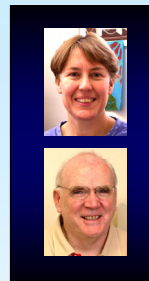




Application of Solvent-free Sample Preparation Methods for MALDI-TOFMS to Organometallic and Coordination Compounds

Laura Hughes, Mark F. Wyatt, Bridget K. Stein and A. Gareth Brenton

EPSRC National Mass Spectrometry Service Centre (NMSSC), Swansea University, Swansea, SA2 8PP, U.K.



Overview

- Recent research has focussed on the use of MALDI-TOFMS to characterize organometallic/coordination/highly conjugated compounds.
- 2-[(2E)-3-(4-tert-butylphenyl)-2-methylprop-2-enylidene]malononitrile (DCTB) matrix is very effective for these compounds, with radical ions observed.
- Solvent-free preparation is required for such compounds that are insoluble, soluble in non-volatile solvents, or unstable in solution.
- First-row transition metal acetylacetonate ($\text{Met}^{\text{III}}\text{acac}_2$ and $\text{Met}^{\text{II}}\text{acac}_2$) complexes were used to develop a working method.
- Several mixing and deposition methods were investigated; the 'ball mill and dab' (BMD) method was considered best overall.
- Case study 1: BMD method was applied to completely insoluble mercury-ferrocene compound. Radical molecular ion was observed.
- Case study 2: BMD method was applied to compounds with poor solution stability. Radical molecular ion was not observed.

Introduction

Matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOFMS) is a very important and successful analytical technique for a wide variety of samples. Generally, sample preparation involves placing a droplet of mixed matrix/sample solution onto a sample target, and then allowing the solvent to evaporate. This technique relies on sample and matrix combinations that are soluble in the same low boiling point solvent or miscible solvents. However, a significant number of samples submitted to the NMSSC are insoluble or only soluble in high boiling point solvents, e.g. DMSO. Additionally, some samples are unstable in solution. The development of solvent-free preparation methods is clearly beneficial to the NMSSC and the wider scientific community. Previous work in this area has focussed mainly on synthetic polymers,¹⁻³ but has also been applied to biochemical samples.^{4,5}

Current Investigation

The following solvent-free mixing methods were studied:

1. Pestle and mortar.¹
2. Steel ball milling.⁶

The following methods of sample deposition were also studied:

1. Pressed disc.¹
2. Pressed disc with KBr.¹
3. Suspension in non-solvent.
4. Dabbing.
5. Double-sided adhesive tape.

Materials and Instrumentation

- $\text{Met}^{\text{II}}\text{acac}_2$ (Met = Fe, Zn) and $\text{Met}^{\text{III}}\text{acac}_3$ (Met = V, Cr) complexes, were purchased from Sigma-Aldrich (U.K.), have been analysed previously.⁷
- DCTB and 7,7,8,8-tetracyanoquinodimethane (TCNQ) charge-transfer matrices were purchased from Fluka (U.K.).
- HPLC-grade water purchased from Fisher (U.K.).
- MALDI-TOFMS measurements obtained with a Voyager DE-STR instrument (Applied Biosystems, U.S.A.).
- Whirlimixer vortex mixer (Fisher, U.K.) was used for milling and creating suspensions.
- 3 mm steel balls were purchased from Wheelies Cycles (Swansea, U.K.).
- Discs were pressed using a C-30 Press (Research and Industrial Instruments Company, London, U.K.).
- Potassium bromide (KBr) was purchased from Sigma-Aldrich (U.K.).

Experimental – Solids Mixing

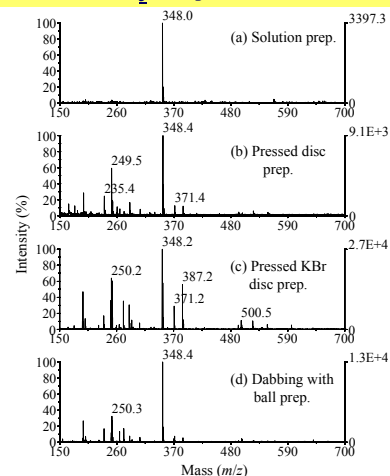
Samples and matrices were mixed in molar ratios of 1:50, 1:75, 1:100, 1:200, 1:500 and 1:1000.

1. Pestle and mortar: Portions of sample and matrix were ground until the mixture was judged by eye to be homogeneous, which was approximately 3 minutes.
2. Steel ball milling: Portions of sample and matrix were added to a plastic, cone-shaped, sample vial. A 3 mm steel ball was added and the mixture ground by agitating the vial with a vortex mixer until judged by eye to be homogeneous, which was approximately 1 minute.

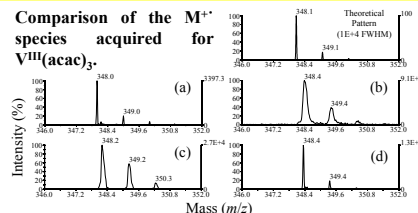
Experimental - Deposition of Mixture onto Plate

1. Pressed disc: Sample/matrix mixture was pressed into a disc utilising a die and press apparatus normally used to press KBr discs for IR measurements. 10 tonnes of pressure were applied. Discs were fixed onto the sample plate with adhesive tape.
2. Pressed disc with KBr: KBr was added to the mixtures in molar ratios of 2:1, 1:1, 0.5:1, 0.25:1 and 0.1:1, with respect to the matrix.
3. Suspension in non-solvent: Sample/matrix mixture was suspended in 0.5 mL of water by using a vortex mixer. 1 μL was then placed onto the sample plate via pipette, and the solvent allowed to evaporate.
4. Dabbing: For pestle and mortar preparations, an aliquot of the mixture was transferred to the sample plate and lightly flattened with a microspatula. For ball milled samples, either the above would occur, or the mixture-coated ball was extracted from the vial with tweezers, and tapped on the plate to transfer mixture, which was then lightly flattened with a microspatula.
5. Double-sided adhesive tape: Tape was fixed to the plate and the sample/matrix mixture was spread onto it with a microspatula. The plate was then brought upright and tapped on the bench to remove excess mixture.

Results – $\text{V}^{\text{III}}\text{acac}_3$ Complex with DCTB Matrix



Results – $\text{V}^{\text{III}}\text{acac}_3$ Complex with DCTB Matrix

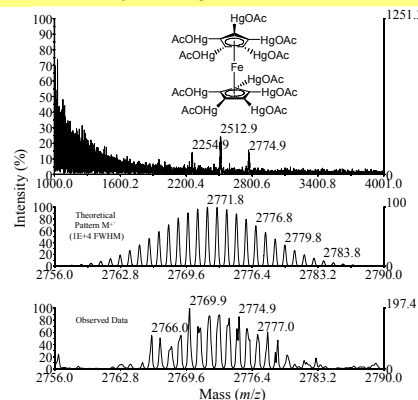


Observations

- The ball milling method of Hanton and Parees uses 2 balls, but 1 appears to be sufficient.
- Comparable data was acquired for each method of solids mixing, regardless of the method of application onto the samples plate. However, ball milling is quicker, cleaner and less labour intensive.
- The pressed discs were very fragile, making handling difficult.
- KBr was added to the mixtures in order to improve the structural integrity of the pressed discs. Additionally, promoting the formation of $[\text{M}^+\text{K}]^+$ species was sought, and these species were observed.
- DCTB considered better matrix, due to TCNQ discs being more fragile and milled TCNQ mixtures adhering to both ball and vial.
- Radical molecular ions were not observed by the suspension and adhesive tape methods.
- Cleanest, highest resolution data obtained with the dabbing method.
- Similar trends were noted in the data obtained for the other acac complexes.
- The method used affected mass accuracy.

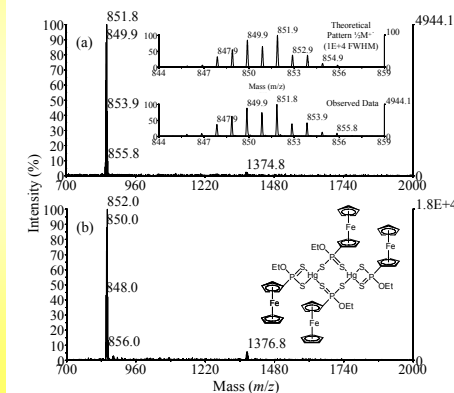
Case Study 1 – Insoluble Compound

Insoluble ferrocene compound⁸ observed as extremely low intensity radical molecular ion, with fragments or impurities also observed.



Case Study 2 – Solvent-degradable Compound

- Proposed structure for solid⁹ was proven by crystallography.
- Radical molecular ion was not observed with solution preparation (a), but observed peaks could be fragments, including apparent $\frac{1}{2}\text{M}^+$.
- The Woolfins group proposed that the sample was degrading in solution.
- Results for solvent-free preparation method (b) were similar to those observed with solution preparation.



Conclusions and Future Work

- Several mixing and deposition methods were investigated; 'ball mill and dab' (BMD) method considered best overall, for ease of application and quality of results.
- Single steel ball was adequate, and DCTB was easier to handle than TCNQ.
- Case study 1: method was applied to completely insoluble mercury-ferrocene compound. Extremely low intensity radical molecular ion was observed.
- Case study 2: method was applied to compounds with poor solution stability. Radical molecular ion was not observed, and results were similar to those obtained in solution.
- Further work required to refine method in order to improve mixture homogeneity, data quality and reproducibility.

References

1. Skelton, R.; Dubois, F.; Zenobi, R. *Anal. Chem.* **2000**, *72*, 1707-1710.
2. Marie, A.; Fournier, F.; Tabet, J. C. *Anal. Chem.* **2000**, *72*, 5106-5114.
3. Trimpin, S.; Rouhanipour, A.; Az, R.; Rader, H. J.; Müllen, K. *Rapid Commun. Mass Spectrom.* **2001**, *15*, 1364-1373.
4. Wang, M. Z.; Fitzgerald, M. C. *Anal. Chem.* **2001**, *73*, 625-631.
5. Trimpin, S.; Deinzer, M. L. *J. Am. Soc. Mass Spectrom.* **2005**, *16*, 542-547.
6. Hanton, S. D.; Parees, D. M. *Proceedings of the 52nd ASMS Conference, Nashville TN, 2004*.
7. Wyatt, M. F.; Havard, S.; Stein, B. K.; Brenton, A. G. *Proceedings of the 52nd ASMS Conference, Nashville TN, 2004*.
8. Sample submitted to the NMSSC by M. Parry and I. R. Butler, University of Wales Bangor, U.K.
9. Sample submitted to the NMSSC by I. P. Gray and J. D. Woolfins, University of St. Andrews, U.K.