SEPARATION OF $N_2@C_{60}$ and $N@C_{60}$

Nitrogen Ion Implantation

ion source

effusion cell

HPLC Separation

column: Buckyprep
eluent: toluene

Absorption / arb. unit

80   100   120   140   160   180
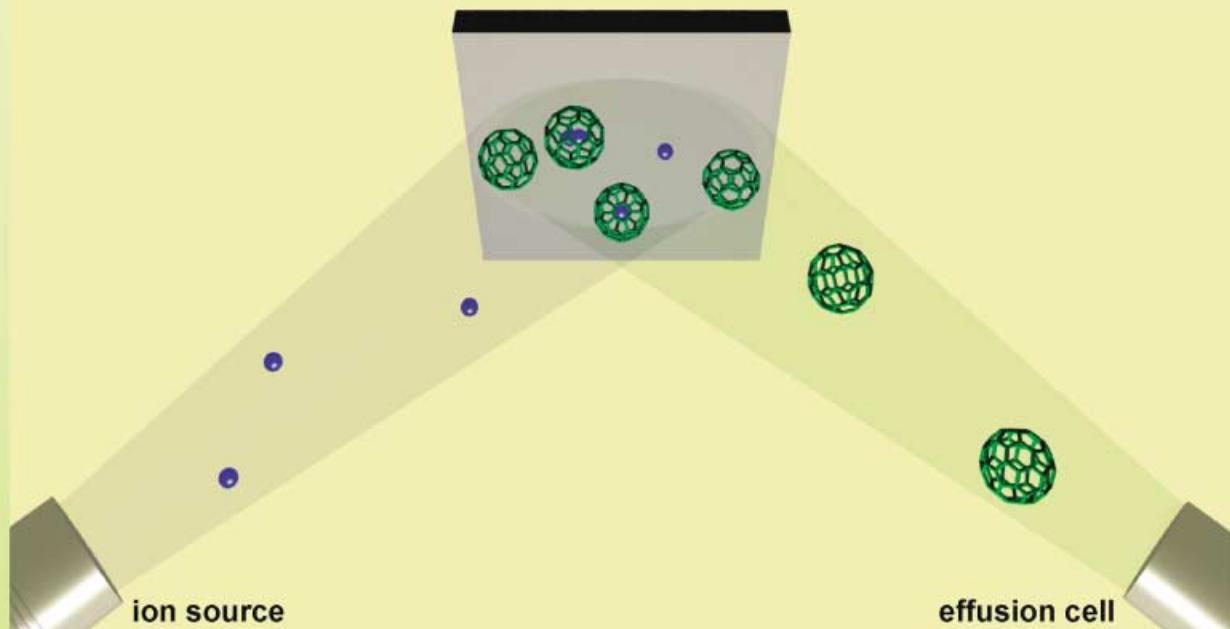
Retention time / min
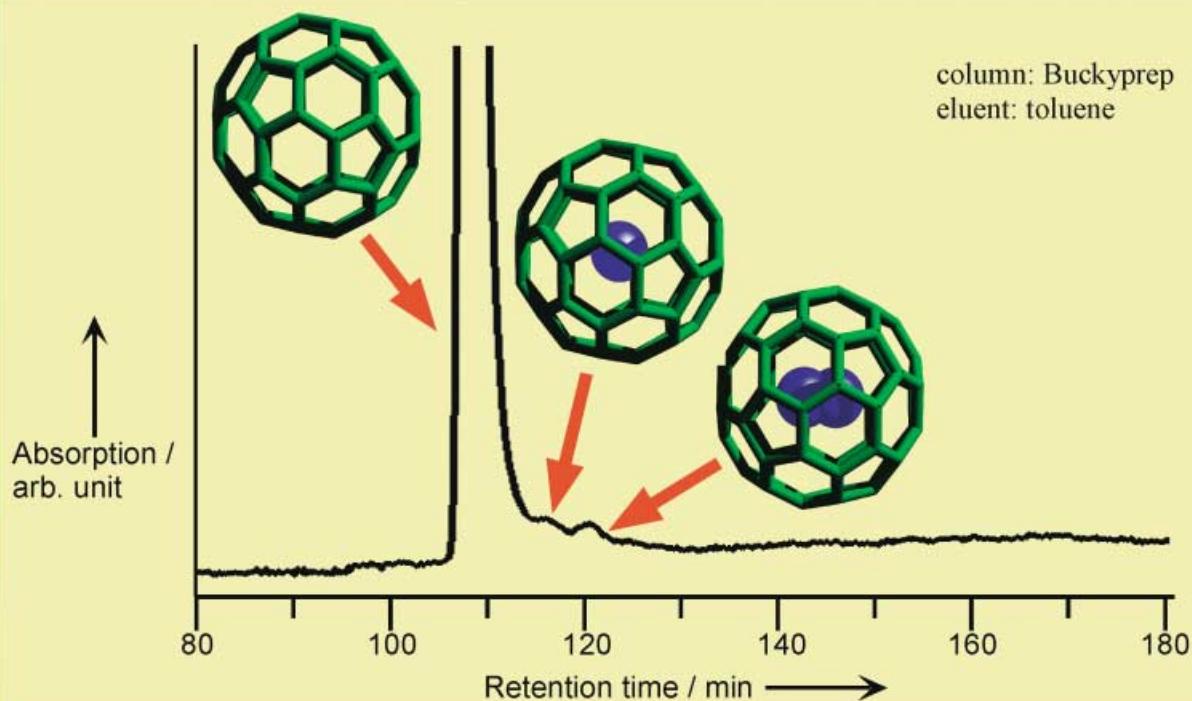
For more information, see the following pages.

Separation of $N_2@C_{60}$ and $N@C_{60}$

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Abstract: We describe the HPLC separation and identification of $N@C_{60}$ and $N_2@C_{60}$. These species were observed after eleven sequential HPLC separations. Their retention times are in the same range as those of the other noninteractive endohedral species of C_{60}, such as noble gas endohedral C_{60}. The separation factors of these endohedrals were evaluated by using a mixture of hexane/toluene as eluent. We note that this is the first evidence for the $N_2@C_{60}$ molecule existing in the form of endohedral C_{60} complex.

Introduction

C_{60}, which has a soccer ball like structure with a hollow interior,[1] can accommodate various atoms or molecules.[2] Up to now several kinds of endohedral fullerenes have been synthesised: metallofullerenes,[3, 4] in which metallic atoms are incorporated inside the cage, noble gas endohedral fullerenes,[5, 6] in which one or two noble gas atoms are inserted in the cage, and endohedral fullerenes with reactive atoms stabilised inside the cage such as nitrogen or phosphorus.[7–9] The endohedral fullerene with a nitrogen atom intercalated (for instance $N@C_{60}$) was first discovered by Murphy et al.[7] This is a unique molecule in which an otherwise extremely reactive nitrogen atom is stabilised in its electronic ground state due to the protection provided by the C_{60} cage. The electronic state of the intercalated atomic nitrogen was probed by EPR[7–12] and ENDOR spectroscopy,[7, 11] but other details of its physicochemical properties have not been studied yet. To determine its properties, it is essential to enrich the sample of $N@C_{60}$ to a high enough concentration. For this purpose we explored the HPLC behaviours of $N@C_{60}$ in order to find the optimum conditions for a large-scale separation, which could provide us with several mg of $N@C_{60}$, enough for the evaluation of its properties.

In general HPLC separations of the endohedral fullerenes are quite difficult. This is because a large amount of the residual C_{60} is present and the peak of C_{60} overlaps with the peaks of the endohedrals and, hence, makes it difficult to evaluate the performances of the HPLC columns. On the other hand, the overlap of the peaks is a clear indication of the endohedral character of the complex and of the inertness of the intercalated species. Moreover, an HPLC detection of an endohedral reaction product could indicate the presence of another endohedral species: this was the case for $N_2@C_{60}$. Its HPLC peak was found during the enrichment of $N@C_{60}$. The presence of a trace amount of dinitrogen endohedral fullerene was recently reported by Cao et al. in a mass spectrometry study of atomic nitrogen implanted in C_{60},[12] but was not discussed in any detail. Here we report the chemical separation of this molecule and show that this molecule exists in the form of an endohedral complex ($N_2@C_{60}$), based on HPLC and EPR data.

To our knowledge, no HPLC peaks of $N@C_{60}$ or $N_2@C_{60}$ have been observed previously, although it has been suggested theoretically,[13] based on EPR experiments, that $N@C_{60}$ should in principle be separated by HPLC. Thus the separation abilities of various columns for $N@C_{60}$ and $N_2@C_{60}$ have not been evaluated. Our attempt was to obtain a sample enriched in $N@C_{60}$ and $N_2@C_{60}$ to such a degree that one can observe their HPLC peaks in only one pass through the column, then evaluate the separation factors at different conditions in order to find the best separation conditions.

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The yield of N@C_{60} after atomic nitrogen ion implantation is about 10^{-5} to 10^{-4}%.[9] Because of the small yield and because C_{60} and N@C_{60} are chemically very similar, the separation/purification of these endohedrals is extremely difficult. In the case of noble gas endohedral C_{60} such as Kr@C_{60}, the yield is about 1.0% when synthesised by catalysis under high temperature and pressure.[14] It was found that the separation factor increases with the size of the intercalated atom, for instance, it is 1.04 for Ar@C_{60}[15] and 1.09 for Kr@C_{60}[16] relative to C_{60} on 5PYE columns. For the latter case we have recently succeeded in establishing the HPLC procedure and obtained milligram amounts of Kr@C_{60} with about 12% enrichment[17] and obtained an X-ray single crystal structure, which shows that, as expected, the Kr atom is located at the centre of C_{60} cage.[18] However, the yields for N@C_{60} and N_2@C_{60} are two to three orders of magnitude lower than that of Kr@C_{60}, making their purification even more difficult. Our work is aiming at establishing the optimum HPLC purification procedure for N@C_{60} and N_2@C_{60} as being an inevitable task for the further study of their physicochemical properties.

Experimental Section

The HPLC separation and analysis were performed on a JASCO GULLIVER 1500 instrument equipped with recycling, an automatic injector, a diode array detector (300–900 nm) and two HPLC pumps with a dynamic mixer for gradient experiments. The columns used were Buckyprep and 5PYE (both 20 × 250 mm) and the separations were performed at 50 °C in order to decrease the peak tailing. The sample was dissolved into toluene, and the injection volume was 2.0 mL, while the flow rate was 6 mL min^{-1} of toluene. Under these conditions the C_{60} eluted at 36.5 min, when using the two columns. We selected to use these two columns because we had found them to be superior to the other columns for the separation of Kr@C_{60}.[17]

For the HPLC analysis we used two analytical Buckyprep columns with various ratios of hexane/toluene (the ratio of hexane was 50–80%) as eluent. The flow rate was 1.0 mL min^{-1}, and the injection volume was 100 µL.

Mass spectrometry was performed on a BRUKER instrument by MALDI-TOF-MS with 9-nitroanthracene as matrix. EPR was done on a JEOL instrument (JES-RE1X) with X band spectrometer (about 9.2 GHz) at room temperature.

A mixture of fullerenes (about 75% C_{60}) was implanted with nitrogen ions at an energy of about 40 eV and a current of about 50 µA according to a procedure described earlier.[19] The product of about 3 g contained mostly C_{60}, trace amounts of N@C_{60} (5.7 × 10^{-5}% by EPR), N_2@C_{60}, and some other impurities such as $C_{60}O$, $C_{60}O_2$, C_{70}, $C_{70}O$, C_{120}, $C_{120}O$, etc. We dissolved this sample in toluene and performed a preliminary HPLC separation in order to remove the higher fullerenes and dimers, whose retention times are significantly different from those of C_{60} compounds. After this coarse separation the sample contained a large amount of C_{60}, trace amounts of N@C_{60} and N_2@C_{60} as well as $C_{60}O$. Subsequently we removed the first half of the C_{60} peak and collected the second half, in which N@C_{60} and N_2@C_{60} were expected to be contained, using the same HPLC setup. The obtained sample (labelled here: after 1st separation) was concentrated and subjected to the same procedure repeatedly. Each time we checked the analytical HPLC and EPR signal of the separated sample. The latter technique showed an increase in the signal intensity of N@C_{60}. After the 11th separation, two additional HPLC peaks besides C_{60} appeared, which were later assigned to N@C_{60} and N_2@C_{60}. The separation of N@C_{60} from N_2@C_{60} was done thereafter on two Buckyprep columns (4.6 × 250 mm) with a toluene/hexane mixture (25:75) as eluent. The flow rate was 1.0 mL min^{-1} and the injection volumes were 100 µL.

Results and Discussion

Eleven sequential separations on two columns (Buckyprep and 5PYE) were necessary to obtain a sample with enough enriched endohedrals so as to detect them in one HPLC run. The HPLC chart for the sample before the 1st separation is shown in the top half of Figure 1 (top), while the one after the
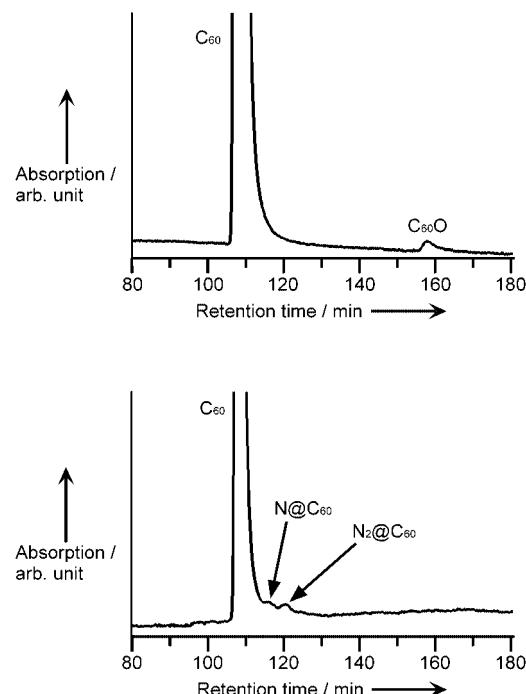

Figure 1. HPLC analysis before separation (top) and after 11th separation (bottom). Columns: two Buckyprep columns; eluent: toluene; flow rate: 1 mL min^{-1}, UV-visible detection: 300–900 nm.

11th separation is shown in the bottom half. In these figures we show the analytical HPLC charts on two Buckyprep columns. Before the 1st separation we did not detect any peaks except those of C_{60} and $C_{60}O$, while after the 11th separation two distinct peaks at 116.0 and 120.5 min (assigned to N@C_{60} and N_2@C_{60}, respectively) became visible. C_{60} eluted under these conditions at 108.0 min. The two peaks were very close to C_{60}, partially overlapping on the tail of their peaks, in agreement with the endohedral character of the two species. However, their presence was clearly indicated and, hence, it should be possible to separate both N@C_{60} and N_2@C_{60} completely if a large amount of the starting material is employed. The MALDI-TOF-MS spectrum of the resulting sample after the 11th separation is shown in Figure 2. In this spectrum one clearly sees two peaks corresponding to N@C_{60} (including a small amount of $C_{60}O$) and N_2@C_{60} apart from that of C_{60}. The isotopic distributions obtained from this spectrum of N@C_{60} and N_2@C_{60} agree well with theoretical ones considering the natural abundances of the elements.

EPR spectra of the sample before and after the separations are shown in Figure 3. A clear increase was observed in the signal of the triplet of ^{14}N near 328 mT after the 11th separation. Based on the EPR experiments we found that the samples are stable at room temperature, in agreement

To further support the identification of the two HPLC peaks appearing in the retention time between C_{60} and $C_{60}O$, we completely separated these two peaks on two Buckyprep columns $(4.6 \times 250 \text{ mm})$ using a toluene/hexane mixture (25:75). At each separation we collected the first fraction from 112.0 to 118.5 min and the second fraction from 118.5 to 125.0 min and performed EPR measurements. The EPR spectrum of the first fraction is shown in the top half of Figure 4 and that of the second fraction is shown in bottom

Figure 2. Mass spectrum of the sample after 11th separation.
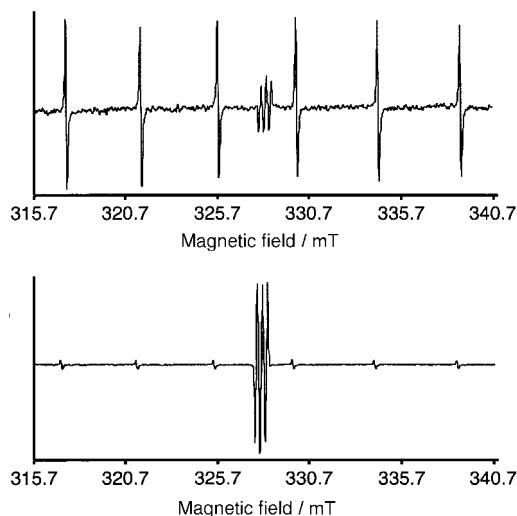
Figure 3. EPR spectra before separation (top) and after 11th separation (bottom).
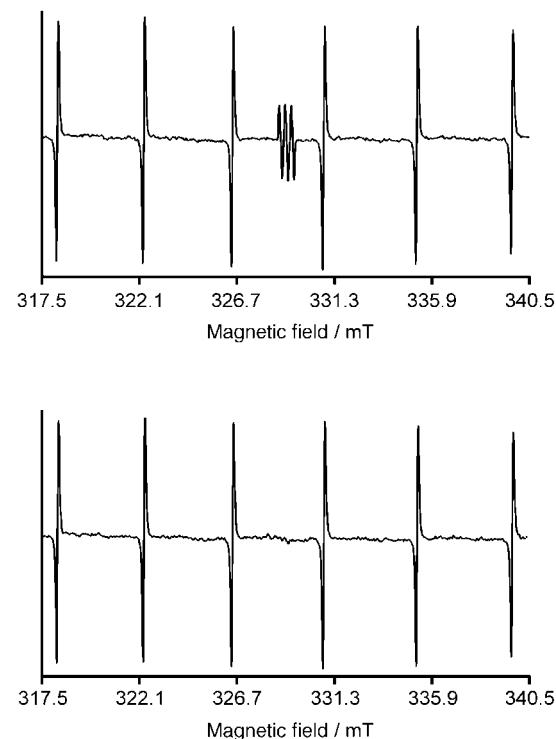

Figure 4. EPR spectra of the first HPLC fraction (top) and the second HPLC fraction (bottom) of Figure 1. The six widely separated lines are due to the standard.

with the observations made by Waiblinger et al.[20] We note that the doublet arising from ^{15}N isotope also became visible after the 11th separation. In both spectra shown in Figure 3, the six widely separated lines belong to Mn^{2+}, which was added as standard in the same amount for the two measurements. The fact that the EPR signal of ^{14}N is observed for the sample collected in the close vicinity of the C_{60} peak in HPLC is the clear evidence of the endohedral nature of atomic nitrogen in the form of $N@C_{60}$. The EPR signal of ^{14}N from $N@C_{60}$ is, apart from a scaling factor, the same as the one from atomic nitrogen in the isolated state,[7] indicating that the very reactive nitrogen atom becomes stable at the electronic ground state as it is protected by the C_{60} cage. The enrichment factor was 92.6 after 11 separations from the comparison of the signal intensities in the two spectra in Figure 3. This is less than one would expect after 22 passes through HPLC. This is because a small loss is inherent at each separation step when collecting a half of the HPLC peak. In spite of a higher concentration of $N@C_{60}$, no broadening of the EPR signal was observed.

half. We could see the triplet from ^{14}N in Figure 4 (top), but could not see any peak from the nitrogen atom in Figure 4 (bottom). These results indicate that the first peak in HPLC is due to $N@C_{60}$ (in which one nitrogen atom is intercalated the cage of C_{60}) and the second peak is due to $N_2@C_{60}$ (in which two nitrogen atoms form a dinitrogen molecule), as could be expected from the influence of mass of the endohedrals on the HPLC separation. The absorption spectra for these samples seems to be identical; because of the very low concentration of the $N@C_{60}$ and $N_2@C_{60}$ an accurate comparison of their UV-visible spectra could not be made.

To study the HPLC separation of $N@C_{60}$ and $N_2@C_{60}$, we performed analytical HPLC on two Buckyprep columns $(4.6 \times 250 \text{ mm})$ using a toluene/hexane mixture as eluent, and checked the separation factor for each run. The ratio of hexane in the mixture ranged from 50 to 80%. The results are summarised in Figure 5. Although the separation factors were very small ($N@C_{60}$: 1.06–1.08, $N_2@C_{60}$: 1.10–1.13), hexane had a positive effect on the separation ability. We can
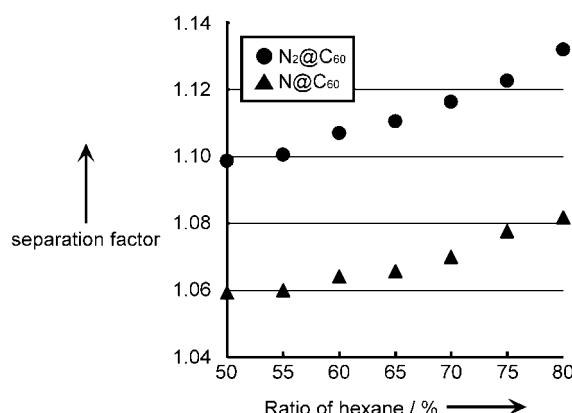
Figure 5. Separation factors for N@C$_{60}$ and N$_2$@C$_{60}$ from C$_{60}$ as a function of the ratio of hexane in the eluent (%).

conclude that the two species, assigned to N@C$_{60}$ and N$_2$@C$_{60}$, are both indeed endohedral complexes, because the separation factors of the two species to C$_{60}$ were very small and depended similarly on the mixture ratio, considering the possibility that the endohedrally intercalated N atom and N$_2$ molecule may have little affect the C$_{60}$ cage. If they were exohedrally bound to C$_{60}$ cage, then chemically they should behave very differently. On the other hand, the separation factor of N$_2$@C$_{60}$ was a little higher than that of N@C$_{60}$. Hence one can suggest that the larger separation factor of N$_2$@C$_{60}$ than N@C$_{60}$ resulted from the larger size of the intercalated atom (or molecule) rather than the electronic reactivity. The addition of hexane to the eluent in fact improves the separation in terms of peak separation. However, this improvement includes longer retention times and much less solubility of the sample (2.8 mg mL^{-1} in toluene and 0.043 mg mL^{-1} in hexane).[21] These results suggest that while the use of a hexane/toluene mixture is a good choice for the analytical HPLC, the use of pure toluene is a better choice for large-scale separation/purification purposes.

In conclusion, from three grams of starting material we obtained about 70 micrograms of sample containing 0.7 % of N@C$_{60}$ and 0.6 % of N$_2$@C$_{60}$. The measurements of the mass and EPR spectra in combination with HPLC data have provided evidence for the existence of N$_2$@C$_{60}$ as well as of N@C$_{60}$ in the endohedral forms. It has also been shown that the isolation of these two species in their pure forms is possible.

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