal (via an input/output port, Hitachi interface card) to the air-driven automatic valve (Rheodyne 7000 L) to switch the flow from main pump B in order to inject the extract from C_1 and C_2 into series connected separation columns, C_3 and C_4, which consist of a 25 × 1.0 cm Perkin-Elmer PL gel, 10-μm, 1000-Å column (C_3) followed by a PL gel 5-μm, 500-Å column (C_4). After a programmable time of 4–5 min, the integrator sends a second control signal to the automatic valve to stop the fullerene injection and isolate the solvent flow into C_3 and C_4. With these conditions, the empty-cage fullerenes (C_{60}, C_{70}, C_{76}, C_{84}) elute mainly during the time interval between 24 and 29 min, while the metallofullerene fractions have slightly longer elution times between 30 and 37 min. After 50 min, the integrator is internally shut off and the fraction collector returns to the initial collection test tube at ~53 min. The injection cycle is repeated, with a signal sent from the ChronTrol controller activating the integrator at chromatographic time, $t = 0$. After six cycles, the fraction collector and main pump B are turned off and the load columns C_1 and C_2 are reloaded when a signal sent from

the ChronTrol controller activates the load pump A (6 min, 4 mL/min), as described above. The six-cycle injection/ elution sequence is then repeated. In this manner, the automated system has operated up to ~16 h unattended (see Figure 2). The UV detector (Hitachi, 340 nm) is continuously utilized in this automated separation mode.

A manual injector valve (see Figure 1) can easily be used for loading small quantities of the extract (via syringe) onto load columns C_1 and C_2. Alternatively, manual syringe injection directly onto separation columns C_3 and C_4 is also possible. An off-line EPR spectrometer, 9.6 GHz (IBM 200D-SRC), was employed to determine the elution times of the paramagnetic metallofullerenes (e.g., Sc_3@C_{82}) fractions. Mass spectra were obtained from a VG 7070E-HF spectrometer (VG Analytical, Manchester, UK) with negative-ion chemical ionization. Metallofullerene samples were evaporated onto the DCI probe filament and heated. The carrier gas was methane.

RESULTS AND DISCUSSION

The automated HPLC system described above allows precisely controlled repetition of a given chromatographic
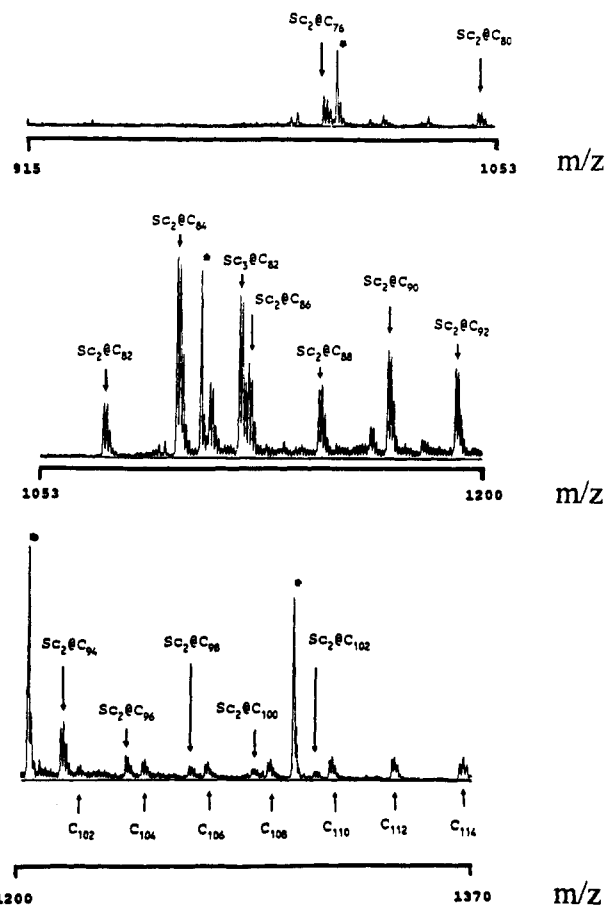
A)

EPR ACTIVE
REGION

B)

C)

D)

0   10   20   30   40   (min)
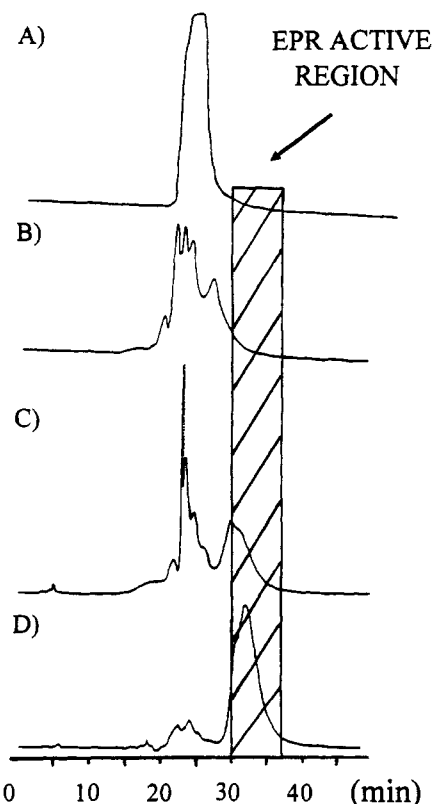
Figure 3. HPLC profile for $Sc@C_{2n}$ sample (UV 340 nm). (A) Profile represents the 30-37-min fraction collected after one pass on the polystyrene column, (B) second pass on the polystyrene column, (C) third pass on the polystyrene column, and (D) fourth pass on the polystyrene column. The chromatographic conditions are the same as noted in Figure 2, and the EPR-active region was defined by off-line EPR (Figure 6a).

$Sc_2@C_{76}$ $Sc_2@C_{80}$

915   1053   m/z

$Sc_2@C_{84}$ $Sc_3@C_{82}$ $Sc_2@C_{86}$ $Sc_2@C_{90}$ $Sc_2@C_{92}$

$Sc_2@C_{82}$ $Sc_2@C_{88}$

1053   1200   m/z

$Sc_2@C_{94}$ $Sc_2@C_{98}$ $Sc_2@C_{102}$

$Sc_2@C_{96}$ $Sc_2@C_{100}$

C_{102} C_{104} C_{106} C_{108} C_{110} C_{112} C_{114}

1200   1370   m/z

Figure 4. Negative-ion CI mass spectrum for the $Sc@C_{2n}$ metallofullerene fraction (fourth pass). The peaks at 1106, 1206, etc. (indicated by stars) are due to the standard, Ultramark 1621.

separation. Peak elution times in the automated mode (Figure 2) deviate only 3–5 s from one injection to the next. The quantity injected is also easily controlled and is highly reproducible. We have also employed this system for other fullerene separations using both normal-phase and reversed-phase chromatographic modes.

Although cursory examination of the UV trace suggests only one broad peak (Figure 2 or 3A), both off-line and on-line[13] EPR detection clearly confirm a significantly longer elution time for the selective detection of the $Sc_3@C_{82}$ species between 28 and 38 min (hatched region, Figure 3). This species has been previously characterized by EPR and has a characteristic 22-line spectrum (Fig. 6A), indicative of hyperfine coupling (6.8 G) of an electron spin to three equivalent Sc nuclei (^{45}Sc, $I = ^{7}/_{2}$) inside the cage.[1,6,8,9] In contrast, pure samples of C_{60} and C_{70} elute at ~24 and 25 min, respectively. To improve the separation, the metallofullerene fraction (30–37 min) was repeatedly recovered and reinjected into the two polystyrene columns. The UV detector traces after the first to fourth passes are shown in Figure 3. The effectiveness of the separation was verified by independent HPLC analysis (reversed-phase Vydac 201TP510, C_{18} col-

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umn) which indicates a significant reduction in the quantity of the empty-cage fullerenes present in the 30–37-min fraction. Specifically, the quantities of the empty-cage fullerenes in the 30–37-min fraction were reduced in a single pass by factors of approximately 28, 18, and 2.3 for C_{60}, C_{70}, and C_{76}, respectively (relative to the original stock solution). Significant quantities of C_{60} and C_{70} still remain after a single injection (Figure 3B), but they are nearly completely removed after the fourth pass (Figure 3D). In each profile (Figure 3A–D), off-line EPR detection was employed to ensure that the metallofullerene fraction (as monitored by the $Sc_3@C_{82}$ species) elutes in the region indicated.

The negative-ion CI mass spectrum for the $Sc@C_{2n}$ fraction obtained after the fourth pass on the polystyrene columns is presented in Figure 4. As already noted, this separation procedure removes most of the empty-cage fullerenes (C_{60} and C_{70}). More importantly, the higher fullerenes (C_{90}, C_{92}, C_{96}), which usually overlap with the metallofullerene fraction,[9] are clearly not present in significant quantities. The two dominant endohedral metallofullerenes present in this fraction are the $Sc_2@C_{84}$ and the paramagnetic $Sc_3@C_{82}$ compounds. The off-line EPR spectrum (Figure 6a) was recorded without concentrating the sample or degassing. Nevertheless, ~19 of the 22 lines in the pattern are observed under these conditions. The smallest endohedral discandium metallofullerenes that have been identifed are $Sc_2@C_{74}$ and $Sc_2@C_{76}$. In addition, every even-carbon-numbered $Sc_2@C_{2n}$ species from $Sc_2@C_{80}$ to $Sc_2@C_{104}$ can be found in the mass spectrum.

A) 30 - 35 MIN.

$Sc_3@C_{82}$

14 G

B) 34 MIN.

$Y@C_{82}$

7 G
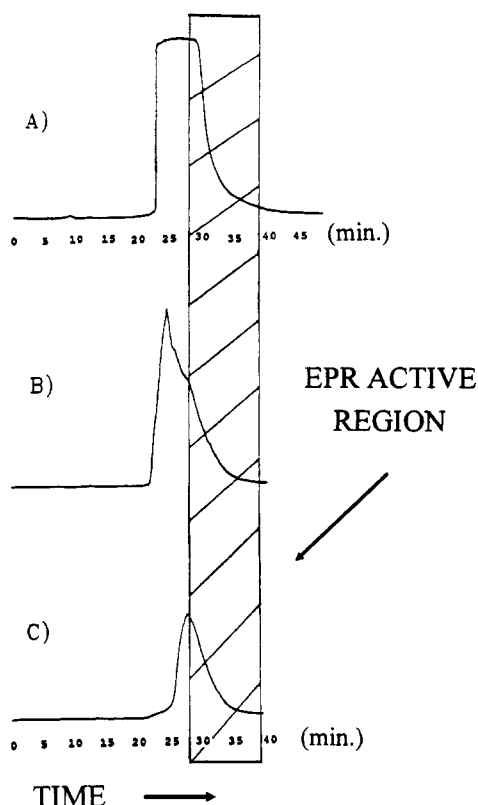
Figure 5. HPLC profile for $Y@C_{2n}$ sample (UV 340 nm). (A) First pass on the polystyrene column, (B) third pass on the polystyrene column, (C) and fifth pass on the polystyrene column. The chromatographic conditions are the same as noted in Figure 2, and the EPR-active region was defined by off-line EPR (Figure 6b).

Figure 6. Off-line EPR spectra, 9.6-GHz. (A) $Sc_3@C_{2n}$ 30–37-min fraction; (B) $Y@C_{2n}$ 28–38-min fraction. EPR spectra were obtained with ~ 10-mW microwave power. The samples were obtained directly from the fraction collector without concentrating (or degassing).

In the present study, the $Sc@C_{2n}$ samples were prepared with a relatively high ratio of three to five scandium atoms per 100 carbons vaporized in the arc-burning procedure. Although, a small quantity of the monoscandium fullerene $Sc@C_{82}$ was detected by examining the EPR spectrum (eight-line pattern) of the starting stock solution,[8,9] the mass spectrum does not indicate the presence of this molecule in the 30–37-min fraction. For a different scandium metallofullerene sample prepared via the same electric arc-burning procedure, but with a lower ratio of scandium/carbon atoms, the $Sc@C_{82}$ species was found in higher preponderance than the $Sc_3@C_{82}$ species. For this sample, it was observed by EPR (off-line analysis) that the $Sc@C_{82}$ species elutes slightly earlier than the 30–37-min fraction and maximizes at 29–30 min. However, the discandium and triscandium metallofullerenes dominate the metallofullerene fraction in the present study vide supra.

As a second example, a $Y@C_{2n}$ extract has been prepared and separated with the same automated HPLC apparatus. In this case, the EPR-active $Y@C_{82}$ compound was monitored on-line[13] and off-line (Figure 6b) to accurately define the retention time of the metallofullerene fraction. As illustrated in Figures 5 and 7, the $Y@C_{2n}$ fraction is effectively separated from the dominant C_{60} and C_{70} fullerenes after five passes on the polystyrene columns. The $Y@C_{82}$ species was found to elute at a retention time of 28–38 min (Figure 6B). In a fashion analogous to the scandium separation, the EPR signal was monitored off-line at each stage in the separation. Once again, the EPR spectrum was recorded without concentrating the samples or degassing. Adequate degassing is essential in
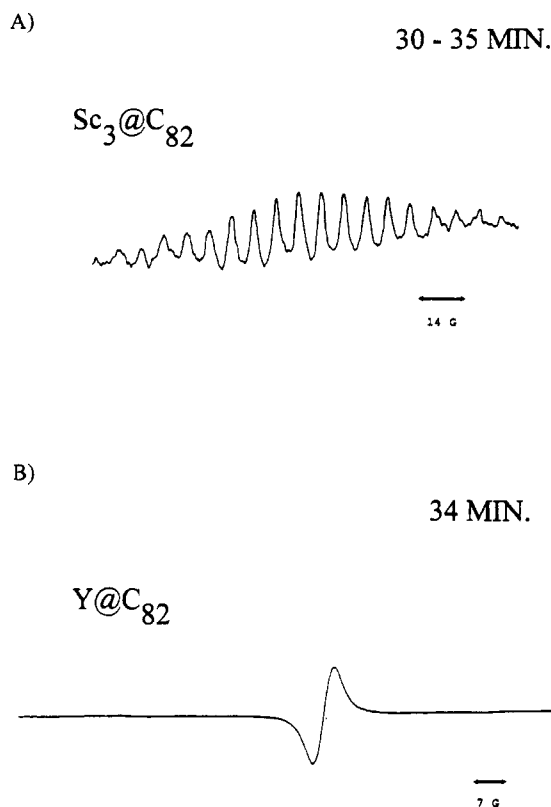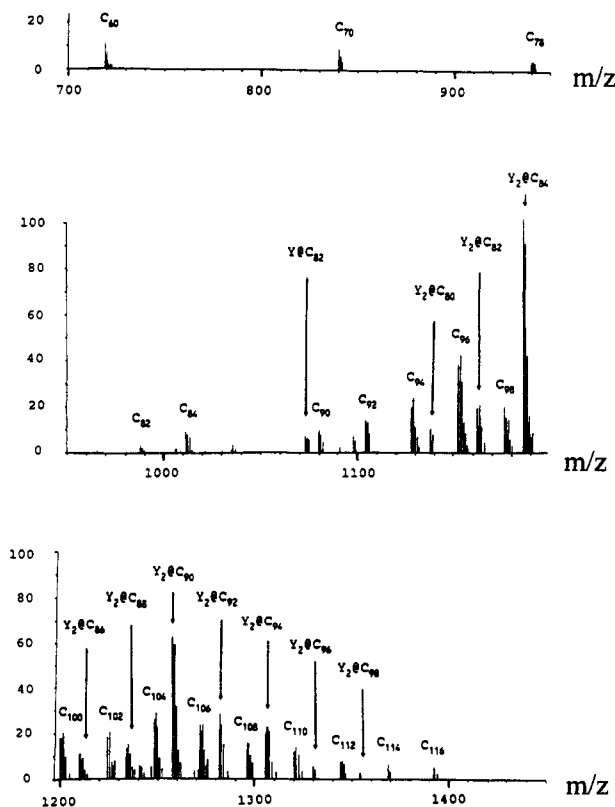
Figure 7. Negative-ion CI mass spectrum for the $Y@C_{2n}$ metallo-fullerene fraction (fifth pass). No standard was employed.

order to observe the small hyperfine doublet (0.48 G) reported for the $Y@C_{82}$ species.[2,3] The importance of conducting all separations under anaerobic conditions is clearly illustrated

by the case of the EPR-active ($Y@C_{82}$) component. We have observed significant irreversible reduction in the EPR intensity for this species after exposure to air. The corresponding negative-ion CI mass spectrum for the separated $Y@C_{2n}$ fraction (Figure 7) shows a range of $Y_2@C_{2n}$ species. The EPR-active $Y@C_{82}$ compound is present together with all even-carbon-numbered diyttrium compounds ranging from $Y_2@C_{82}$ to $Y_2@C_{104}$. Although the empty-caged fullerenes (C_{60}, C_{70}, C_{84}, C_{92}, C_{94}) are largely removed after five passes, significant quantities of $C_{98}-C_{106}$ are still present. In contrast to the scandium case, there is no mass spectral evidence for either triatomic yttrium species ($Y_3@C_{82}$) or the smaller carbon-cage dimetal species ($Y_2@C_{74}$ or $Y_2@C_{76}$) for this sample.

The effectiveness of the separation described above is undoubtedly a result of the weak $\pi-\pi$ interactions between the metallofullerene outer cage and the chromatographic polystyrene substrate. In general, the polystyrene support is a more effective weak complexing substrate for a given metallofullerene than it is for the corresponding empty-cage analog, as demonstrated by the large differences in their retention times ($\sim5-10$ min). In a separation study of empty-cage fullerenes,[11] it was shown that polystyrene columns do not always exhibit a "size exclusion" based separation (e.g., C_{84} elution before C_{60}). This is also consistent with the importance of the weak $\pi-\pi$ interaction separation mechanism for the $Sc@C_{2n}$ and $Y@C_{2n}$ metallofullerene fractions.

CONCLUSIONS

An automated HPLC system employing polystyrene columns can be very effective in isolating metallofullerenes from the more abundant empty-cage fullerenes. The automated approach using polystyrene columns and a solvent system permitting high solubility provide relatively large single injections ($\sim8-15$ mg). This allows separation of $\sim125-$ 200 mg of metallofullerene-containing extract in a 16-h time period. Thus, with this apparatus, $\sim1-2$ mg of the $Sc@C_{2n}$ fraction can be isolated from ~400 mg of the soluble fullerene and metallofullerene extract in 2-4 days. The apparatus has the notable advantage that all operations are conveniently accomplished under an inert nitrogen gas atmosphere. The nearly complete removal of the empty-cage fullerenes facilitates further chromatographic separation of the pure metallofullerene compounds. As described by Shinohara,[9] separation of the $Sc@C_{2n}$ fraction into individual purified compounds (e.g., $Sc_2@C_{84}$) is feasible by utilizing a more selective chromatographic phase.[14] The EPR detection of active "marker" metallofullerenes (e.g., $Sc_3@C_{82}$) is also clearly advantageous in cases where they are present. For all separations, the CI mass spectrometry provides off-line identification of the full range of fullerenes and metallofullerenes present in a given fraction.

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