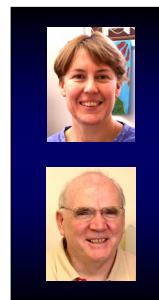


# Accurate mass measurement of negative radical ions by MALDI-TOFMS: Application to functionalized fullerenes

Shao Zhecheng, Mark F. Wyatt, Bridget K. Stein and A. Gareth Brenton

EPSRC National Mass Spectrometry Service Centre (NMSSC), Institute of Mass Spectrometry (IMS), School of Medicine, Swansea University, Singleton Park, Swansea, SA2 8PP, U.K.



## Overview

- Functionalized fullerenes can easily be characterised as negative radical ions by MALDI-TOFMS with DCTB matrix.
- Accurate mass measurement increases the certainty of analyte identification, but a MALDI-TOFMS method needed to be developed.
- Combination of higher order fullerenes and fluorinated porphyrin standards have been identified and accurate mass measurements were made for six functionalized fullerene analytes.
- Mean mass accuracy errors ranged from -1.3 to 3.9 ppm or from -1.3 to 2.9 ppm, depending upon acquisition delay time.
- Poor precision of individual measurements indicates the current measurement methodology is slightly capricious and further refinement of the acquisition protocols is required in order to achieve reliable accurate mass measurement.

## Introduction

Matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOFMS) is the premier mass spectrometry technique for characterizing functionalized fullerenes.<sup>1</sup> Samples are generally observed as negative radical ions and the usual matrix is 2-[2(E)-3-(4-*tert*-Butylphenyl)-2-methylprop-2-enylidene]malononitrile (DCTB). Accurate mass measurement is a very important application of mass spectrometry, allowing the elemental formulae of small molecules to be determined, and general operational guidelines are available.<sup>2,3</sup> At the NMSSC, computer-assisted peak matching on a magnetic sector instrument with an electrospray (ESI) source<sup>4</sup> is preferred for the wide range of samples received. Alternative sources are used where ESI is not appropriate, and while these systems generally give excellent results, some samples specifically require MALDI ionization. MALDI-TOFMS is infrequently used for accurate mass measurements, but we have been developing methods in this area recently,<sup>5,6</sup> and a method for functionalized fullerenes would be highly beneficial given that most other ionization techniques perform poorly. Tetra-alkyl/alkylaryl *meso*-substituted porphyrin standards<sup>7</sup> developed for the successful accurate mass measurement of positive radical ions were found to ionize poorly in negative ion mode, thus new calibration materials had to be identified. A combination of higher order fullerenes and fluorinated porphyrin standards have been identified as potential negative ion mode standard reference materials covering the 720-1270 mass range. Here we present accurate mass measurements made for six functionalized fullerene analytes, using these calibrants and established acquisition protocols.<sup>5,6</sup>

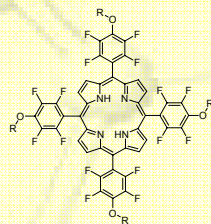
## Materials and Instrumentation

- DCTB was purchased from Fluka (U.K.).
- HPLC-grade dichloromethane (DCM) was purchased from Fisher (U.K.).
- 5,10,15,20-tetra(pentafluorophenyl)porphyrin (TF<sub>4</sub>PP), HPLC-grade toluene, reagent grade (>99%) dimethyl formamide, ethanol, propan-1-ol, butan-1-ol, pentan-1-ol, phenol, sodium, C<sub>70</sub>, and fullerene soot were purchased from Sigma-Aldrich (U.K.).
- Functionalized fullerene samples (**1-6**) were submitted to the NMSSC by Prof. M. Schroder, University of Nottingham, U.K.
- Fullerene standards C<sub>60</sub>, C<sub>70</sub>, and C<sub>86</sub> were kindly donated by Prof. G. A. D. Briggs, University of Oxford, U.K.
- MALDI-TOFMS measurements were acquired with a Voyager DE-STR instrument (Applied Biosystems, U.S.A.). Data were acquired using positive ion, reflectron mode. Delay time = 150 or 100 ns, acceleration voltage = 20 kV, and grid voltage = 65.5%. Every acquisition was an accumulation of 2 × 25 laser shots, and the laser power was optimised for each.

## Calibration Standards A-K

- A** Fullerene soot (predominantly C<sub>60</sub> and C<sub>70</sub>).  
Exact mass (M<sup>-</sup>) = 720.0005 and 840.0005.
- B** Fullerene C<sub>70</sub>.  
Exact mass (M<sup>-</sup>) = 840.0005.
- C** Fullerene C<sub>76</sub>.  
Exact mass (M<sup>-</sup>) = 912.0005.
- D** TF<sub>4</sub>PP; Formula = C<sub>44</sub>H<sub>10</sub>N<sub>4</sub>F<sub>20</sub>.  
Exact mass (M<sup>-</sup>) = 974.0592.
- E** Fullerene C<sub>84</sub>.  
Exact mass (M<sup>-</sup>) = 1008.0005.
- F** Fullerene C<sub>86</sub>.  
Exact mass (M<sup>-</sup>) = 1032.0005.

- G** R = Et; Formula = C<sub>52</sub>H<sub>30</sub>N<sub>4</sub>O<sub>4</sub>F<sub>16</sub>.  
Exact mass (M<sup>-</sup>) = 1078.2017.
- H** R = Pr; Formula = C<sub>54</sub>H<sub>32</sub>N<sub>4</sub>O<sub>4</sub>F<sub>16</sub>.  
Exact mass (M<sup>-</sup>) = 1134.2643.
- I** R = Bu; Formula = C<sub>60</sub>H<sub>46</sub>N<sub>4</sub>O<sub>4</sub>F<sub>16</sub>.  
Exact mass (M<sup>-</sup>) = 1190.3269.
- J** R = Pent; Formula = C<sub>64</sub>H<sub>54</sub>N<sub>4</sub>O<sub>4</sub>F<sub>16</sub>.  
Exact mass (M<sup>-</sup>) = 1246.3895.
- K** R = Ph; Formula = C<sub>68</sub>H<sub>30</sub>N<sub>4</sub>O<sub>4</sub>F<sub>16</sub>.  
Exact mass (M<sup>-</sup>) = 1270.2017.



Standards **G-K** were synthesised by derivatizing **D** with the appropriate alcohol, according to literature methods.<sup>8</sup>

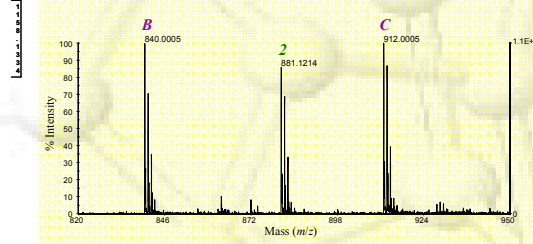
## Samples 1-6

- 1** R = Formula = C<sub>70</sub>H<sub>8</sub>N<sub>2</sub>. Exact mass (M<sup>-</sup>) = 876.0693.
- 2** R = Formula = C<sub>71</sub>H<sub>15</sub>N. Exact mass (M<sup>-</sup>) = 881.1210.
- 3** R = Formula = C<sub>74</sub>H<sub>16</sub>N<sub>2</sub>. Exact mass (M<sup>-</sup>) = 926.0849.
- 4** R = Formula = C<sub>80</sub>H<sub>39</sub>NO. Exact mass (M<sup>-</sup>) = 1029.3037.
- 5** R = Formula = C<sub>86</sub>H<sub>24</sub>O<sub>4</sub>. Exact mass (M<sup>-</sup>) = 1120.1680.
- 6** R = Formula = C<sub>88</sub>H<sub>18</sub>N<sub>4</sub>O<sub>4</sub>. Exact mass (M<sup>-</sup>) = 1158.1334.

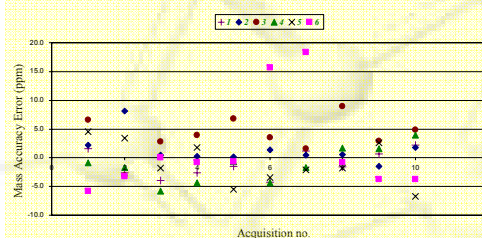
## Accurate Mass Measurement

- Samples **1-6** and Standards **A-K** were dissolved in DCM or toluene.
- Each sample was mixed with DCTB matrix (20 mg mL<sup>-1</sup> in DCM) and the appropriate high and low mass calibrants, e.g. Sample **1** was calibrated by bracketing Standards **B** and **C**.
- Minimum acceptable resolution = 12000 FWHM; minimum acceptable signal:noise = 200.
- Post-acquisition calibration was applied using Data Explorer V4.0 software supplied by Applied Biosystems.
- No data processing was performed, as this can adversely affect peak shapes.
- The three species used in a measurement should all have comparable ion statistics; too weak and the peak shape is poor, too strong and detector echoes form a high-mass shoulder, altering the centroid.
- The correct sample/standards/matrix ratio was crucial to good peak shapes, and therefore mass accuracy, and ionization efficiencies must be considered.

## Example Data for 2



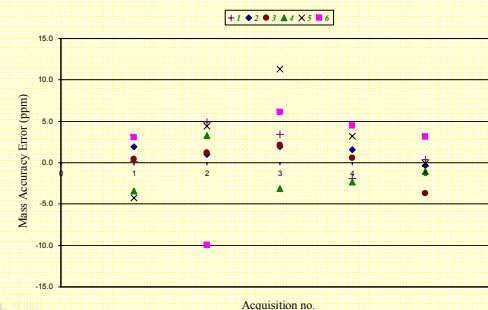
## Mass Accuracy Data (150 ns Delay Time)



Sample	1	2	3	4	5	6
Mean Accurate Mass Value (m/z)	876.0683	881.1222	926.0885	1029.3024	1120.1670	1158.1352
Mean Mass Error (ppm)	-1.1	1.4	3.9	-1.3	-0.9	1.5
RMS Mass Error (ppm)	2.5	2.8	5.0	3.2	3.8	8.1

Delay time was changed to 100 ns to try to obtain more reproducible accurate mass measurements.

## Mass Accuracy Data (100 ns Delay Time)



Sample	1	2	3	4	5	6
Mean Accurate Mass Value (m/z)	876.0705	881.1221	926.0850	1029.3023	1120.1713	1158.1350
Mean Mass Error (ppm)	1.4	1.2	0.1	-1.3	2.9	1.4
RMS Mass Error (ppm)	2.8	1.5	2.0	2.8	5.9	5.9

## Conclusions

- Fullerene and fluoroporphyrin calibration standards have been used for the accurate mass measurement of six functionalized fullerenes by negative ion MALDI-TOFMS.
- Mean mass accuracy errors ranged from -1.3 to 3.9 ppm or from -1.3 to 2.9 ppm, depending upon acquisition delay time.
- Poor precision of individual measurements indicates the current measurement methodology does not give rise to repeatable results.
- Unlike positive radical ion measurement, outlying results could not be explained by data acquisition acceptance criteria.
- Further refinement of the technique is required in order to achieve reliable accurate mass measurement.

## References

- Ulmer, L.; Mattay, J.; Torres-Garcia, H. G.; Luftmann, H. *Eur. J. Mass Spectrom.* **2000**, *6*, 49-52.
- Bristow, A. W. T.; Webb, K. S. *J. Am. Soc. Mass Spectrom.* **2003**, *14*, 1086-1098.
- [http://www.bmss.org.uk/Docs/VIMMS\\_guide.pdf](http://www.bmss.org.uk/Docs/VIMMS_guide.pdf).
- D'Agostino, P. A.; Hancock, J. R.; Provost, L. R.; Semchuk, P. D.; Hodges, R. S. *Rapid Commun. Mass Spectrom.* **1995**, *9*, 597-603.
- Wyatt, M. F.; Stein, B. K.; Brenton, A. G. *J. Am. Soc. Mass Spectrom.* **2006**, *17*, 672-675.
- Griffiths, N. W.; Wyatt, M. F.; Stein, B. K.; Brenton, K. S. *manuscript in preparation*.
- Smith, B. M.; Kean, S. D.; Wyatt, M. F.; Graham, A. E. *submitted to Synlett*.
- Battioni, P.; Brigaud, O.; Desvaux, H.; Mansuy, D.; Traylor, T. G. *Tetrahedron Letters* **1991**, *32*, 2893-2896.