New Insights Relative to Matrices in Mass Spectrometry

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Purpose

Binary matrix mixtures can be tailored to improve MAI and MALDI ionization. Understanding the mechanism of enhancements is the long-range goal of this research.

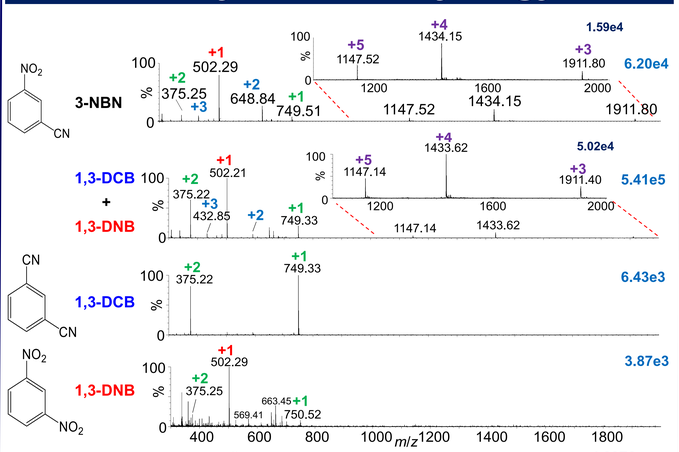
Introduction

All ionization methods used in mass spectrometry (**MS**) capable of converting nonvolatile compounds to gas-phase ions with good sensitivity do so through use of a small molecule matrix compound. Despite the importance of matrices in the success of MS, the mechanism by which they work is poorly understood. Matrix-assisted Ionization (**MAI**), and more particularly, vacuum (**v**) MAI, offer insights into how matrices function to convert even large nonvolatile compounds into gas-phase ions. As with solvent matrices in electrospray ionization (**ESI**) and solvent-assisted ionization (**SAI**), no laser is necessary, thus for nonvolatile and high-mass compounds, excellent sensitivity is achieved without resorting to either a photochemical or high temperature thermal ionization models. Here we discuss results obtained with binary matrix mixtures.

Methods

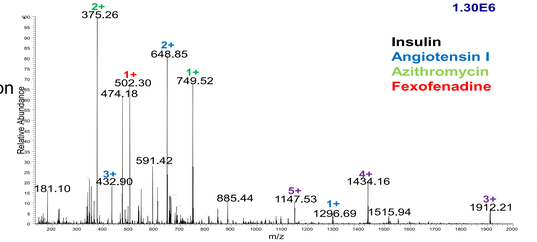
Small molecule compounds including MAI and MALDI matrices, as well as compounds that sublime but do not show matrix properties were mixed in various ratios to make binary matrices (**Fig. 1-5**). The matrix solutions were mixed with analyte solution on a sample plate (either metal or glass), briefly dried and exposed to the vacuum of the mass spectrometer using a novel approach for vacuum ionization (**Fig. 6**). Sample introduction to vacuum can be achieved in *ca.* 2 sec and with multiple samples on a plate, we achieve 4 sec/sample using vMAI and 1 sec/sample using MALDI. For vMAI, no laser or heat is applied and only a low voltage to extract the ions from near the sample surface. For MALDI a nitrogen laser was used to ablate the sample and produce ions. Various analytes were used including a mixture of fexofenadine (3 nM), azithromycin (3 nM), angiotensin I (5 nM), and insulin (5 nM). Analyte and matrix solutions were mixed 1:1.

Binary Matrices Synergy



DCB:DNB 3:2 Ratio gave best Ion abundance.

Fig.1: Comparison of 4-component mixture using 3-NBN, DCB, DNB, and mixtures of DCB and DNB.



vMAI Binary Matrices for Proteins

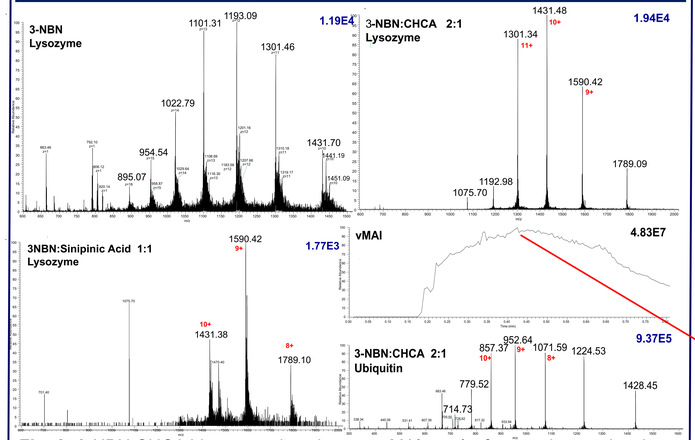
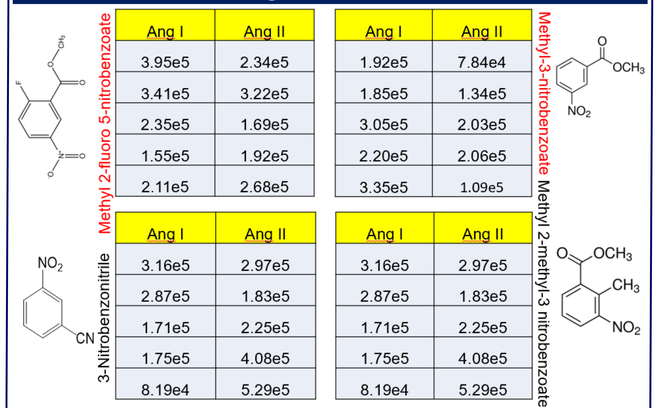


Fig. 2: 3-NBN:CHCA binary matrix enhances vMAI results for certain proteins, but does not appear to help or hurt with peptides and small proteins such as insulin and ubiquitin which ionize well with 3-NBN alone. However, the binary matrix 3-NBN:sinapinic acid decreases ion abundance for proteins. The mechanism of enhancement by CHCA is currently unknown.

3-NBN Binary Matrices with MAI



Ang I	Ang II
3.95e5	2.34e5
3.41e5	3.22e5
2.35e5	1.69e5
1.55e5	1.92e5
2.11e5	2.68e5

Ang I	Ang II
1.92e5	7.84e4
1.85e5	1.34e5
3.05e5	2.03e5
2.20e5	2.06e5
3.35e5	1.09e5

Ang I	Ang II
3.16e5	2.97e5
2.87e5	1.83e5
1.71e5	2.25e5
1.75e5	4.08e5
8.19e4	5.29e5

Ang I	Ang II
3.16e5	2.97e5
2.87e5	1.83e5
1.71e5	2.25e5
1.75e5	4.08e5
8.19e4	5.29e5

Fig. 3: Solutions of 3 benzoate compounds were mixed with a 3-NBN solution in a 1:4 molar ratio and this solution mixed 1:1 with a 0.5 µM solution containing Ang I and II. The MS™ Ionique automated platform was used to inject 0.2 uL Of the dried matrix:analyte sample into the inlet of a Q-Exactive Focus mass spectrometer. The two compounds (top in red letters) are not MAI matrices, but because are more volatile than 3-NBN, the speed of ionization is increased.

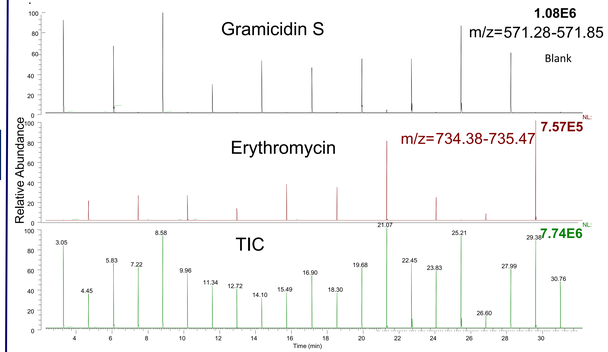


Fig. 4: Binary matrix containing 3-NBN and methyl 3-nitrobenzoate, 1:3 molar ratio, using MS™'s Ionique automated platform to mix and dispense 0.2 µL matrix solution and 0.2 µL solution of 0.5 µM gramicidin S and erythromycin. The volatility of the binary matrix results in little carryover between samples.

Single Acquisition of Ubiquitin

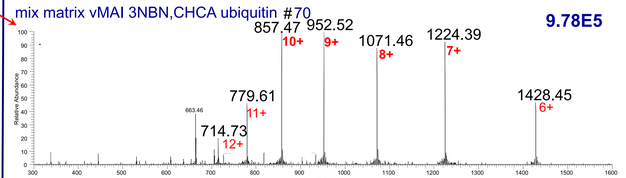
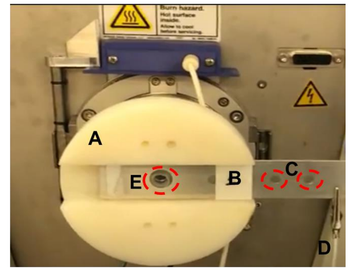
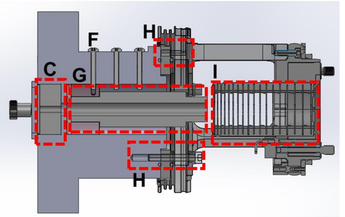


Fig. 5: Single acquisition using vMAI with 3-NBN:CHCA binary matrix representing <10 fmol of ubiquitin.

Fig. 6: MS™ multimode high throughput concept source for high sensitivity and robust ionization. Interchanging the source with the ESI source is accomplished in <1 hr plus pump down. Top source view: A) flange, B) glass slide, C) metal valve plate, D) Q-Exactive Focus, E) entrance to MS covered by glass slide. Bottom view: flange with focusing lens interfaced to Thermo 'S'-lens assembly.

For MALDI results and movies see: Trimpin, ThOA am 08:30 Multi-functional Vacuum Ionization Source for MAI, LSI, and MALDI: Operational from AP for Comprehensive, Low-Cost Data-Mining in Mass Spectrometry.





Conclusions

- Binary matrix combinations can enhance analyses in MAI and MALDI (data not presented)
- A MAI matrix transfers analyte into the gas phase in charged particles which upon loss of matrix produces bare analyte ions as is also the case with ESI, SAI, and likely MALDI
- In MAI, matrix particles are expelled into the gas phase through a sublimation driven process.
- The rate of sublimation or the charge separation process can be altered through additives or binary matrix combinations to achieve a desired outcome,
- But, we don't yet understand how certain matrix combinations produce enhanced ionization.

Acknowledgements

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- MAI, SAI, and vSAI are protected under US patent 2013/0214154 (A1) and the vMAI/MALDI high throughput method through provisional application (PA #63003299).