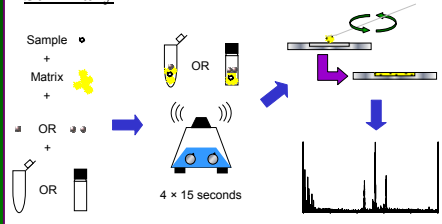


Further Developments in the Application of Solvent-free Preparation Methods for MALDI-TOFMS

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Summary



Introduction

Matrix-assisted laser desorption/ionisation time-of-flight mass spectrometry (MALDI-TOFMS) is an important and successful analytical technique for a wide variety of compounds. A traditional dried-droplet sample preparation technique relies on sample and matrix combinations that are soluble in the same volatile solvent or miscible solvents. However, many samples, submitted to our Centre are insoluble or only soluble in less volatile solvents e.g. dimethyl sulphoxide. Development of a solvent-free MALDI method with specific reference to organometallic or coordination compounds complements other potentially solvent-free ionisation techniques, such as electron ionisation (EI), offering a softer method of ionisation. In addition, the method facilitates the analysis of compounds not suited to these other techniques.

Previous Work & Current Study

- Previous work in this area has focused mainly on synthetic polymers,^{1,2} but has also been applied to biochemical samples.^{3,4}
- Preliminary investigations into the analysis of various first-row transition metal acetylacetonate complexes using assorted literature and NMSSC solvent-free methods were summarised in a recent conference poster.⁵
- $V(acac)_3$ is partially soluble in dichloromethane and 1,1,1,3,3,3-hexafluoroisopropanol, and, together with related complexes, was the subject of a recent MALDI-TOF investigation within this laboratory.⁶
- Intense positive radical ions were observed using 2-[(2E)-3-(4-*tert*-butylphenyl)-2-methylprop-2-enylidene] malononitrile (DCTB) matrix.
- The preparation method of ball-milling the sample and matrix mixture, followed by 'smearing' onto the target plate,⁷ including our own variations,⁵ is evaluated here for vanadium (III) acetylacetonate ($V(acac)_3$), in order to establish a reliable protocol.
- In addition, two insoluble samples submitted to the NMSSC were examined as case studies. Compound **1** was ferrocene fully substituted with mercury acetate groups, while compound **2** was an erbium complex with three bis(pentafluorophenyl) phosphinate ligands (ErL_3).

Materials

- $V(acac)_3$, 2,3,4,5,6-pentafluorobenzoic acid (PFBA) and 2,3,4,5,6-pentafluorocinnamic acid (PFCA), were purchased from Sigma-Aldrich (Dorset, U.K.).
- DCTB was purchased from Fluka (Dorset, U.K.).

Solvent-free Sample Preparation

- For $V(acac)_3$, 0.1, 0.2, 0.5, and 1 mg were added to both tapered plastic and flat-bottomed glass sample vials.
- 10 mg of DCTB matrix was added to each vial.
- One 3 mm steel ball was added to the plastic vials (Method A), and two balls to the glass vials (Method B).
- Each vial was agitated for 4 x 15 seconds to ensure complete homogenisation, using a vortex mixer.
- After each round of mixing, material was dislodged from vial crevices with a thin, stiff object *i.e.* a microspatula or straightened paperclip.
- A small amount of loose material from each vial was then placed onto a stainless steel sample plate and smeared with the flat side of a microspatula.
- Material stuck to the ball from the plastic vials (Method C) was dabbed onto the plate and then smeared.
- Gentle pressure and a circular motion were used to smear until no loose powder remained.
- For **1**, 1 mg was mixed with 10 mg of DCTB matrix.
- For **2**, 0.3 mg was mixed with 10 mg each of DCTB, PFBA, and PFCA matrices.
- Method B was followed for all case study preparations.

Mass Spectrometry

- MALDI-TOFMS spectra were acquired using an Applied Biosystems Voyager DE-STR spectrometer, which is equipped with a nitrogen laser ($\lambda = 337$ nm).
- The instrument was operated in positive or 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, but were kept constant for all data acquisitions for the $V(acac)_3$ complex.

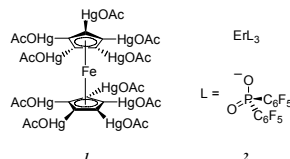
Results – $V(acac)_3$

Positive ion MALDI-TOFMS data for the various preparations of $V(acac)_3$

Preparation Method	Sample (mg)	Relative Intensity (%)	
		$V(acac)_3$ (M^{2+})	DCTB (M^{2+})
A	0.1	16	100
	0.2	46	100
	0.5	100	43
	1	100	9
B	0.1	30	100
	0.2	45	100
	0.5	84	37
	1	100	27
C	0.1	6	100
	0.2	23	100
	0.5	92	100
	1	100	40

- Radical ions for the sample (m/z 348.1) were observed for all preparations, showing that for a range of sample loadings, any milling/mixing method and method of sampling each mixture for analysis, may be viable.
- The relative intensities of ions were moderately consistent between preparations with identical sample amounts.
- The total ion count fluctuated randomly between all preparations, despite every acquisition parameter remaining constant, and taking 'hot spots' into consideration.
- Re-analysis of the prepared plate revealed that a large increase in ion count and improvement in signal-to-noise ratio was observed when the laser was aimed at more translucent areas of the preparation, compared to opaque, powdery areas.
- Translucent areas for a dried-droplet preparation usually indicate a low sample-to-matrix ratio.
- This data clearly indicates that the smearing stage of the preparation is more critical than the milling/mixing stage.

Case Study Compounds



Case Study Results - I

