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Application Note No 9

Analysis of functionalised fullerenes by MALDI-TOFMS, including accurate mass measurement

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Introduction

Functionalised fullerenes are becoming an increasingly important class of compound, with (potential) applications in fields such as nanotechnology, electronics, optics, materials science, medicine, and architecture. Functionalized fullerenes may be divided into two classes: exohedral with substituents on the outer surface of the cage, generically depicted in Figure 1, and endohedral fullerenes with trapped molecules or ions inside the cage.

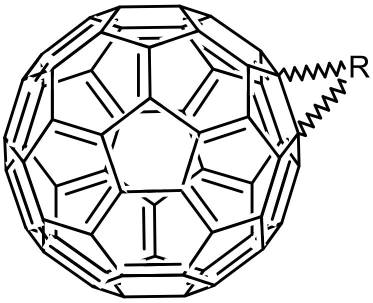

Figure 1. *Generic exohedral mono-functionalized fullerene.*

While simple fullerenes may be observed by mass spectrometry using electron ionisation (EI), the high energy involved in the technique renders it non-amenable to observing intact species for functionalised analogues. Electrospray ionisation (ESI) is less energetic, but not really suited to such non-polar molecules, so a successful analysis is very much dependant upon the nature of the functional group attached. However, matrix-assisted laser desorption/ionisation time-of-flight mass spectrometry (MALDI-TOFMS) allows the analysis of functionalised fullerenes with minimal fragmentation. In early experiments, the traditional polar acidic matrices did not perform well, and it was only with the development of the matrix 2-[(2*E*)-3-(4-*tert*-butylphenyl)-2-methylprop-2-enylidene]malononitrile (DCTB) that molecular ions were observed, irrespective of the analyte functional group.[1] DCTB is an aprotic, electron-transfer matrix, so its' properties are similar to those of fullerenes, and may be used in both positive and negative ion modes. More recently, sulphur (S_8),[2] 9-nitroanthracene,[3] *trans*-4-tert-butyl-40-nitrostilbene (TBNS)[4] and trihyroxyanthracene[5] have all given acceptable data, but in our experience DCTB in negative ion mode gives consistent clean spectra with very good signal-to-noise ratios. Further details of the technique, including a description of accurate mass measurement, together with example spectra are given below.

Experimental methodology

Chemicals

Functionalised fullerene analytes (**1**-**6**) were submitted for analysis to the NMSSC. DCTB was purchased from Fluka (Dorset, UK). HPLC-grade dichloromethane (DCM) was purchased from Fisher Scientific (Loughborough, UK). HPLC-grade toluene, was purchased from Sigma-Aldrich (Dorset, UK). Standard reference materials (SRMs) for accurate mass measurements are described in detail elsewhere.[6]

MALDI Sample Preparation

DCTB matrix solution was prepared to a concentration of 20 mg mL^{-1} in DCM. For fullerene analytes **1**-**6** and fullerene SRMs, saturated toluene solutions were used, while fluorinated porphyrin SRM solutions were made to a concentration of 1 mg mL^{-1} in DCM. For a standard analysis, volumes within the range of 0.1 to 2 µL of sample and 49 µL of DCTB solutions were vortex-mixed in a plastic, snap-top lid sample vial. 0.5 µL of the final mixture was spotted (see ***TIP!*** in NMSSC Application Note No. 1) onto a sample plate (gold-plated, deep-welled plates are advantageous for organic solvent based mixtures) and allowed to dry, leaving an opaque crystal layer. For accurate mass measurements, additional amounts (to ensure that all three species have > 50% relative intensity when analysed) of SRM solutions that bracket the analyte mass were vortex-mixed prior to deposition onto the sample plate.

Mass Spectrometry

MALDI-TOFMS spectra were acquired using an Applied Biosystems Voyager DE-STR spectrometer (Framingham, MA, USA), which is equipped with a nitrogen laser (λ = 337 nm). The instrument was operated in negative ion, reflectron mode. The accelerating voltage was 20 kV, while the grid voltage was maintained at 65.5 %. The delay time and laser fluence were optimised for each sample. Post-acquisition calibration was applied using Data Explorer V4.0 software supplied by Applied Biosystems.

Results and discussion

Figure 2 shows a typical spectrum for a functionalised fullerene, acquired with DCTB in negative ion mode. The base peak is the negative radical ion ($M^{-\bullet}$), with excellent signal-to-noise, no dimerisation and little fragmentation. The low solubility of most functionalised fullerenes means an appropriate sample-to-matrix ratio is much lower than for fully soluble samples. CS_2 (smelly!) is another satisfactory solvent, while the larger or greater in number the substituent, the more likely that DCM or MeOH may prove an adequate solvent.

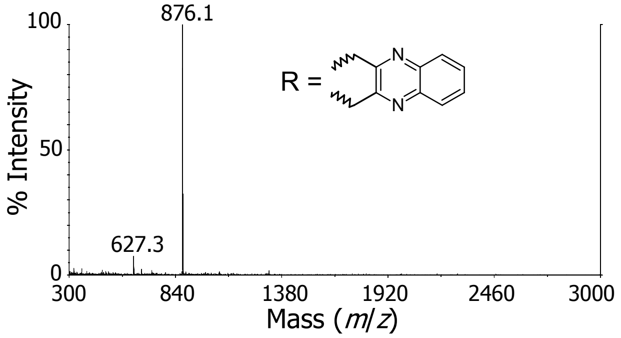

Figure 2. *Negative ion MALDI-TOFMS spectra for **1** (MW = 876) acquired with DCTB matrix.*

We have recently developed a method for accurate mass measurement by MALDI-TOFMS, which is described in detail elsewhere.[6] Essentially, the method requires two internal SRMs that bracket the analyte in mass, and which ionise by the same mechanism as the analyte. Sample preparation is critical, as all three species should be mixed to ensure they give similar ion intensities, as shown in Figure 3.

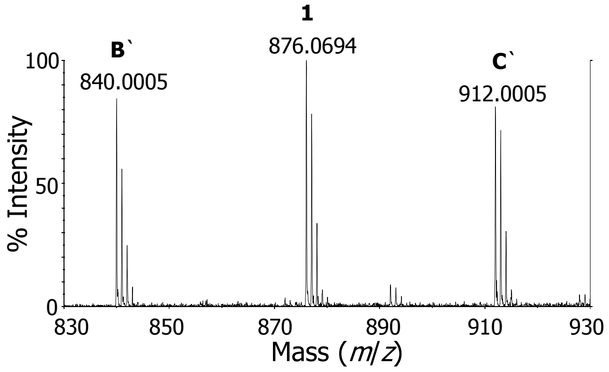

Figure 3. *MALDI-TOF accurate mass measurement spectrum for **1** using SRMs B` and C` (mass measurement error = 1.6 ppm).*

Greater confidence in a result is always obtained through acquiring multiple measurements and subsequent averaging. Table 3 gives accurate mass measurement data for five measurements of functionalised fullerenes **1**-**6**. The method compares well with other accurate mass measurement techniques, and was also subjected to rigorous statistical analysis, which showed it was free from systematic error.

Analyte (formula)	Exact mass ($M^{-\bullet}$)	aaMMA[a] (ppm)	RMS (ppm)
1 ($C_{70}H_8N_2$)	876.0693	2.1	2.8
2 ($C_{71}H_{15}N$)	881.1210	1.4	1.5
3 ($C_{74}H_{10}N_2$)	926.0849	1.6	2.0
4 ($C_{80}H_{39}NO$)	1029.3037	2.6	2.8
5 ($C_{86}H_{24}O_4$)	1120.1680	1.3	1.7
6 ($C_{85}H_{18}N_4O_4$)	1158.1334	1.2	1.4

Table 1. *MALDI-TOFMS accurate mass measurement data for functionalised fullerenes **1**-**6**. [a]Average absolute mass measurement accuracy.*

Conclusions

Here we demonstrate that excellent mass spectrometry data can be acquired for functionalised fullerenes MALDI-TOFMS in negative ion mode with DCTB matrix. With the addition of appropriate internal SRMs that bracket the analyte, accurate mass measurements are also possible.

References

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