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Short communication

Determination of C_{60} and C_{70} fullerenes in geologic materials by high-performance liquid chromatography

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Abstract

It is demonstrated that high-performance liquid chromatography (HPLC) is an excellent and reliable analytical method for the determination of the C_{60} and C_{70} fullerenes extracted from geologic samples because the analysis is carried out at room temperature, is non-destructive (fullerenes can be recovered), and is easily calibrated with synthetic fullerenes. The determination of fullerenes in geologic materials is greatly empowered by the pre-separation of fullerenes from organics on a “big” HPLC column.

1. Introduction

When fullerenes were first discovered to be formed spontaneously in condensing carbon vapors [1], it was suggested that they might be widely distributed in the Universe. Subsequent searches for fullerenes in interstellar media and meteorites [2,3–5] have thus far been unsuccessful, but C_{60} and C_{70} have been reported to occur in the ejecta associated with an impact crater on the Long Duration Exposure Facility Spacecraft [6]. On the earth, C_{60} and C_{70} have been reported to occur in certain samples of shungite, a meta-anthracite coal from a deposit near Shunga, Russia [7], and in “fulgurite”, a substance formed when lightning strikes certain soils or rocks [8]. Also in clay from the Cretaceous–Tertiary (K–T) boundary [9,10] and in car-

bonaceous matter from the Sudbury impact structure, Ontario, Canada [11].

Analyses of fullerenes have generally used mass spectrometry of either laser-vaporized or thermally heated samples. While this is often a good technique, we are concerned that, given the levels of a few parts-per-billion (ppb) [9,11], or parts-per-million (ppm) [11] at which fullerenes occur in geologic materials, the fullerenes may not volatilize efficiently from the surrounding matter, and that the high temperature and gas-phase conditions required in the analysis may inadvertently produce fullerenes that were not originally present in the sample. Instead we have used high-performance liquid chromatography (HPLC) of toluene extracts together with direct photo diode array (PDA) detection of the UV–visible absorption spectrum of the separated fullerenes.

HPLC is carried out at room temperature, hence the probability that fullerenes are formed

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during the measurement is nil. Moreover, the retention times and peak areas, both salient properties of the substance to be determined, can be easily calibrated with standard solutions of synthetic C_{60} and C_{70} . The analysis is non-destructive and the fullerenes can be recovered after they emerge from the instrument. Whereas these positive aspects of HPLC have been evident to us for quite some time, we have encountered considerable skepticism, especially with regards to the sensitivity of the method. To engender a better understanding of the application of HPLC to the search for fullerenes in geologic samples we present here our cumulative experience of several years together with new results on the pre-separation of fullerenes from large amounts of organic substances.

2. Experimental

For our search for fullerenes in geologic materials we have assumed that significant fractions of C_{60} and C_{70} , if present, can be extracted from the materials by treatment with solvents such as toluene, and that the yields would probably increase upon the crushing, powdering, and ultimately, the demineralization of samples. Yet, even after the dissolution of inorganic minerals by repeated treatment with HCl and HF (i.e. demineralization), we can only recover those fullerene molecules which are not encased in chemical structures which prevent their dissolution in toluene. Hence, a failure to find fullerenes does not necessarily mean that these are not present in the sample.

Both non-demineralized and demineralized samples were placed in glassware, adequate amounts of HPLC-grade toluene were added, and the slurries were then sonicated for at least four hours. Next, the slurries were transferred to a Soxhlet thimble (the emerging fluids were, of course, collected) and the solids were washed by Soxhlet treatment using the collected fluids themselves for the washing. This procedure also brought about a good first separation of fluid and solids. Only centrifugation was used for additional separation when needed.

It has long been known that fullerenes are vulnerable to ultraviolet radiation [12], heating in air [13], and exposure to ozone [14]. We have therefore avoided strong irradiation and heating above 60°C of any solid sample, or any toluene extract, which means that reductions of fluid volumes by evaporation were done at reduced pressure. However, we have used the vulnerability to heating in air for cleaning all glassware at 500°C for at least 12 hours.

The end product of the treatment of a geologic sample was always a volume between 0.1 and 1.0 ml of filtered toluene, with, or without fullerenes, but nearly always with some organics dissolved in it. The HPLC instrument into which 25 μ l of this solution was injected consisted of two Waters 510 pumps, an U6K injector, a 3.9×300 mm Nova-Pak silica-bonded C-18 column (called the "small" column) and a 996 photo diode array (PDA) taking UV-Vis absorption spectra of the fluid emerging from the column every 2 seconds. The PDA can take spectra in the range 210–640 nm, but our spectra were taken only in the range 310–430 nm (or sometimes 300 to 450 nm) because the toluene in the mobile phase absorbs too strongly below 310 nm and because the absorption by amounts of fullerene from geologic samples above 430 nm was negligibly small.

The mobile phase was commonly a 40:60 toluene-methanol mix flowing at 2.00 ml/min. For these conditions, the retention times of C_{60} and C_{70} were around 6.50 and 11.3 min respectively. Higher proportions of toluene were also used, depending on the sample. These separations with a stronger mobile phase gave the usual decrease in run time, narrower peaks and reduced resolution.

3. Results and discussion

3.1. Instrument performance

Fig. 1 shows chromatograms of the injection of: (A) 0.99 nanogram synthetic C_{60} and 1.06 ng synthetic C_{70} ; (B) 4.95 and 5.32 ng of the fullerenes; and (C) of 49.5 and 53.2 ng of the

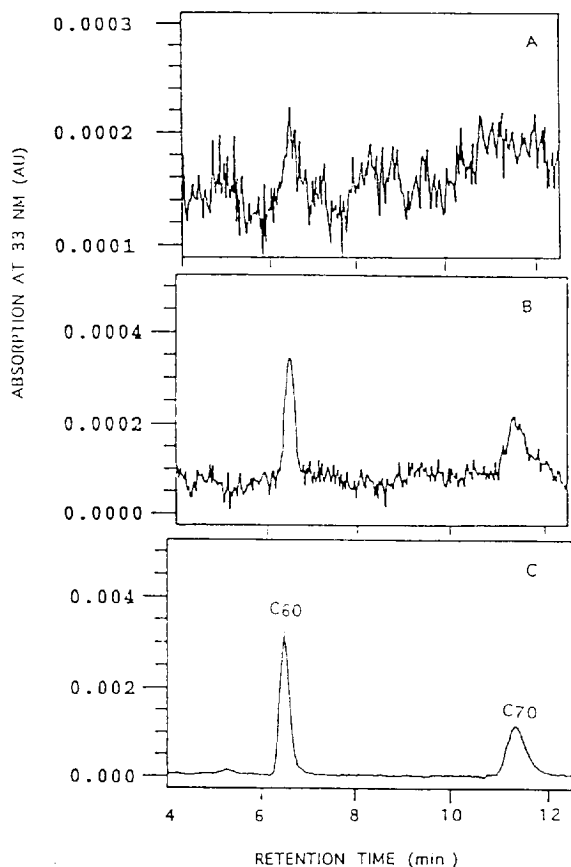


Fig. 1. Chromatograms of 25 μ l-aliquots of increasingly concentrated solutions of synthetic fullerenes in toluene. The amounts are given in the text. All three chromatograms (A, B, and C) were generated under identical conditions. The small column was used. The mobile phase was 1.20 ml/min methanol and 0.80 ml/min toluene. An absorption spectrum was taken by the PDA detector every 2 s in the range 300 to 450 nm, but the chromatograms were constructed from absorption data at 330 nm, where the C_{60} spectrum has a local maximum. The chromatograms are shown from 4 to 12.5 min because only a meaningless peak corresponding to the dead time of the column is present between 0 and 4 min.

fullerenes. The first four minutes of the chromatograms are not shown as they only contain a peak at 1.2 min, which has no practical significance as it corresponds to the dead time of the column.

Fig. 2A and 2B show the absorption spectra of C_{60} and C_{70} in the range 300 to 450 nm, obtained at 6.47 min and 11.35 min from the injection of about 50 ng of fullerenes. Fig. 2C shows the C_{60}

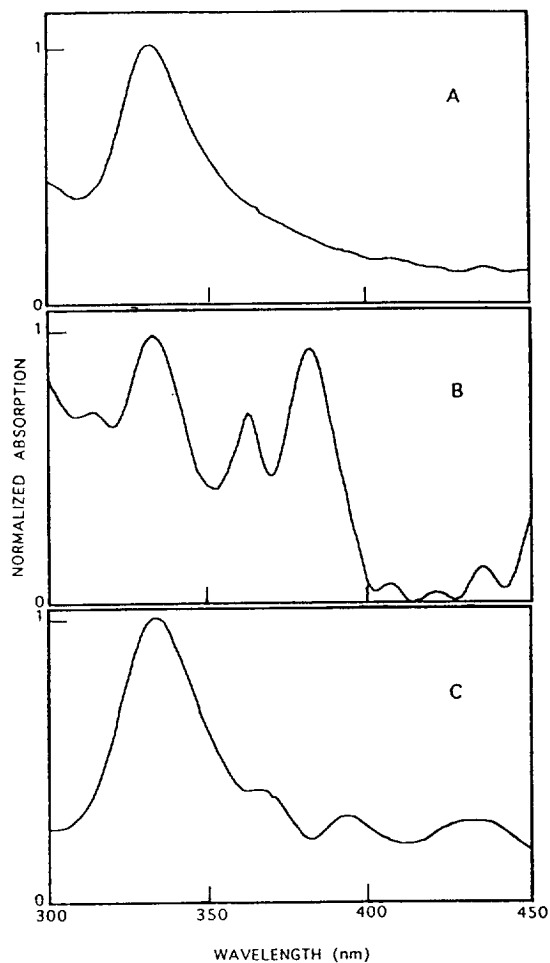


Fig. 2. The identification of fullerenes based on retention times and absorption spectra. A and B are absorption spectra in the wavelength range 300 to 450 nm at 6.47 and 11.35 min, the known retention times of C_{60} and C_{70} , for the injection of about 50 ng of each of the fullerenes. C is the absorption spectrum at the retention time of C_{60} for the injection of about 5 ng of that fullerene. The noise level of the PDA prevents the obtaining of a good C_{70} spectrum from the injection of 5 ng of that fullerene. The spectra are normalized such that absorption at maximum is 1.000. Actually the specific absorption of C_{70} at 330 nm is only about 0.6 times that of C_{60} . For identification of the fullerenes by both retention time and convincing absorption spectra, the minimum amounts required for injection are about 5 ng C_{60} and 20 ng C_{70} .

spectrum at 6.46 min from the injection of about 5 ng of fullerenes. The absorption spectrum for C_{70} for the injection of 5.32 ng of that fullerene

is greatly distorted by the noise of the PDA, hence is not shown. The C_{60} spectrum is greatly distorted by PDA noise for the injection of about 1 ng. Therefore, whereas the limit of detection from chromatograms alone is about 1 ng for C_{60} and 5 ng for C_{70} , when one requires that convincing absorption spectra must be paired with retention times, these limits must be raised to about 5 ng and 20 ng respectively. Fortunately we have been able to extract significantly larger amounts of fullerenes from our geologic samples.

Fig. 3 shows that the response of the PDA at 330 nm is linear in the range of concentrations of 20 to 20 000 ng per ml. A linear regression fit to the data yielded an integrated C_{60} peak area of $2237 \pm 180 \mu\text{Vs}$ (micro-volt-second) per ng injected. The fit for C_{70} (data not shown) yielded $1342 \pm 121 \mu\text{Vs}$ per ng injected. These areas are roughly 20, respectively 10 times larger than typical blank values obtained by the injection of pure toluene at the salient retention times. Ten replicate analyses of the 2000 ng/ml standard (50 ng injected) have yielded a standard deviation from the mean of $\pm 8\%$ of the peak area.

3.2. Pre-separation

Toluene extracts of geologic samples always contain from small to large amounts of organic substances, including pure hydrocarbons to compounds which contain O, N, S, and other elements. Polar compounds such as alcohols, ketones, aldehydes, acids, etc. tend to have shorter retention times than the C_{60} and C_{70} fullerenes on the C_{18} -bonded column used, and non-polar molecules tend to have increasingly longer retention times as their molecular weights increase. A major problem arises with all of these substances when their cumulative absorptions compete with those of the fullerenes.

Let us consider in some detail the example of a clay from the Cretaceous–Tertiary (K–T) boundary at Woodside Creek, New Zealand. It had long been known that the basal layer of the boundary seam at that K–T locality, which is about 2 cm thick, was rich in iridium and other platinum-group metals [15], as well as in various forms of elemental carbon, including soot [16,17], all putatively due to the after-effects of a

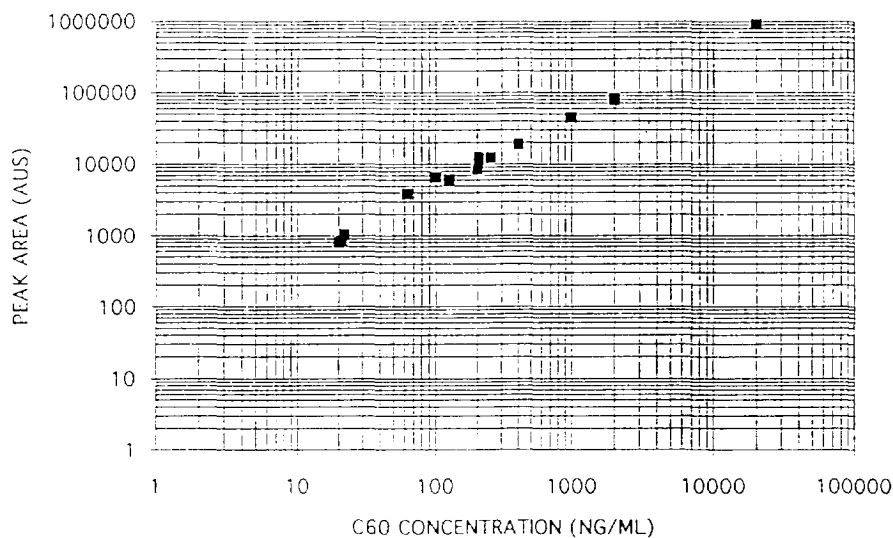


Fig. 3. Peak area as a function of C_{60} concentration in toluene solutions as determined with $25 \mu\text{l}$ -injections. Multiple injections were done for every value of concentration. A least-squares fit for this particular calibration yielded: $2237 \pm 180 \mu\text{Vs}$ per ng injected for C_{60} and $1342 \pm 121 \mu\text{Vs}$ per ng injected for C_{70} (analogous plot not shown).

gigantic crater-forming impact at Chicxulub, Yucatan, Mexico, some 65 Ma ago. Since the soot was thought to have formed by world-wide wildfires [16,17], we speculated that it might be attended by a complement of fullerenes.

Six samples of clay, ranging in weight from 68 to 201 gram, were treated with toluene. The extracts were pale yellow. Upon HPLC analysis the clays turned out to contain extractable C_{60} in the range 0.32 to 5.4 ppb. Fig. 4A shows a typical chromatogram of one of these extracts.

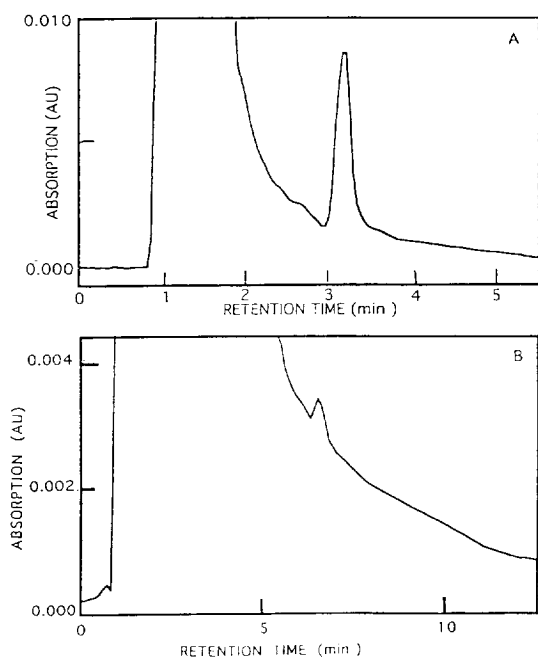


Fig. 4. A: Chromatogram from an extract of a clay sample from the Cretaceous–Tertiary boundary site of Woodside Creek, New Zealand. With a mobile phase of 1.00 ml/min methanol and 1.00 ml/min toluene, the retention times of C_{60} and C_{70} (little, if any present in the extract) were 3.18 min and 4.48 min respectively. The large and broad peak which crests at 1.5 min is due to organics also extracted from the clay. B: Chromatogram from an extract of a demineralized clay sample from the Cretaceous–Tertiary boundary site of Woodside Creek, New Zealand. The mobile phase used was 1.20 ml/min methanol and 0.80 ml/min toluene. The organics peak is huge, and baseline is not reached even after 12.5 min. The C_{60} peak at 6.48 min is riding on a substantial tail of the organics peak. The C_{70} peak was expected at 11.3 min. No peak was detected at that time.

The rather broad peak cresting at 1.5 min is due to organics extracted along with the C_{60} , whose peak occurs at 3.18 min. The C_{70} peak was expected at 4.48 min. Apparently this sample contained little, if any C_{70} . Although the C_{60} peak is riding on the tail of the organics, their interference with determining the peak area is only modest. However, when we succeeded recently in destroying most of the inorganic minerals of another clay sample of 250 gram by treatment with HCl and HF, and when we treated the carbon-rich residue with toluene, we obtained a strongly orange colored solution, obviously richer in organics than any we had previously obtained. We shall not be concerned here with the causes for the increased yield of organics, but with their effect on the detection of the fullerenes. Fig. 4B shows the chromatogram of the extract. It is overwhelmingly dominated by the organics peak and its huge tail. It is seen that the extract contained just enough C_{60} for its peak (6.48 min) to stick out above the organics background, but small amounts of C_{70} (expected at 11.3 min), if present, could be totally masked by the tail of the organics peak.

3.3. Preparative study

In order to deal with problems such as this, we obtained a column with the same stationary C-18 phase, but with internal dimensions of 19×300 mm (the “big” column). As mobile phase we used 10 ml/min each of methanol and toluene. Up to 2.0 ml of extract could be injected into the system. To test the efficacy of separation and recovery, we spiked fullerene-free organics obtained by the extraction of soot produced by the burning of a crude fossil oil from the Brent field in the North Sea with 396 ng C_{60} and 426 ng C_{70} . A sample of this mixture was analyzed on the small column (Fig. 5A). The remainder was then injected into the big (prep) column and the emerging fluids were collected from one minute before to one minute after the retention times of C_{60} and C_{70} , sufficient to catch more than 90% of the emerging fullerenes (Fig. 5B). The volume of the combined fullerene fluids was then

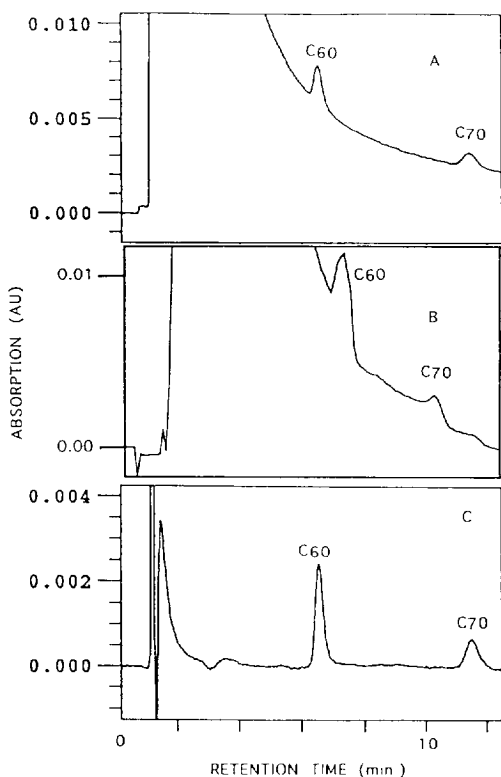


Fig. 5. Three chromatograms which illustrate the testing of the big column. A mixture was made of fullerene-free organics with 396 ng C_{60} and 426 ng C_{70} in toluene. Chromatogram A was obtained from an injection of this mixture. The amounts of the fullerenes were sufficiently large so that their peaks are seen to ride on the tail of the organics peak. Chromatogram B was taken while the organics and fullerenes progresses through the big column. Chromatogram C was taken after separation. Note the injection peak and the very modest organics peak (the negative excursion between the two peaks is quite common). By 4 min into the analysis, the PDA is back to zero level. The fullerene peaks are unencumbered by organics. Their absorption spectra (not shown) are identical to those of C_{60} and C_{70} .

reduced to less than 0.5 ml by evaporation at reduced pressure. A sample of this fluid was injected for analysis into the small column (Fig. 5C). Fig. 5 shows from A to C the succession of salient chromatograms. At the top is the chromatogram of the non-separated mix, showing the fullerene peaks riding on a the tail of a large organics peak similar to the one in Fig. 4B. The sample was so rich in organics that the PDA became saturated. The next chromatogram was

recorded as the substances migrated down the big column. One can see the peaks of C_{60} and C_{70} at about 7 and 10 min riding on the tail of the organics peak. The PDA was saturated for this injection as well. The chromatogram at the bottom shows the extract after separation. There is still a small organics peak at the beginning of the chromatogram, but zero baseline is reached after about 4 min and the peaks of the fullerenes are unencumbered by organics. The recoveries were 365 ng C_{60} and 390 ng C_{70} , which correspond to very good yields of 92% and 91%, respectively.

With the test successful, we proceeded to treat the Woodside Creek sample in analogous fashion. Fig. 6A shows the progression of organics and fullerenes through the big column and the bottom chromatogram (4 to 12.5 min only, because the organics peak is very small) is from the injection of the combined recovered C_{60} and C_{70} fractions evaporated to a volume of 0.12 ml.

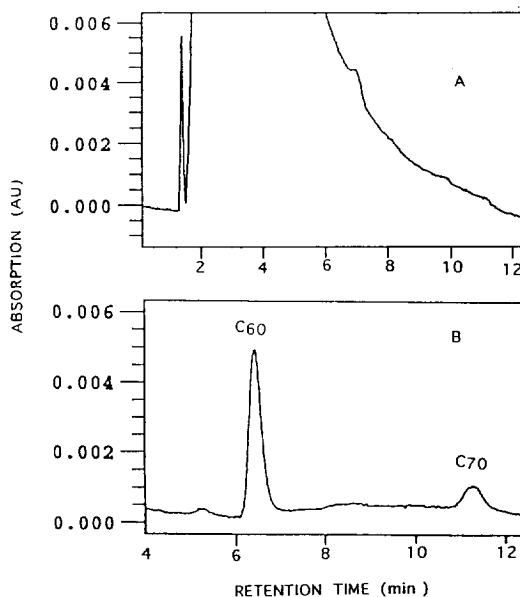


Fig. 6. These chromatograms are analogues of chromatograms B and C of Fig. 5, but for the extract from the demineralized Woodside Creek sample. The chromatogram of the separated extract is shown only from 4 to 12.5 min. The organics peak in it is very small. The peak at 6.44 min was identified by its spectrum as C_{60} . The peak at 11.3 min gave only a distorted C_{70} spectrum. The substance of the peak is, however, most likely C_{70} .

With an assumed recovery of 90%, the amounts of extracted fullerenes were 496 ng C₆₀ and 171 ng C₇₀, which correspond to contents of 2.0 ppm and 0.68 ppb in the clay, and a C₇₀/C₆₀ ratio of 0.34. Perhaps the most remarkable aspect of this result is that we had not been able to reveal C₇₀ in the chromatograms of the earlier extracts of clay samples from Woodside Creek. One possible reason is that the separation as carried out here has removed organics to the extent that they no longer mask the C₇₀ presence in the chromatogram.

A word of caution is in order. We found that the sensitivity of the PDA detector decreased substantially, by almost a factor of two, after large amounts of organics (the peak at 1.5 min!) had passed through it. We think that some organic compounds were partially decomposed photochemically when they passed through the PDA detector, hence that a thin absorbing film of products was deposited on the walls of the analytical cell. We were able to restore the sensitivity to about 0.8 times its original value by washing the detector with methanol and acetone. We conclude that the safest procedure is to determine the retention times of the fullerenes on the large column with synthetic, pure C₆₀ and C₇₀, and to run organics-rich samples with the PDA turned off. The times when sample collection should begin or end depend, of course, on the widths of the fullerene peaks.

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