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Solid Phase Extraction as a Simple Method for the Enrichment of Endohedral Metallofullerenes

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Abstract: A simple cleanup procedure involving Solid Phase Extraction with C_{18}-bonded silica removes about 22 to 30% of empty fullerenes (C_{60}-C_{84}) from a chlorobenzene solution containing the empty fullerenes and di-scandium metallofullerenes (mainly $Sc_2@C_{84}$); the metallofullerene content is not reduced significantly. Copyright © 1996 Elsevier Science Ltd

The endohedral metallofullerenes have been the subject of intense research activities in recent years.[1-2] The existence of these interesting compounds was first reported in 1985,[3] and macroscopic quantities of these materials have been produced since 1991.[4] The isolation of individual endohedral metallofullerene of high purity has, however, not been straightforward and has invariably involved solvent extraction of the empty and metallofullerenes followed by tedious chromatographic separations.[5-9] In general, the difficulty of HPLC purification of endohedral metallofullerenes lies in the lack of selectivity between these compounds and the higher fullerenes. In this paper, we describe our preliminary results of using solid phase extraction (SPE) as a new and simple method for the selective removal of some of the empty fullerenes. Although SPE is essentially a chromatographic technique, it is often used as a simple cleanup procedure in lieu of the conventional liquid-liquid extraction.[10]

Unlike the empty fullerenes, the endohedral metallofullerenes are thought to be rather polar and often have large dipole moments.[11] The use of a polar medium, namely carbon disulfide-methanol azeotrope, has resulted in improved extractions of different metallofullerenes.[9] Based on the same principle, we predicted that empty fullerenes would be preferentially adsorbed by a non-polar sorbent. We thus investigated the possibility of removing empty fullerenes with octadecyl silica from a chlorobenzene solution containing both empty fullerenes and di-scandium metallofullerenes (mainly $Sc_2@C_{84}$).

The relevant metallofullerene-containing soot was prepared as described previously.[5a] The soot was Soxhlet-extracted with carbon disulfide; after solvent removal, the extract was stored under Argon until use. In this work, one mg of this extract was added to 4 mL of chlorobenzene and the resulting solution was ultrasonicated for 30 min. before filtration. The filtrate was subjected to the following SPE procedure.

The SPE tube, LC-18 (Supelco Inc., Bellefonte, PA, USA) each containing 100 mg of the endcapped C_{18}-bonded silica (about 10% carbon), was first washed with acetone (2 x 1 mL) and then chlorobenzene (2 x 1 mL). The level of chlorobenzene was maintained just above the sorbent in order to keep it from drying. One mL of the sample containing empty fullerenes and metallofullerenes was poured into the tube. With the help of a SPE vacuum manifold (Supelco Inc., Bellefonte, PA, USA), the solution was allowed to pass through in a dropwise manner under a slightly-negative pressure and the coloured eluate was collected. The volume of the collected eluate was greater than 0.92 mL. The entire SPE procedure took less than 15 min.

The samples before and after SPE were first analyzed by negative atmospheric pressure chemical ionization (APCI) mass spectrometry in the selected ion monitoring (SIM) mode. Five μL of each of the two samples were injected via a sample loop into a mobile phase of chlorobenzene/toluene (1:4 v/v) at 0.5 mL/min. Their ion signals at m/z 840 (for C_{70}) and m/z 1098 (for $Sc_2@C_{84}$) were measured.[12] Figures 1a and 1b are the respective ion chronograms[13] for multiple injections of the starting solution and the collected eluate. After SPE, the average integrated signal for C_{70} decreased by 14%, whereas that for $Sc_2@C_{84}$ increased by 187% or nearly two-fold. Since it is unlikely that the present cleanup procedure would increase the concentration of $Sc_2@C_{84}$, we believe the sharp increase observed for m/z 1098 was due to a partial removal of empty fullerenes such as C_{70}, leading to an enhanced formation of the $Sc_2@C_{84}$ ion under a competing ionization environment.[14]

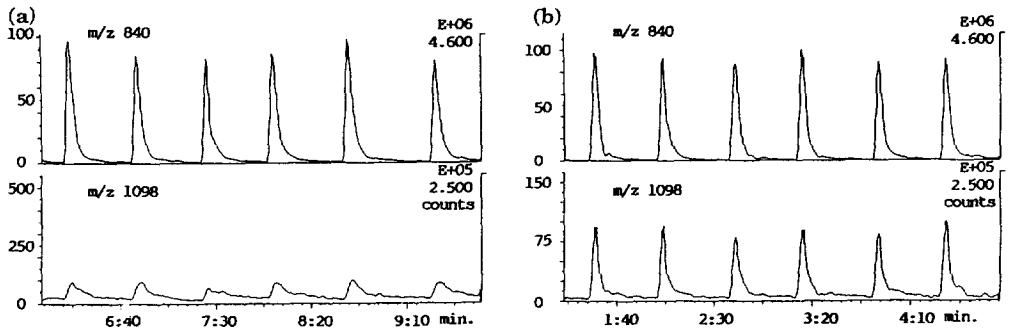
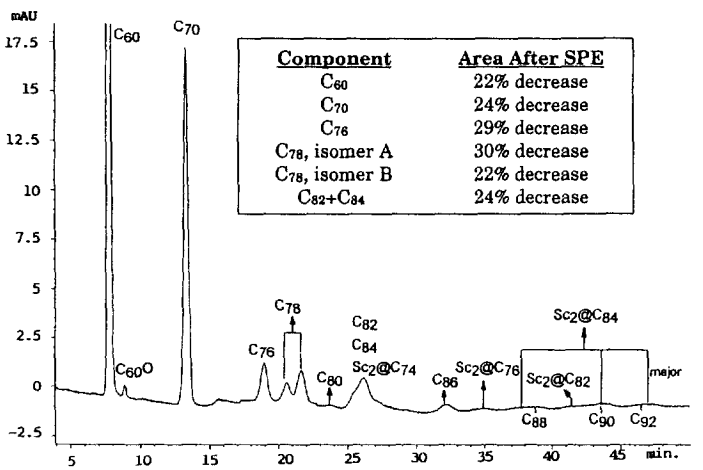


Fig. 1. Negative APCI ion chronograms monitoring m/z 840 (upper traces) and m/z 1098 (lower traces): (a) from six injections of the sample before SPE and (b) from six injections of the sample after SPE.

Next, the two samples were analyzed by LC using a Cosmosil Buckyprep® 25 cm L x 4.6 mm i.d. column together with a 10 mm L x 4.6 mm i.d. guard column (Nacalai Tesque, Inc., Kyoto, Japan). The mobile phase was toluene (distilled) at 1 mL/min. Detection was at 313 nm. The volume of injected samples was 10 μL in each case. The resulting chromatograms for the samples before and after SPE appeared to be rather similar; the one for the starting solution is given in Figure 2.



Component	Area After SPE
C_{60}	22% decrease
C_{70}	24% decrease
C_{76}	29% decrease
C_{78}, isomer A	30% decrease
C_{78}, isomer B	22% decrease
C_{82}+C_{84}	24% decrease

Fig. 2. LC/UV chromatogram of the sample before SPE.

To identify the individual components of empty fullerenes and di-scandium metallofullerenes in the LC chromatograms, on-line LC/MS analysis was also performed. The LC condition was the same as for LC/UV, except that 5 μL of each sample was injected. The mass spectra were recorded under negative APCI condition.[15] Extracted ion chromatograms were used to identify the retention times of individual fullerenes and metallofullerenes. In particular, the m/z 1098 extracted ion chromatograms (Figure 3)[13] showed three separate peaks in each case, with their intensities sharply increased after SPE cleanup. These peaks correspond to the isomers of $Sc_2@C_{84}$, and their retention times had good agreement with those reported earlier.[16] Such an increase could be explained by a preferential removal of the overlapping higher fullerenes (i.e. C_{88}, C_{90} and C_{92} respectively) by the SPE method.

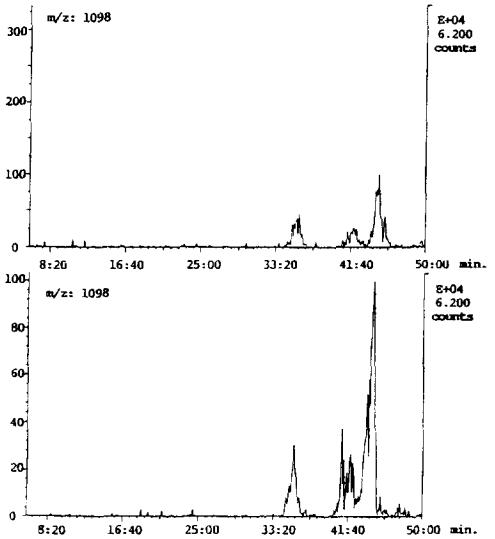


Fig. 3. Ion chromatograms at m/z 1098 extracted from the LC/MS analyses of the samples before SPE (top graph) and after SPE (bottom graph).

Although the LC/MS runs were found to be slightly faster, the peaks (or expected positions of individual components) in the LC/UV chromatograms were easily identified by means of a relative retention time (RRT) correlation. The results are also given in Figure 2. The integrated areas of the identified peaks in the LC/UV chromatograms were then compared, and the results are summarized in the inset of Figure 2.[17] Adding this information with the negative APCI/SIM data, we concluded that the present SPE method had selectively removed a fraction of the empty fullerenes, and there was no indication of a significant reduction in the metallofullerene content by this simple cleanup procedure.[18]

The concentrations of C_{60} in the two samples were determined by LC/UV, using a two-point calibration curve constructed with 25 and 100 μg/mL of >99.5% pure C_{60} as standards (Strem Chemicals, Newburyport, MA, USA). The C_{60} concentration was found to be 56.4 μg/mL for the starting solution and 43.5 μg/mL after SPE.

In conclusion, we have demonstrated the potential of using SPE as a fast and simple procedure for the enrichment of endohedral metallofullerenes. Although the present work was conducted in a small scale, SPE techniques can be automated or scaled-up conveniently. The method just described did not result in the removal of a substantial proportion of the empty fullerenes, however, the sample loading has not been optimized.

Moreover, since a stronger solvophobic association of the empty fullerenes, particularly the higher ones, with hydrophobic species in solution was observed when the polarity of the medium was increased,[19] such removal should be possible with a more polar solvent, and this might even favour the selective removal of the troublesome but more hydrophobic higher fullerenes.[20] Work is in progress in our group towards these problems. The method's applicability to other metallofullerene mixtures is also being investigated.

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12. The scan times for m/z 840 and m/z 1098 were 0.1 and 0.8 s respectively.
13. Absolute intensity scales are on the right. The left vertical scales have arbitrary units.
14. A similar negative APCI/SIM experiment has confirmed a substantial increase in the ion signal of m/z 1002 (for $Sc_2@C_{76}$) after SPE.
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17. According to our LC/MS results, the amount of the overlapping $Sc_2@C_{74}$ was insignificant compared to C_{82} and C_{84}.
18. Another LC/negative APCI/SIM experiment was performed with a 25-cm Vydac® 201TP546 C18 column (Separation Group, Hesperia, CA, USA), 50% acetonitrile in chlorobenzene as mobile phase (1 mL/min.), and using 8 (instead of 1) mg of a different batch of the $Sc_2@C_{84}$ containing extract. The scan times for m/z 1098 and m/z 720 were 0.5 and 0.1 s respectively. The integrated areas for the separated $Sc_2@C_{84}$ signal (eluted at about 2.7 min.) were 4.36 E+04 counts before SPE and 6.01 E+04 counts after SPE, whereas those for the separated C_{60} signal (eluted at about 4.0 min.) were 2.55 E+08 counts before SPE and 2.23 E+08 counts after SPE. Details will be reported elsewhere.
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20. Using a more concentrated sample, more higher fullerenes were removed by SPE than the C_{60} or C_{70}.

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