

Contract No:

This document was prepared in conjunction with work accomplished under Contract No. DE-AC09-08SR22470 with the U.S. Department of Energy (DOE) Office of Environmental Management (EM).

Disclaimer:

This work was prepared under an agreement with and funded by the U.S. Government. Neither the U. S. Government or its employees, nor any of its contractors, subcontractors or their employees, makes any express or implied:

- 1) warranty or assumes any legal liability for the accuracy, completeness, or for the use or results of such use of any information, product, or process disclosed; or
- 2) representation that such use or results of such use would not infringe privately owned rights; or
- 3) endorsement or recommendation of any specifically identified commercial product, process, or service.

Any views and opinions of authors expressed in this work do not necessarily state or reflect those of the United States Government, or its contractors, or subcontractors.

Development of Direct Injection/Ionization Mass Spectrometry Methods for Whole Molecule Characterization

The objective of this work is to adapt ambient ionization mass spectrometry (AMS) techniques for the rapid analysis of intact uranium complexes, stable strontium, cerium, and explosive compounds. The methods used were “soft ionization” techniques, which facilitate the detection of whole molecule complexes. The soft ionization mass spectrometry (MS) techniques that were investigated include paper spray ionization (PSI), matrix-assisted ionization (MAI), electrospray ionization (ESI) and direct analysis in real time (DART). For the first time, PSI-MS methods were successfully developed for whole molecule uranium-containing analytes (uranyl acetate, uranyl nitrate, and uranyl-tributylphosphate complexes). This was also the first demonstration of uranium complex detection and characterization and one of the few examples of inorganic analysis using MAI techniques. Proof of concept experiments also putatively identified matrix-derived ions and ion complexes that have not previously been described in the literature. Additionally, PSI-MS on cotton swipe samples doped with a multi-element standard containing μg levels of U, Bi, Pb, Cd, Fe, and Zn were directly analyzed without purification, representing a major improvement over existing methods. Both PSI and MAI methods demonstrated limits of detection (LODs) in the 10 - 100's ng for various uranyl species within a range of 10's ppm - 100's ppb, dependent on analytical method and analyte species. AMS methods were also developed for other inorganics, including Ce and Sr, and organic explosive residues to address specific challenges in environmental monitoring and forensics. Further refinement and qualification of the AMS techniques developed within this effort would lead to significant cost reduction and timeliness by facilitating the triage and queueing of samples for subsequent more sensitive and time-consuming analyses.

Awards and Recognition

One manuscript has been submitted to the peer-reviewed *Journal of the American Society for Mass Spectrometry* titled “Matrix Assisted Ionization of Molecular Uranium Species”. A manuscript on the rapid detection of inorganic Strontium and Cerium species by ambient mass spectrometry techniques is in preparation and will be submitted to *Journal of the American Society for Mass Spectrometry*.

Intellectual Property Review

This report has been reviewed by SRNL Legal Counsel for intellectual property considerations and is approved to be publicly published in its current form.

SRNL Legal Signature

Signature

Date

Development of Direct Injection/Ionization Mass Spectrometry Methods for Whole Molecule Characterization

Project Team: W. Kuhne (Primary), K. Lawrence, A. Swindle, D. Mannion, R. Smith, J. Mannion, C. Shick, M. Wellons

Collaborators: C. Cody (JEOL USA Inc.)

Thrust Area: National Security

Project Start Date: October 1, 2019

Project End Date: September 30, 2020

Soft ionization methods for mass spectrometry allow the detection of partial or whole molecular species to enable rapid, unambiguous identification of species relevant to environmental remediation and nuclear safeguards challenges. In this project, the trace detection of whole molecule uranyl complexes, including uranyl acetate, uranyl nitrate, uranyl chloride, and uranyl oxalate were successfully demonstrated using matrix-assisted ionization mass spectrometry (MAI-MS). Detection and characterization of uranium analytes using 3-

nitrobenzlnitrile (3-NBN) as the ion generating matrix is the first demonstration of uranium complex analysis using MAI techniques.. Paper spray ionization mass spectrometry (PSI-MS) and Electrospray ionization mass spectrometry (ESI-MS) of strontium and cerium species led to ppm limits of detection (LOD) with fast (<1 min) measurement times and no sample preparation. Additionally, the detection and characterization of explosive compounds were carried out using standards for RDX and TNT to develop and optimize methods for concentrating samples swiped from surfaces. This work has demonstrated a new characterization modality for convenient and rapid analysis with minimal sample preparation.

FY2020 Objectives

- Development of matrix-assisted ionization techniques for uranium complexes;
- Optimization and quantification of Sr and Ce species detection using ESI-MS and PSI-MS
- Development of AMS methods for explosive material detection and characterization

Introduction

The rapid characterization of trace inorganic molecules and complexes is important for a litany of applications, including forensic analysis, nuclear safeguards monitoring,¹⁻² environmental monitoring³⁻⁴ and geochronical measurements.⁵ For nuclear safeguards applications, the quantification of inorganic isotopes from environmental samples typically involves extensive chemical and physical processing prior to analysis. Harsh sample preparation results in the loss of the original chemical speciation (i.e. what the metal was bonded to/with when found in the environment) as well as the mixing of anthropogenic uranium with background material contained within the collection media (e.g. a swipe or soil). To overcome these challenges, soft ionization techniques using ambient mass spectrometry (AMS) techniques, including paper spray ionization (PSI), matrix assisted ionization (MAI), and electrospray ionization (ESI) were employed for the rapid analysis of intact uranium complexes, strontium and cerium analytes, and explosives compounds for national security applications (**Figure 1**).

Matrix assisted ionization (MAI) uses a chemical matrix that spontaneously produces analyte ions when exposed to the vacuum of a mass spectrometer. Unlike the other AMS techniques, MAI does not require the use of photons, electron beams, high voltage or added heat to initiate ionization to produce a mass spectrum. Instead, it is dependent on the choice of matrix and vacuum conditions.⁶ The actual mechanism by which MAI produces analyte ions is unknown, and this technique is often referred to as “magic ionization.” MAI is typically carried out by introducing solid matrix crystals directly into vacuum; however the introduction of liquid solutions containing saturated 3-NBN concentrations has yielded rapid results for inorganic species.⁷ Though detection limits comparable to ESI-MS have been demonstrated for a variety of organic analytes using MAI-MS,⁸⁻¹¹ no reports exist in the literature of utilizing MAI for inorganic analysis. This method was employed for uranium complexes, including uranyl nitrate, uranyl chloride, uranyl acetate, and uranyl oxalate with ng LODs for most species and analysis times of <5 seconds.

FY20 data built upon initial studies from FY19 and focused on the quantification of other inorganic species as well as explosives compounds.

Approach

The goal of this work was to develop analytical techniques for the identification and quantification of inorganic and explosives compounds. Initial studies began in late FY19 for the detection and quantification of uranyl complexes, RDX and TNT and extended into FY20 with an additional \$50K to continue LOD optimization and to leverage against a planned field exercise. All samples were analyzed with an AccuTOF™ DART® 4G mass spectrometer from JEOL (Boston, MA, USA).

MAI analysis for inorganic elements and complexes with uranium ions In FY20, MAI analysis was carried out using a common ionization matrix material, 3 nitrobenzonitrile (3-NBN), to investigate the direct ionization method for uranyl hydrate complexes of nitrate, chloride, acetate, or oxalate speciation. Samples were analyzed in negative ion mode, as no spectra corresponding to uranyl was detected in positive ion mode. Stock solutions of uranyl chloride, uranyl acetate, and uranyl oxalate salts composed of natural uranium (International Bio-Analytical Industries, Boca Raton, FL, USA) were used to make solutions of 1, 10, 50, 100, 250, and 500 µg/mL of each uranyl salt in ASTM Type II water. A stock solution of depleted uranium certified reference material U005 (New Brunswick Laboratory, Argonne, IL) was used to make solutions of 0.1, 0.5, 1, 10, 50, and 100 µg/mL uranyl nitrate in 2% nitric acid. Samples were

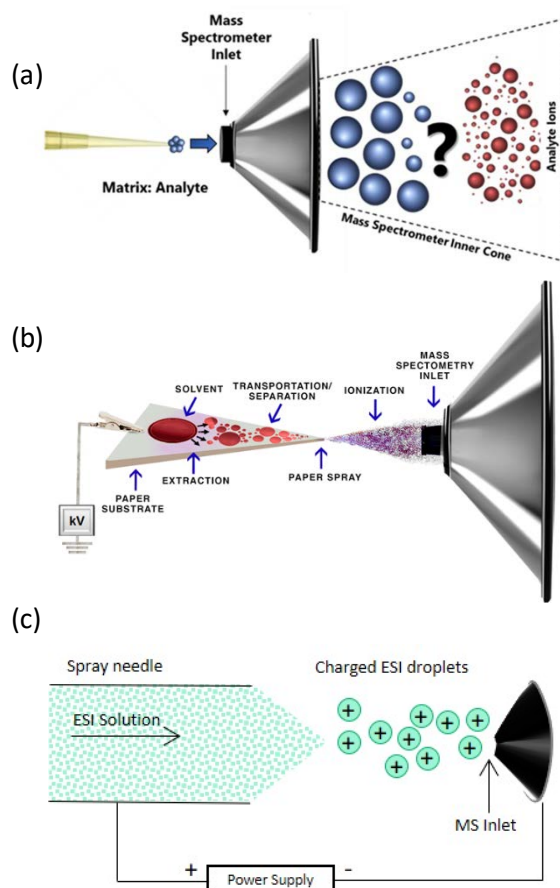


Figure 1. Overview of the different ionization modes that were used: (a) MAI-MS; (b) PSI-MS; (c) ESI-MS

prepared for MAI analysis by mixing each sample with methanol (ACS Reagent Grade, Sigma-Aldrich, St. Louis, MI, USA) to create 50:50 sample/methanol mixtures.

PSI-MS and ESI-MS for inorganic species of Ce and Sr

All samples were analyzed in positive ion mode. The radiofrequency quadrupole ion guide was set to an amplitude of 50 – 1000 V for cerium analysis and 250 – 500 V for strontium analysis, the cone (“orifice 1”) voltage was set to 180 V, the needle voltage was set to +3.0 kV and the detector voltage was set to 2.5 kV. For PSI-MS, the spectrometer was equipped with a JEOL paper spray ion source, which consists of a copper clamp, safety interlock and manual positioning system. The paper spray source was held at +3.0 kV for positive-ion mode. JEOL version 4 was used in msAxel software for data acquisition, mass calibration, and to export spectrum data from regions of interest in the extracted ion current chromatogram (EICC). Peak deconvolution was carried out in OriginPro 2016 using second order derivatives to obtain peak locations and intensities. For the calibration curves, the intensities were extracted from raw data using RStudio with MALDIquant and MALDIquantForeign packages.

RDX and TNT Explosives Detection

Calibration curves were created using explosive standards in acetonitrile and methanol in negative ion mode using DART. Analytes were measured by introducing the sample into the gas stream using a capillary tube and ~5 µl of sample.

Results/Discussion

MAI-MS analysis of inorganic uranyl complexes ions

Ionized forms of the four uranyl species investigated were putatively identified. The calculated LODs were found to be 5 ng of uranyl nitrate, 10 ng of uranyl oxalate, 100 ng of uranyl acetate, and 200 ng of uranyl chloride. Additionally, six anion peaks were observed in all mass spectra containing 3-NBN. Subsequent analysis of the matrix by GC/Q-TOF suggest these ions are created from the matrix during the ionization process and are not matrix contaminants. Two of these matrix derived anions were found to form detectable ion complexes with the uranyl analyte.

The uranyl ligands analyzed were detected in negative ion mode and when aspirating liquid sample saturated with suspended 3-NBN crystals. The four uranyl ligand species examined were all detected as negative ion complexes composed of one uranyl cation in conjugation with anions totaling three negative charge equivalents. For example, uranyl nitrate was primarily observed as uranyl complexed with three nitrates, though other ion complexes were observed that were the result of oxide, hydroxide, and matrix-derived adducts (Figure 2).

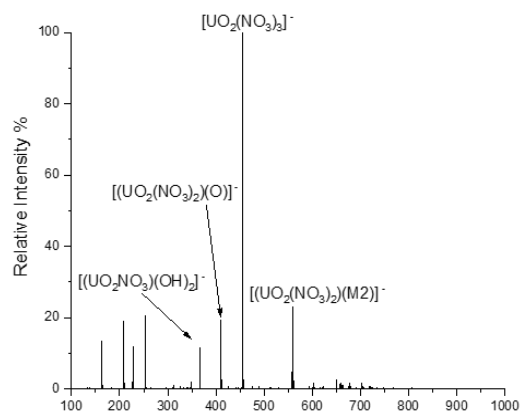


Figure 2. Negative ion MAI mass spectra of uranyl nitrate.

PSI-MS and ESI-MS for inorganic species of Ce and Sr

The detection of inorganic species, strontium nitrate and cerium nitrate, using internal standards was successfully carried out in both ESI-MS and PSI-MS with relatively low error and detection limits observed

in the ppm range in FY19. Adducts produced via these ionization modes, to include adducts from the original nitrate molecules, have been observed. For cerium adducts, the PSI-MS had lower LOD at 100 and 250 V but had higher LOD at the higher voltages. LOD for Sr was higher with PSI-MS than ESI-MS. Isotopic abundancies were measured with less than 10% error for Sr using ESI-MS and less than 25% error for PSI-MS at the lowest abundant isotope (0.56% abundance for ^{84}Sr). The measured abundancies for Ce isotopes were around 1% or lower for ESI-MS but up to 25% on PSI-MS. The higher percent error for PSI-MS compared to ESI-MS is attributed to a generally lower signal. In FY20, a more thorough comparison of ESI-MS and PSI-MS for the detection of Sr and Ce species was performed to investigate associated error within the measurements. A drinking water standard was also analyzed to validate the calibration curve. As shown in **Table 1**, the concentration of Ce in a drinking water standard containing other metal analytes was determined to have the lowest calculated percent error when measured with 100 V. With the appropriate selection of internal standards, PSI-MS is a viable method for the rapid detection and quantification of inorganic species, Sr and Ce, with isotopic discrimination requiring minimal sample preparation efforts.

Table 1. Measured concentration and percent error for a standard sample containing Ce from the obtained calibration curve

ICP-MS-TS-21 Standard Containing Ce (Internal Standard Gd) using PSI-MS Expected Value: 0.26 ppm		
Voltage (V)	Calculated [CeO ⁺] (ppm)	% error
100	0.23	9.97
250	0.22	14.8
500	0.29	11.6
1000	0.21	19.0

RDX and TNT Explosives Detection

Measurement of TNT and RDX were carried out using ESI-MS. Contaminants associated with the solvents methanol and acetonitrile, including palmitic acid-H at m/z 255.24, steric acid-H at m/z 283.48, and myristic acid-H at m/z 227.38 were identified. Initial findings showed that [TNT-H]⁻ (m/z = 226.01) and [RDX-H]⁻ (m/z = 221.12) do not mutually ionize at the same instance, however there does seem to be a relationship between their ionization and that of palmitic acid, indicating there is a matrix effect as a result of solvent impurities. A stronger relationship seemed to be present for TNT compared to RDX. Additional work was required to fully understand this relationship.

FY2020 Accomplishments

FY20 accomplishments were primarily focused on

- Method development using ESI-MS and PSI-MS were developed for the direct analysis of inorganic Strontium and Cerium with ppm to ppb LODs.
- Successful analysis of uranyl complexes using MAI providing <5 second analysis times with the identification of potentially novel matrix-derived anions.

Over three years, this project has employed four junior SRNL scientific staff and supported two DOE SULI interns with statistical/data analysis experience, thus building the technical expertise of the next generation. Work products from the efforts in FY20 include a manuscript submitted to a peer-reviewed journal on uranyl complex analysis using MAI-MS, and a manuscript in preparation for a peer-reviewed journal on Sr and Ce detection using PSI-MS.

Future Directions

- A Discovery Science LDRD was granted for FY21 to build upon the experiments conducted on MAI uranium analysis to investigate the fundamentals of the inorganic ionization mechanism. A full proposal was requested and submitted to the NNSA Defense Nuclear Nonproliferation Research and Development program for the FY21 call for proposals entitled “FY21-SR-PD1Ac-P39: Triboluminescent Matrices for Rapid Trace Detection and Characterization of Actinides”
- A full proposal was requested and submitted on topics related to PSI-MS method development to an external USG Research, Development, and Integration (RDI) Fund for a FY21 call for proposals in collaboration with an external USG partner.

FY20 Publications/Presentations

1. Mannion, Danielle R.; Mannion, Joseph M.; Kuhne, Wendy W.; and Wellons, Matthew S. (2020). Matrix Assisted Ionization of Molecular Uranium Species. *Journal of the American Society for Mass Spectrometry*. Submitted.
2. Coopersmith, Kaitlin; Swindle, Ashlee; Dunbar, Joshua; Kuhne, Wendy W.; and Wellons, Matthew S. (2020). Rapid Detection of Inorganic Strontium and Cerium Molecular Species by Ambient Mass Spectrometry Techniques. *Journal of the American Society for Mass Spectrometry*. (In Preparation)

References

1. Lamouroux, C.; Moulin, C.; Tabet, J. C.; Jankowski, C. K., Characterization of zirconium complexes of interest in spent nuclear fuel reprocessing by electrospray ionization mass spectrometry. *Rapid Communications in Mass Spectrometry* **2000**, *14* (19), 1869-1877.
2. Zheng, J.; Tagami, K.; Watanabe, Y.; Uchida, S.; Aono, T.; Ishii, N.; Yoshida, S.; Kubota, Y.; Fuma, S.; Ihara, S., Isotopic evidence of plutonium release into the environment from the Fukushima DNPP accident. *Scientific Reports* **2012**, *2* (304), 1-8.
3. Liu, W.; Dai, X.; Bai, Z.; Wang, Y.; Yang, Z.; Zhang, L.; Xu, L.; Chen, L.; Li, Y.; Gui, D.; Diwu, J.; Wang, J.; Zhou, R.; Chai, Z.; Wang, S., Highly Sensitive and Selective Uranium Detection in Natural Water Systems Using a Luminescent Mesoporous Metal–Organic Framework Equipped with Abundant Lewis Basic Sites: A Combined Batch, X-ray Absorption Spectroscopy, and First Principles Simulation Investigation. *Environmental Science & Technology* **2017**, *51* (7), 3911-3921.
4. Xiao, S. J.; Zuo, J.; Zhu, Z. Q.; Ouyang, Y. Z.; Zhang, X. L.; Chen, H. W.; Zhang, L., Highly sensitive DNAzyme sensor for selective detection of trace uranium in ore and natural water samples. *Sens. Actuators B* **2015**, *210*, 656-660.
5. Spano, T. L.; Simonetti, A.; Balboni, E.; Dorais, C.; Burns, P. C., Trace element and U isotope analysis of uraninite and ore concentrate: Applications for nuclear forensic investigations. *Applied Geochemistry* **2017**, *84*, 277-285.
6. Lee, C.; Inutan, E. D.; Chen, J. L.; Mukeku, M. M.; Weidner, S. M.; Trimpin, S.; Ni, C.-K., Toward understanding the ionization mechanism of matrix-assisted ionization using mass spectrometry experiment and theory. *Rapid Communications in Mass Spectrometry* **0** (0).

7. Cody, R. B., Ambient Profiling of Phenolic Content in Tea Infusions by Matrix-Assisted Ionization in Vacuum. *J. Am. Soc. Mass Spectrom.* **2018**, 29 (8), 1594-1600.
8. Inutan, E. D.; Trimpin, S., Matrix assisted ionization vacuum (MAIV), a new ionization method for biological materials analysis using mass spectrometry. *Molecular & Cellular Proteomics* **2013**, 12 (3), 792-796.
9. Trimpin, S.; Inutan, E. D., Matrix assisted ionization in vacuum, a sensitive and widely applicable ionization method for mass spectrometry. *Journal of The American Society for Mass Spectrometry* **2013**, 24 (5), 722-732.
10. Trimpin, S.; Inutan, E. D., New ionization method for analysis on atmospheric pressure ionization mass spectrometers requiring only vacuum and matrix assistance. *Analytical chemistry* **2013**, 85 (4), 2005-2009.
11. Wang, B.; Dearing, C. L.; Wager-Miller, J.; Mackie, K.; Trimpin, S., Drug detection and quantification directly from tissue using novel ionization methods for mass spectrometry. *European Journal of Mass Spectrometry* **2015**, 21 (3), 201-210.

Acronyms

AMS: Ambient Mass Spectrometry
DART: Direct analysis in real time
EICC: Extracted ion current chromatogram
ESI-MS: Electrospray Ionization Mass Spectrometry
LOD: Limit of detection
MAI: Matrix Assisted Ionization
MS: Mass Spectrometry
 m/z : mass to charge ratio
RDX: 1,3,5-Trinitroperhydro-1,3,5-triazine
3-NBN: 3-Nitrobenzonitrile
PSI-MS: Paper Spray Ionization Mass Spectrometry
S/N: Signal to noise ratio
TBP: Tributylphosphate
TNT: Trinitrotoluene

Intellectual Property

There is no intellectual property to report for this effort.

Total Number of Post-Doctoral Researchers

None in FY20

Total Number of Student Researchers

None

External Collaborators (Universities, etc.)

None