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Liquid Sampling–Atmospheric Pressure Glow Discharge Ionization Source Coupled to an Ultra High Resolution Orbitrap Mass Spectrometer for Diverse Spectrochemical Analysis

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LIQUID SAMPLING – ATMOSPHERIC PRESSURE GLOW DISCHARGE IONIZATION SOURCE COUPLED TO AN ULTRA-HIGH RESOLUTION ORBITRAP MASS SPECTROMETER FOR DIVERSE SPECTROCHEMICAL ANALYSIS

A Thesis
Presented to
the Graduate School of
Clemson University

In Partial Fulfillment
of the Requirements for the Degree
Master of Science
Chemistry

by
Jacob Ryan Bills
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Accepted by:
Dr. R. Kenneth Marcus, Committee Chair
Dr. Carlos D. Garcia
Dr. George Chumanov
ABSTRACT

The liquid sampling – atmospheric pressure glow discharge (LS-APGD) coupled to an ultra-high resolution Orbitrap mass spectrometer has demonstrated expanded abilities for the uranium isotope ratios and molecular analysis by adding low polarity polyaromatic hydrocarbons (PAHs) to its already impressive repertoire. The LS-APGD/Orbitrap combination has shown the ability to analyze all three natural isotopes of uranium, $^{234}\text{U}$, $^{235}\text{U}$, and $^{238}\text{U}$, simultaneously. This is different from traditional instruments use a scanning type mass analyzer, but the Orbitrap analyzes all analytes simultaneously. Traditionally, in order to analyze both uranium and PAHs, two entirely different instruments would be required, typically an inductively coupled plasma mass spectrometer (ICP-MS) for uranium analysis and an atmospheric pressure chemical ionization source coupled to a mass spectrometer. However, with the LS-APGD, a simple switch in the carrier solution allows for these analyses. The International Atomic Energy Agency (IAEA) sets international target values (ITVs) for measurement uncertainty for uranium analysis. The LS-APGD/Orbitrap has shown the ability to meet these international target values, upon the addition of an external data acquisition system (DAQ), this pairing expanded on this analysis by adding in the ability to measure $^{234}\text{UO}_2$, while still maintaining the high precision measurement of $^{235}\text{UO}_2$. On top of this, the external DAQ allowed for a resolution improvement of 10x that of the standard system to be afforded and a limit of detection (LOD) of $<13 \text{ pg mL}^{-1}$ has been realized. By simply switching to MeOH:H$_2$O from the standard 2% HNO$_3$, the analysis of PAHs was realized, and more interesting, the observation of a protonated molecular ion was seen. This protonated molecular ion was not
expected as there is not traditional site for protonation on these molecules that would afford facile protonation as do small molecular species. It was found that plasma conditions that result in higher rotational temperatures provide more protonation of these molecules, suggesting that more energy is necessary for the protonation. Along with the protonation being investigated, LODs from 110 pg mL$^{-1}$ to 28 ng mL$^{-1}$ were found. These LODs are comparable to those that are listed in EPA method 610.
DEDICATION

This thesis is dedicated to my friends and family who helped me throughout this journey.
ACKNOWLEDGMENTS

I would like to acknowledge Dr. Marcus for his mentorship during my time at Clemson and the opportunities he afforded me during my time in his lab. The ability to attend conferences, give presentations, and collaborate with Spectroswiss is greatly appreciated and was very beneficial to my education.

I want to thank my labmates, Tyler Williams, Katja Hall, and Brandon Smiddy for the help in editing papers, bouncing ideas, and just being there in lab whenever I needed help with something. Lastly, I want to thank my thesis committee Dr. Chumanov and Dr. Garcia.
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CHAPTER ONE
INTRODUCTION

Mass spectrometry (MS) is a sophisticated analytical technique with the ability to analyze a wide array of analyte from elemental species all the way to large macromolecules.\textsuperscript{1, 2} Mass spectrometry has its roots in atomic analysis dating back to using magnetic sectors to separate uranium isotopes for the production of nuclear weapons and has evolved to be dominated by proteomic and other biological analysis.\textsuperscript{3} While there have been a great number of advancements in the realm of mass analyzers, the primary reason for this wide diversity of analyte is the development of a vast array of ion sources.\textsuperscript{2, 4-7} Originally, the ion sources used only allowed for small volatile organic molecules or atomic species.\textsuperscript{1} This is due to the necessity for the analyte to be in the gas phase prior to entering the ionization source in regards to electron ionization (EI) and the ability to desorb easily in the case of thermal ionization (TI)\textsuperscript{1} One of the largest advancements in sampling in the realm of mass spectrometry is the development of liquid sampling/atmospheric pressure ionization sources. The biggest of these being inductively coupled plasma (ICP) for atomic mass spectrometry and electrospray ionization (ESI) for molecular and biological mass spectrometry.\textsuperscript{2, 7} Liquid sampling greatly expanded the types of analyte that can be analyzed via mass spectrometry as volatility is no longer a requirement for ionization sources as it was with EI and TI, allowing larger molecules like molecules >300 $m/z$ including large organic molecules, proteins, and peptides.\textsuperscript{2, 4, 6, 8} Along with increasing the types of analyte that can be analyzed, the ability to interface these MS techniques to
other instrumentation including liquid chromatography (LC) and capillary electrophoresis (CE).¹

For atomic mass spectrometry, the development of ICP-MS was one of the most important advancements in the field. ICP-MS is an atmospheric pressure liquid sampling ionization source that is capable of ionizing atomic species with great efficiency and with little to no sample preparation in comparison with TI. ICP is capable of being interfaced with the same high resolution mass spectrometers as TI including magnetic sector mass analyzers. This allows for the same high precision measurements that were previously achieved with TIMS to be achieved with a higher throughput and less complex ionization source.¹

One aspect of atomic mass spectrometry that is of extreme interest is isotope ratio mass spectrometry (IRMS). IRMS is important for geochronological dating, geolocation, and nuclear nonproliferation. Originally dominated by TIMS, IRMS has more recently have been conducted using ICP-MS. These instruments are extremely expensive and in the case of TIMS require high skill levels to operate, however, they result in extremely high precision measurements. One drawback of ICP-MS is the amount of consumables required for operation, up to 14 L min⁻¹ of argon gas and over 1 mL min⁻¹ of solution flow rates make not only the instrument expensive to run, but also expensive to operate. While these ion sources dominate the field of atomic mass spectrometry, there has been advancements in the development of microplasma ionization sources.⁷,⁹

Work by Cserfalvi et al. with the development of electrolyte-as-cathode glow discharge (ELCAD) have shown the ability to analyze atomic species originally developed
for optical emission spectrometry (OES), but have the capability to be utilized for MS.\textsuperscript{6} Along with ELCAD the development of the solution cathode glow discharge (SCGD) and the liquid sampling – atmospheric pressure glow discharge (LS-APGD) have shown great promise for the use with OES and MS.\textsuperscript{5, 8} These ionization sources have shown great analytical capabilities in regards to atomic mass spectrometry, specifically the sub part-per-billion detection limits of the LS-APGD.\textsuperscript{10-15} Along with these analytical abilities, another attractive feature of these sources is the low consumables needed to operate. The SCGD not requiring a gas flow and the LS-APGD utilizing low gas flow rates <1 L and low solution flow rates <200 µL min\textsuperscript{-1}.\textsuperscript{5, 8, 10-21} Along with the low consumable usage, these ionization sources can be interfaced with any instrument that utilizes an atmospheric pressure interface (API) which means it can be interfaced with instruments that are much cheaper than a traditional ICP-MS. This along with the ability to manufacture these ionization sources for much less than an ICP provide a great alternative for atomic mass spectrometry.\textsuperscript{11-14, 18}

Along with the LS-APGD’s ability to compete with ICP for the analysis of atomic species, recently, it has shown the ability to analyze small organic polar species. The only change required to go from atomic analysis to molecular analysis is switching from the 2% nitric acid electrolytic carrier solution to a carrier solution of 70:30 MeOH:H\textsubscript{2}O.\textsuperscript{19-22} This change results in spectra where the [M+H]\textsuperscript{+} species is observed for many of these small molecular compounds. This shows that while the LS-APGD was originally designed for atomic analysis, it can also operate similarly to ESI, thus expanding the diversity of sampling that is not seen with many other ion sources. Along with small polar compounds,
recently, work by Williams et al has shown the LS-APGD’s capability to analyze low polar compounds that are not typically analyzed by ESI. This means the LS-APGD has the ability to compete with a wide array of ion sources.\textsuperscript{19, 20, 22}

While the development of additional ionization sources has been crucial to the analysis of diverse samples, advancements in mass analyzers have allowed for an increase in resolution and sensitivity in different fields of mass spectrometry. One of the benefits of increased resolution is the ability to separate more isobars with increasing resolution. One problem with ICP-MS is the formation of oxides which can cause isobars which must be removed via reaction cells due to the mass analyzers being utilized by ICP, while providing great precision, do not provide sufficient resolution to separate many isobars.\textsuperscript{7, 12} The development of Fourier Transform mass spectrometry (FTMS) has shown the ability to obtain ultra-high resolution spectra, initially with a Fourier Transform – ion cyclotron resonance (FT-ICR) and more recently orbitrap mass spectrometers.\textsuperscript{23, 24} Orbitrap mass spectrometers of high interest due to these instruments being of benchtop size and low consumable cost whereas FT-ICR instruments are large and require a high consumable cost due to the need to cool the instrument with liquid helium.

\begin{figure}
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\includegraphics[width=\textwidth]{figure1.png}
\caption{Schematic of the LS-APGD/Orbitrap pairing.}
\end{figure}
The LS-APGD has been previously paired with Orbitrap mass spectrometers for IRMS and shown tremendous abilities in high precision measurements across many different elements. The LS-APGD/Orbitrap pairing is shown in Fig 1.1.\textsuperscript{11-14} Recently, this pairing has shown the ability to meet the International Atomic Energy Agency’s (IAEA) international target values (ITV’s) for measurement precision for uranium isotope ratios.\textsuperscript{11-14} While this is impressive, this pairing to this point has only been able to observe the natural uranium isotopes $^{235}$U and $^{238}$U. The inability to observe $^{234}$U in a sample of natural uranium with this pairing is due to the way the in-built data acquisition system performs the FT and averages mass spectra. It is thought that if this data system could be bypassed that this $^{234}$U isotope could be observed.\textsuperscript{11-14} The LS-APGD has always been thought to operate between atmospheric pressure chemical ionization (APCI) and ESI when operating in molecular analysis mode, but with the observation of $[\text{M+H}]^+$ species of low polarity molecules has shown that the ionization mechanism could be more similar to that of APCI or atmospheric pressure photoionization (APPI).\textsuperscript{19} Knowing how to obtain the most powerful data from the orbitrap mass spectrometer and having a better understanding of the ionization mechanism of the LS-APGD could lead to a better understanding of the total capabilities of this pairing.
References


7. Fassel, V. A.; Kniseley, R. N., INDUCTIVELY COUPLED PLASMAS. *Analytical Chemistry* 1974, 46 (13), 1155-&.


CHAPTER TWO

IMPROVED URANIUM ISOTOPE RATIO ANALYSIS IN LIQUID SAMPLING – ATMOSPHERIC PRESSURE GLOW DISCHARGE/ORBITRAP FTMS COUPLING THROUGH THE USE OF AN EXTERNAL DATA ACQUISITION SYSTEM
(Reprinted with permission from the Journal of the American Society of Mass Spectrometry)

Thermal ionization mass spectrometry (TIMS) and inductively coupled plasma-mass spectrometry (ICP-MS), particularly on multicollector, sector-field platforms, have long been the gold standards for isotope ratio (IR) analysis due to their abilities to obtain high precision measurements.\textsuperscript{1−4} Unfortunately, whereas these instruments provide high precision measurements, each method is not without its drawbacks. TIMS instruments are large, complex, and expensive while having low throughput as a result of often-tedious sample preparation processes.\textsuperscript{1,5,6} Extensive chemical separations are required in most cases to minimize chemical matrix effects in the form of ionization suppression and complex-ion formation as well as to alleviate potential isobaric interferences. ICP-MS instruments require large capital input, both in up-front costs and consumables, requiring argon flow rates of up to 14 L min\textsuperscript{−1}. While the sample preparation for ICP-MS is not as extensive as for TIMS, chemical separations are often necessary to alleviate isobaric interferences, even in the case of high resolution (m/ Δm ≈ 10000) sector-field instruments.\textsuperscript{7−9} An additional drawback in the case of ICP-MS is due to the relatively large volumes of sample that are required for analysis due to high sample introduction rates (>0.1 mL min\textsuperscript{−1}) and extended analysis times.\textsuperscript{1,10} In recent years, the application of microplasma sources for spectrochemical analysis has garnered general attention in hopes of reducing operational overhead, sample and waste volumes, and the potential for instrument
transportability. The liquid sampling–atmospheric pressure glow discharge (LS-APGD) microplasma source has been developed by Marcus and co-workers at Clemson University, with mass spectrometric advances begun in collaboration with Koppenaal et al. at the Pacific Northwest National Laboratory. The LS-APGD is a low cost, low power (<50 W), and low consumption ionization source with gas flow rates of <1 L min\(^{-1}\) and solution flow rates of <50 μL min\(^{-1}\), while operating with total consumption of analyte-containing solutions. In addition to its use in atomic (elemental) mass spectrometry, the LS-APGD has been demonstrated to serve as a combined atomic and molecular (CAM) ionization source, effectively ionizing small polar molecules, polyaromatic hydrocarbons, and proteins using the same hardware; indeed doing so simultaneously. Of direct relevance to the effort here, prior implementations have demonstrated the capabilities of the LS-APGD coupled to Orbitrap Fourier transform mass spectrometers (FTMS) for uranium isotope ratio (IR) analysis, with UO\(_2\) being the measured species (illustrated in Appendix Figure 1). In each of these works, the system was found to meet the International Atomic Energy Agency’s (IAEA) International Target Values (ITV) for measurement uncertainty for various enrichment levels of uranium. However, only the \(^{235}\text{UO}_2/^{238}\text{UO}_2\) IR has been measured to date for natural abundance uranium \(^{235}\text{U}/^{238}\text{U}\approx 0.0073\) using the LS-APGD/Orbitrap combination. As previously described, in addition to the detrimental space charge effects which are especially pronounced for measurements across a high spectral dynamic range (3 orders of magnitude), the standard signal processing method employed in the commercial Q Exactive Focus Orbitrap instrument may limit the accuracy of the \(^{235}\text{UO}_2/^{238}\text{UO}_2\) IR in natural and \(^{235}\text{U}\)-depleted
Additionally, the system-imposed restrictions in the dynamic range prevent the simultaneous measurement of the lower (natural) abundance $^{234}\text{U}$ and $^{235}\text{U}$ isotopes. The orbitrap mass analyzer is an electrostatic ion trap that operates under ultrahigh ($10^{-10}$ mbar pressure) vacuum to measure high-frequency oscillations of trapped ions. The oscillating ions in the orbitrap induce an AC current through a pair of trapping (detection) electrodes. The signals for each ion species of different m/z occur at unique frequencies, with all ions within the trap being detected simultaneously in a combined broadband signal, an important aspect in any IR measurement approach. The thus-generated time-domain waveform, known as a transient, can be processed with the Fourier transform (FT) to obtain frequency data which can then be converted to m/z. Fourier transforms can be performed using multiple modes, including magnitude mode (mFT), absorption mode (aFT), and the enhanced mode (eFT) which is a combination of both aFT and mFT. The outcomes of the aFT and eFT approaches are graphically presented in Appendix Figure 2, Supporting Information. By definition, mass spectra represented in the mFT and eFT modes, prior to noise thresholding processes, are supposed to have all positive values resulting in a mean noise level above zero (full profile notation). On the contrary, the aFT mode (full-profile) mass spectra, prior to noise thresholding, have both positive and negative values that result in the mean noise level being centered around zero (Appendix Figure 2). Therefore, if several mass spectra are summed or averaged (coadded), this difference in the noise characteristics will render data processed using aFT providing higher sensitivity and dynamic range, which is a more similar result to coaddition of transients. In another point of comparison, both the eFT and aFT modes provide
about twice the spectral resolution in comparison to mFT from the same transient signals, even for a single measurement.\textsuperscript{40–42} However, the generation of aFT mass spectra is technologically challenging due to the necessity of accurately knowing the initial phase of ions as a function of m/z. Artifacts in the determination and characteristics of the phase function, meaning a pronounced dispersion of ion initial phases along m/z, could result in large mass spectral baseline deviations and peak distortions, especially effecting low abundance species. The commercial Orbitrap FTMS data acquisition (DAQ) system and software operate in the eFT mode, with which only the top portion of the peaks in a mass spectrum is analogous to an aFT representation whereas the baseline of the mass spectra resembles the mFT in that they have only positive values.\textsuperscript{37} Typical eFT spectral data provided to the Q Exactive Orbitrap end-user are referred to as “reduced profile mode mass spectra”. The reduced-profile mass spectra are produced by performing a noise thresholding step which removes data points in mass spectra having intensities of less than a certain threshold, usually at around 4–5 standard deviations of the spectral noise in that mass window, Appendix Figure 2.\textsuperscript{41} To overcome the spectral reduction restrictions and to enhance the dynamic range, Orbitraps can perform averaging (or summation) of the time-domain ion signals (transients) prior to Fourier transform and noise thresholding. In this case, a single scan mass spectrum will include several so-called microscans. By definition, a microscan in the Orbitrap FTMS is the result of measuring the transient generated from a single packet of ions injected into the mass analyzer followed by acquisition of a single transient. That packet of ions is subsequently “quenched” from the cell, followed by introduction of a new packet and a repeat of the process. The single (micro)scan spectral
dynamic range of modern Orbitrap instrumentation is specified to reach the 1:5000 (~0.02% abundance) level with respect to the highest abundance peak in the mass spectrum/spectral window.\textsuperscript{45} This limited spectral range, resulting from the noise thresholding, prevents the detection of \textsuperscript{234}U (~0.0053%) in a single microscan that includes the \textsuperscript{238}UO\textsubscript{2} isotopologue, Appendix Figure 1. Naturally, summation or averaging of many single (micro)scan reduced profile mass spectra will not enable the \textsuperscript{234}UO\textsubscript{2} isotope detection. Therefore, to observe lower abundance ions, such as \textsuperscript{234}UO\textsubscript{2} in the case of uranium IR analysis, there are different acquisition/processing options. For example, coadding a large number of transients (increased number of microscans) and then performing mFT, eFT, or aFT or coadding aFT mass spectra obtained from individual (or multiple, averaged) microscan transients may be potentially more efficient than averaging of individual mass spectra. However, the commercial instrument software limitation on the maximum number of microscans (up to 10 for a Q Exactive Focus Orbitrap) prohibits detection of the extremely low abundance species of interest, the \textsuperscript{234}UO\textsubscript{2} isotope. Furthermore, the true aFT mass spectra are not provided by the contemporary Orbitrap DAQ system and software. Recently, Tsybin and co-workers have demonstrated improvements on the built-in Orbitrap DAQ system, with a new generation high-performance DAQ system, the FTMS Booster, expanding the analytical capabilities of FTMS generally, and Orbitrap FTMS in particular.\textsuperscript{46,47} This is achieved by obtaining accurately phased time-domain ion signals (transients) from the Orbitrap FTMS via advanced hardware and digital signal processing, and performing aFT processing to obtain mass spectra inclusive of low abundance ion signals, so-called full-profile aFT mass
spectra (Appendix Figure 2). Owing to the high-performance architecture of the external DAQ system, it also permits the extended monitoring of ion transients during the entirety of the time ions are trapped in the mass analyzer, providing substantial improvements in the obtained mass resolution. Presented in this work is the LSAPGD/Orbitrap combination (specifically a Q Exactive Focus platform) outfit with the advanced DAQ system, FTMS Booster X2, yielding much-improved performance toward uranium IR analysis employing the microplasma as the ionization source. The enhanced spectral resolution and increased dynamic range also have substantial implications in the Orbitrap FTMS use for high sensitivity, multielement analysis. Ultimately, it is hoped that this combination will yield an analytical platform which delivers high quality IR and elemental analysis while addressing many of the practical challenges presented in TIMS and ICP-MS analysis performed on multicollector, sector-field instruments.

Figure 1.1: Diagrammatic representation of the components of the LS-APGD/Orbitrap coupling along with the advanced DAQ system. The discharge conditions employed throughout these studies were: Discharge current = 30 mA, liquid flow = 30 μL min⁻¹, He sheath gas flow = 500 mL min⁻¹, electrode gap = 0.5 mm, and sampling distance = 1.0 mm.
EXPERIMENTAL

Liquid Sampling-Atmospheric Pressure Glow Discharge. The experimental apparatus employed in the present effort was based on a standard Q Exactive Focus Orbitrap (Thermo Scientific, San Jose, CA), Figure 2.1. The coupling of the LS-APGD microplasma to Orbitrap instruments has been described in detail previously.\textsuperscript{16,23,28} The source consists of a solution electrode (cathode) and a stainless steel counter electrode (anode) between which the discharge is generated. The solution electrode is made of a stainless steel outer capillary (316 SS, 0.8 mm i.d., 1.6 mm o.d., McMaster-Carr, Elmhurst, IL) through which a helium (99.99\%) sheath gas is delivered, and a fused silica inner capillary (i.d. 250 μm, o.d. 360 μm, Molex, Lisle, IL) through which an electrolytic solution carrying the analyte is introduced. The discharge current (30 mA), gas flow rate (0.5 mL min\textsuperscript{-1}) and liquid flow (30 μL min\textsuperscript{-1} of constant analyte solution) are delivered through an umbilical cord assembly and controlled via a custom-built control box (GAA Custom Electronics; Kennewick, WA).\textsuperscript{19,20} The discharge conditions were held constant throughout these studies. The LS-APGD is mounted to the Orbitrap FTMS by removing the equipped electrospray source and attaching the LS-APGD using the standard lever pins. The multielement solution used for resolution tests consisted of 1 μg mL\textsuperscript{-1} (each) of Rb, Tl (High Purity Standards; Charleston, SC) and Ag (Sigma-Aldrich; St. Louis, MO). Uranium test solutions were prepared from a NIST-traceable (SRM 3164) 9.93 μg mL\textsuperscript{-1} natural-abundance $^{238}$U concentration standard (CRM 238U10, High Purity Standards; Charleston, SC), prepared from a uranium isotopic standard (CRM 129-A, New Brunswick
Laboratories, Argonne, IL) of a known $^{234}$U abundance of 0.0052962% and $^{235}$U abundance of 0.72087% which was certified by TIMS. Analytical response curves were generated using serial dilutions in 2% nitric acid of the same standard to obtain total-uranium concentrations of 250 ng mL$^{-1}$, 500 ng mL$^{-1}$, 1 μg mL$^{-1}$, and 10 μg mL$^{-1}$. Orbitrap/Advanced Data Acquisition System Interface. The Q Exactive Focus Orbitrap was outfitted with the advanced data acquisition system FTMS Booster X2 (Spectroswiss, Lausanne, Switzerland) as depicted in Figure 2.1. The mass spectrometer was controlled using Thermo Xcalibur Instrument Setup software. The spectral acquisition method began with an all-ion fragmentation (AIF) scan which allows the higher energy collisional dissociation (HCD) cell to be used to remove concomitant ion signals from the mass spectrum. The AIF settings were as follows: in-source collision induced dissociation (isCID) = 90 eV, scan range = 255–305 m/z, HCD = 120 eV, and an automatic gain control (AGC) target = $2 \times 10^4$ or $1 \times 10^6$. The chosen mass range includes all of the UO$_2$ isotopologues. The AGC target is the desired number of charges that are to be collected in the C-trap and injected into the Orbitrap. The two employed AGC values, lower ($2 \times 10^4$) and higher ($1 \times 10^6$), were used to reveal the influence of potential space charge effects. The analytically useful AIF scan was followed by a “dummy” scan, a scan covering a mass range where no plasma-generated ions are anticipated to be present, which forces the instrument to accumulate ions in the C-trap for an extended period of time (to reach the AGC target value) and is run in the full MS mode. The full MS settings for the dummy scan were as follows: mass resolution = 17.5k, scan range = 1008.8–1009.2 m/z, an AGC target = $2 \times 10^4$, and maximum injection time ($IT_{\text{max}}$) = 1000 ms. The $IT_{\text{max}}$ can be varied.
by the user based on the desired transient length in the analytical scan, with the dummy scan having a narrow mass range so as to not reach the AGC target, allowing for a long actual $T_{\text{scan}} = IT_{\text{max}}$ of the dummy scan. This scan sequence was developed in order to collect longer transients than are typically allowed in the acquisition methods employing the standard data acquisition system, similar to a previous implementation of this approach on a Q Exactive GC Orbitrap.\textsuperscript{47} Because a new ion packet is not subsequently injected until either the AGC target or the $IT_{\text{max}}$ value is reached, the initial ion packet continues to oscillate within the orbitrap mass analyzer, generating time domain data that are then collected by the FTMS Booster X2. The time-domain data collected using the FTMS Booster X2 were then processed, including conditional transient averaging, user-controlled transient truncation, and generation of the aFT mass spectra, with the Peak-by-Peak software (Spectroswiss). Conditional coadding of transients employed the following conditions: a user-defined signal-to-noise-ratio (SNR) range and a mass error range of a target peak - the base peak, $^{238}\text{UO}_2$. The parameters and characteristics of the aFT mass spectra were further exported and analyzed using Python (with Peak-by-Peak package, Spectroswiss) or Excel. These included, for example, the extracted peak areas used in the IR calculations. In this work, precision is defined as the percent relative standard deviation (%RSD) of determined values, and error is defined as the percentage deviation from the accepted IR values, as done in previous efforts for uranium isotope ratio analysis.\textsuperscript{23,28}
Results and Discussion

Improvements in Mass Resolution

The advanced DAQ system (FTMS Booster X2) allows the collection of ion signal transients of an arbitrary length (not limited to a 2-fold transient length increment step) and for extended periods of time, resulting in resolution beyond what is implemented using the standard acquisition/processing system. As a demonstration of the enhanced resolution, a mass spectrum from the multielement solution is presented in Figure 2.2 (data obtained with the standard built-in DAQ are presented in black font, with data obtained with the advanced DAQ in red font).

Figure 2.2 a) Acquired signal transients and b) corresponding spectral characteristics for the standard workflow (black font) and advanced DAQ (red font) systems for the same ion packets. c) Expanded mass spectra for the $^{205}$Tl isotope for the two acquisition systems. The test solution was 1 μg mL$^{-1}$ each of rubidium, silver, and thallium.
advanced DAQ appearing in red font). It is essential to point out that the advanced DAQ system operates completely in parallel with the standard acquisition system, and thus, the mass spectra are derived from the same ion populations. Figure 2.2a depicts a transient acquired by the external DAQ (FTMS Booster X2), with the acquisition time for standard DAQ system indicated with the first vertical dashed line. The standard system is programmed to provide \( \sim 70k \text{ m}/\Delta \text{m} \) maximum resolving power at 200 m/z that is achieved with a transient length of 256 ms, whereas the advanced DAQ continues monitoring what are clearly useful signals for a total of 3.5 s (\( \sim 14X \) longer). The product mass spectra from the two transient periods (the standard 256 ms period, eFT processing, and the extended 3500 ms period, aFT processing) are presented in Figure 2.2b. The spectral features and relative analyte intensities do not differ substantially according to the processing method (aFT versus eFT), but as indicated, the derived resolution is \( >10 \times \) higher for the advanced DAQ for each isotopic pair (Rb, Ag, and Tl). A zoomed-in portion of the mass spectrum which shows the \(^{205}\text{Tl}\) isotopic signal, Figure 2.2c, clearly demonstrates the difference in the peak widths between the transient lengths supported by the standard (at the maximum possible resolution) and the advanced DAQ systems. This large increase in resolution expands the qualitative abilities of this Orbitrap for atomic (and molecular) analysis, as the resolution of this magnitude allows for the separation of nearly all potential elemental isobars. The value of attaining resolution in excess of the admittedly high resolution of the base Q Exactive Focus has been demonstrated on a high-field (D20 mass analyzer, 2 s transient) Fusion Lumos Orbitrap 1 M previously in the separation of the geologically important \(^{87}\text{Sr}:^{87}\text{Rb}\) pair (a mass difference of only 0.3 mDa) with the achieved resolution
of 1.7 M.\textsuperscript{19} The implementation of the advanced DAQ provides near equivalent resolution of a high field orbitrap (with a 1 M option enabled, 2 s transient) on the lower-field (D30 mass analyzer, 3.5 s transient), lower cost, Q Exactive platform.\textsuperscript{47} Resolution of this level practically eliminates any need for chemical separations required to remove potential isobars that would otherwise be problematic on sector-field instruments. A classic example in this case would be the common interference of various lead-containing polyatomic species which are very problematic in uranium and plutonium isotopic analyses.\textsuperscript{8} We shall note that the demonstrated performance remains a function of the orbitrap manufacturing quality and instrument parameters tuning.

**Improvements in Dynamic Range.**

![Graph](image)

*Figure 2.3. a) Transients for the standard workflow (black font) and advanced DAQ (red font) acquisition systems along with the product mass spectrum for the major 235UO₂ species. b) Spectral overlays of the respective target UO₂ species for the standard workflow (black) and advanced DAQ (red) systems, where*
The blue line represents the expected peak height based on natural abundance and using the $^{235}\text{UO}_2$ based peak. A test solution of 1 µg mL$^{-1}$ of natural abundance uranium in 2% HNO$_3$ was used.

The dynamic range of the standard Orbitrap system is limited by the automatic data reduction step, restricting the ability to observe and accurately quantify low abundance (e.g., <0.5% relative abundance) ions. Because the data reduction step typically removes signals below ~0.02% relative abundance in the spectral window (a function of the instrument and SNR of a base peak under specific experimental parameters), the full isotopic analysis of natural-abundance uranium is not possible as the $^{234}\text{UO}_2$ isotope has an abundance of 0.0055%, Appendix Figure 1. Beyond this process, regardless of this data reduction step, the standard workflow employing the eFT would result in the $^{234}\text{UO}_2$ peak not being present. Simply put, the maximum allowed setting of 10 microscan averaging on the Q Exactive Focus does not provide the SNR necessary to draw the $^{234}\text{UO}_2$ out of the background noise.$^{23,24}$ To illustrate this concept, Figure 3 presents the spectral responses for the target UO$_2$ isotopic species, along with the minor $^{16}\text{O}^{17}\text{O}$ form of the $^{238}\text{U}$ dioxide, based on the standard and advanced DAQ systems. The $^{238}\text{U}^{16}\text{O}^{17}\text{O}$ provides a useful analyte signature at an intensity level intermediate between $^{234}\text{UO}_2$ and $^{235}\text{UO}_2$. Figure 2.3a shows representative mass spectra for both the standard (eFT, black) and the advanced (aFT, red) data acquisition and processing approaches for the 1 µg mL$^{-1}$ natural abundance uranium solution. The depicted transient shows a comparison of the transient lengths acquired with the standard DAQ system (black) and the advanced DAQ system (red). To de-emphasize the demonstrated enhancement in resolution versus the differences in dynamic range, a transient of only ~2× longer than the standard system (500 ms) was
acquired with the advanced DAQ. The broadband aFT mass spectrum depicts a very small peak representing the $^{235}$UO$_2$ species within the black square highlighted on this scale. The spectral profiles for the $^{238}$UO$_2$ species are overlaid (to the right) at 100% relative abundance, illustrating the $\sim 2\times$ better mass resolution realized with the advanced DAQ, reflective of the longer transient length.

The extracted isotopic signals for the three minor UO$_2$ species are presented in Figure 2.3b, where the dashed blue line in each spectrum represents the expected peak intensity based on the natural uranium isotopic abundances versus the $^{238}$UO$_2$ response. The line for $^{238}$U$^{17}$O$^{16}$O species is a theoretical value due to this species possibly being composed of solution or plasma-originating oxygen species, and therefore, the oxygen IR composition is not certified. That said, the comparison of signal recoveries is interesting as it provides insight into the IR characteristics (at three different orders of magnitude) relative to the effects of the FT parameters/modalities (eFT vs aFT and spectral vs transient coadding). As in the case of the most abundant $^{238}$UO$_2$, the intermediate $^{235}$UO$_2$ and $^{238}$U$^{17}$O$^{16}$O signals, which are present at relative abundances of $\sim 0.6$ and $\sim 0.06$, display a 2-fold increase in resolution for the advanced DAQ method. In both instances, less than unity recovery versus the expected responses is seen, which is slightly more pronounced for the standard system response of the lower abundance $^{238}$U$^{17}$O$^{16}$O species. This phenomenon is attributed in part to the data reduction step and in-part to space charge effects (depression of lower abundance species by the higher abundance ones),$^{49}$ though the extent does not appear as severe as in previous reports.$^{23,24}$ Improvements regarding recoveries of low-abundance species may be due to more appropriate selection of the
experimental parameters and improved instrument tuning. Most notable in the presented
mass spectra is the observation of the $^{234}\text{UO}_2$ signal in the case of the advanced DAQ data,
where a “flat line” response (black line) is obtained from the standard system. In fact, two
peaks are clearly resolved in the case of the advanced DAQ, with the available frequency
measurement accuracy allowing certain identification of the target isotope. The ability to
determine $^{234}\text{U}$, $^{235}\text{U}$, and $^{238}\text{U}$ (an isotopic span of $\sim 5$ orders of magnitude) in a single data
acquisition is a step-function improvement in the realm of isotopic analysis in the LS-
APGD/Orbitrap coupling and makes the approach even more competitive with existing
TIMS and ICP-MS methods for uranium isotopic analyses.$^{27}$

On a fundamental level, because of the low isotopic abundance of $^{234}\text{UO}_2$, the
number of ions being analyzed in a single injection/scan must be considered. As the
limiting AGC target was set to $2 \times 10^4$ to minimize ion–ion interactions, and assuming that
through the limited quadrupole scan range only U-related (as dioxide) species are entering
the C-trap, statistically only a single $^{234}\text{UO}_2$ ion is present in a given scan. The ability to
repeatably measure (by virtue of the observed signal integrity) a single ion suggests that
the dynamic range is limited not at the low end, but only at the high end which is
determined by the number of charges injected into the Orbitrap (set by the AGC). By
coadding transients, which improves ion counting statistics, the SNR improves and allows
for the identification and better quantification of the $^{234}\text{UO}_2$ signal.$^{41,47}$ The improvement
in counting statistics is detailed in Figure 4 where the $^{234}\text{UO}_2$ SNR is shown to increase as
a function of number of transients coadded, with a minimum of $\sim 1000$ transients being
required for its distinction from the background with an AGC target of $1 \times 10^6$. This implies
that averaging any number of microscans less than 1000 would not result in detectable ion signals, as is the case with the standard Q Exactive Orbitrap workflows. Also plotted are the SNR characteristics of the other monitored species, reflecting two different sets of dependencies. In the beginning of the experiment, all species are seen to follow the expected SNR improvement that is proportional to the square root of the number of scans \((n^{1/2})\). However, starting from the middle of the experiment the SNR trends do not follow this relationship, being more proportional to the number of transients \((n)\). This effect can be attributed to changes in the mass spectral composition of consecutive scans (intensity decrease of chemical background, HCD ion fragments, etc.) developed during the long-term acquisition, influencing ion accumulation conditions. Specifically, more ions for the uranium oxide species were accumulating with the background intensity decrease at the same AGC value, yielding the UO\(_2\) species SNR dependence change. The extracted mass spectra shown in Figure 2.4 demonstrate the products of increased numbers of coadds and the resulting improvement in the SNR for the \(^{234}\text{UO}_2\) and \(^{238}\text{U}^{16}\text{O}^{17}\text{O}\) species, where the horizontal dashed blue lines represent a level of 5\(\sigma\) above the background spectral noise. Both of the target signals are easily discernible from the background, with an increased number of transients coadded, clearly exhibiting improved SNR characteristics.
Figure 2.4: Signal-to-noise ratios (SNR) for the $^{234}\text{UO}_2$, $^{235}\text{UO}_2$, $^{238}\text{U}^{17}\text{O}^{15}\text{O}$, and $^{238}\text{UO}_2$ responses as a function of the number of transients coadded. Expanded mass spectra inclusive of the $^{234}\text{UO}_2$ and $^{238}\text{U}^{17}\text{O}^{15}\text{O}$ species for representative numbers of coadds. Dashed blue line represents $5\sigma$ of the spectral noise. Test solution of 1 µg mL$^{-1}$ of natural abundance uranium in 2% HNO$_3$. Acquisition transient length = 0.5 s and AGC = 1x10$^6$.

The ability to observe $^{234}\text{UO}_2$ in the broadband mass spectrum (Figure 3) also reveals an interferent peak at the same nominal mass. The identity of this species was not explicitly determined but is most likely a cluster ion involving H$_2$O and HNO$_3$. This interferent peak at m/z = 266.036 would cause positively biased IR values (due to overlap or interference) for any mass analyzer having lesser mass resolution capabilities (i.e., sector-field instruments). Simply put, with the added sensitivity to observe this low abundance isotope comes the need for greater mass resolution and accuracy. In the case of FTMS analysis, Liebisch and coworkers have shown that spectral deconvolution methods can help minimize quantitative errors in the case of isobaric overlaps for complex lipid systems, so long as the nature of the species is known.$^{50}$ Based on the relative intensity of the two signals, the reported isotope ratio involving $^{234}\text{U}$ would be positively biased by a factor of three on virtually any other non-FT MS instrument. The ability of the advanced
DAQ to collect longer transients than the standard Q Exactive Orbitrap hardware and software alleviates the isobaric interference that appears with $^{234}$UO$_2$. As an illustration of the role of resolution on the recovery of the target ion, Appendix Figure 3 provides the spectral profiles across the pair as a function of the transient acquisition lengths (aFT processing, half-window Kaiser function apodization). As can be seen, the isobars become baseline-resolved in the case of the 0.5 s transient (~2× the length of the standard workflow), representing a resolution of 109k m/Δm. While longer transients provide a more defined separation of the two peaks, the intensity of the peaks is slightly diminished due to the preferential decay of these low abundance ion packets in the course of longer transients; however, the actual reduction in SNR across the 0.5 to 1.5 s transients is only ~2× (18 to 8).

**Evaluation of Acquisition Parameters Affecting Uranium IR Performance.**

![Graphs showing the relationship between various parameters and peak intensity and area.](image-url)
Figure 2.5: Isotope ratios of $^{235}$UO$_2$/^{238}$UO$_2$, $^{238}$U$^{17}$O$^{16}$/^{238}$UO$_2$, and $^{234}$UO$_2$/^{238}$UO$_2$ using both peak area (red circles) and peak intensity (grey triangles). a) IR values as a function of transient length (8160 coadds). b) IR values as a function of the number of transients coadded (0.5 s transients). AGC = 1x10$^6$.

With the ability to simultaneously detect the target uranium isotopes within a single mass spectrum, the data acquisition parameters were further evaluated relative to their effects on U IR characteristics. In order to achieve the best overall performance with the advanced DAQ, the scan parameters of the transient length (detection period) and the number of transients coadded are key parameters to optimize. It would be expected that ioncounting statistics should improve by coaddition of larger numbers of transients and by increasing transient lengths (up to the point where ion signals have diminished into the noise). When looking at IR analysis, accuracy and precision are the primary figures of merit. The sources of imprecision and error, throughout the measurement process, are of fundamental concern. In general, poor quantitative accuracy can be remedied using a bracketed analysis of isotopic standards to correct for what is colloquially termed “mass bias” in the case of sector-field spectrometry. Despite this, it is pertinent to look at the effects that these parameters have on the “raw” IR accuracy. The effects of transient lengths on the accuracy of the IRs, using peak heights and peak areas are shown in upper portion of Figure 2.5 using a nominal value of 8140 transient coadditions; a number yielding high-fidelity signals for the very minor isotopes as presented in Figure 2.4. As would be expected, for each of the ion pairs, the ratios based on the peak intensities tend to decrease steadily with increasing transient times as the lower abundance ions are lost from the stored ion packets at a faster rate than the more abundant ones ($^{238}$UO$_2$). In fact, this phenomenon was a key aspect in recent studies described by Hofmann and co-workers in
the use of Orbitrap MS to characterize the isotopic composition ($^{13}$C/$^{12}$C) of organic compounds. Figure 4 of that work illustrates the basic concept wherein ions of lower abundance are susceptible to loss of coherence due to space charge effects inflicted by the higher abundance species, resulting in preferential losses in recovery. Clearly, this phenomenon would be expected be more pronounced in the case of $^{235}$UO$_2$/238UO$_2$ (here) versus $^{13}$C/$^{12}$C (in that case) based on the disparity in the relative abundances in the two isotopic pairs. Using peak areas in the case of the most abundant $^{235}$UO$_2$/238UO$_2$ ratio, the raw accuracy is slightly improved over previous works on this instrument using the standard acquisition system where the best $^{235}$UO$_2$/238UO$_2$ values were generally biased to lower values of 0.0066–0.0068. The variation in the determined values is minimal across the longer transients but then deviates in the negative direction at more extended transient lengths, most likely due to the decay in the ion packet density in the orbitrap. Perhaps the lower field orbitrap cell employed here, accompanied by its tuning state, is unable to hold the ions in a coherent packet for as long as a high field orbitrap. As such, the quality of the signal (actually the SNR) diminishes at transient times of greater than 1 s, though still much improved over the best cases of previous efforts using the standard DAQ system on this instrument. The transient length has a much greater effect on the middle-valued peak area isotope ratio, $^{238}$U$^{17}$O$^{16}$O/238UO$_2$, than it has on the $^{235}$UO$_2$/238UO$_2$. Here, the area-based values are sporadic up to the 0.5 s transient time, with a distinct crossover in improved performance seen after $\sim$0.75 s transient lengths. The accuracy of the $^{234}$UO$_2$/238UO$_2$ values is far more sensitive to changes in the transient observation times. At the shorter transient lengths, there is insufficient resolution to isolate
the $^{234}$UO$_2$ from the interferent peak causing the skewing of the IR, more so in the case of peak area than peak height. In this case, the ability to resolve the target analyte and the isobaric interferent at transient lengths of $>$0.5 s is very clear, for both the peak height and peak area values. As in the case of the other two ratio pairs, the use of peak areas yields appreciably better IR accuracy at the longer ($>$0.75 s) transient times. As a point of comparison, the trends in IR accuracy as a function of transient times are consistent for the $2 \times 10^4$ AGC setting (Appendix Figure 4).

As suggested above, the steady decline in peak height-based IR values as a function of transient time can be attributed to the preferential loss in ion number density (peak height) of the lower abundance isotopes. Interestingly, at the same time, the SNRs of all of the target isotope measurements actually increase as a function of detection period as shown in Appendix Figure 5 for each of the monitored species at AGC settings of $1 \times 10^6$ and $2 \times 10^4$. Overall, the spectral noise, which is a reflection of the signal variability across the entirety of the mass spectrum, decreases at a much faster rate than the individual analyte ion signals as observation time evolves. Two distinct differences are seen between the two AGC settings. In the first case, the higher trapping capacity maintains a consistent improvement in SNR with transient times, albeit with lower absolute values than the lower capacity setting. On the other hand, the lower capacity also shows distinct negative curvature at longer transient lengths, particularly for the lowest abundance ions. In this case, there are simply insufficient numbers of ions to maintain high SNR characteristics. It is important to appreciate the fact that there are other, low abundance ion species (e.g., HNO$_3$/H$_2$O clusters) in the orbitrap which contribute to the noise experienced by the
analytes. Being of low abundance, their contributions to the noise decrease quickly as a function of transient time, thus the improvement in SNR. The contributions of these minor species are more pronounced for the case where the higher trap capacity is employed.

Returning to the responses in Figure 2.5a, the SNR characteristics explain the trade-off between the peak height versus peak area-based isotope ratio accuracy. At short transient times, where there are strong analyte signals, that metric yields the more accurate IRs. As raw intensities decrease at longer detection times, while at the same time SNR values improve, the peak areas yield more uniform and accurate results. This concept is fairly straightforward in the case of the $^{235}\text{UO}_2/^{238}\text{UO}_2$ as both isotopes have relatively strong responses (while still differing by more than 3 orders of magnitude). The peak intensity IR calculations for both the $^{235}\text{UO}_2/^{238}\text{UO}_2$ and $^{238}\text{U}^{17}\text{O}^{16}/^{238}\text{UO}_2$ follow similar trends. For these IRs, at shorter transient lengths the IR for peak area and peak intensity calculations are similar; however, at the extended transient lengths, the peak intensity IRs decrease far more so than peak area IR values. These IRs are biased-low at the extended transients due to the faster signal decay of the minor isotope. In the case of the ratios based on $^{234}\text{UO}_2$, and $^{238}\text{U}^{17}\text{O}^{16}$ to a lesser extent, IR values based on area start to be biased fairly low and with much scatter, their inherently low raw ion signals require enough transient time length ($\sim0.75$ s) to yield sufficiently high SNR ratios, allowing for higher accuracy via the area measurements. Here again, the time necessary to affect peak resolution of the $^{234}\text{UO}_2$ also comes into consideration as at the shortest times the analyte signal response is not discernible.
As demonstrated above (Figure 2.4), the coaddition of transients can have a definitive effect on the SNR for a given ion species, as such it would be expected to affect IR accuracy and precision. Figure 2.5b presents the determined isotope ratios as a function of the number of transients coadded for the $^{235}\text{UO}_2^{238}\text{UO}_2$, $^{238}\text{U}^{17}\text{O}^{16}\text{O}^{238}\text{UO}_2$, and $^{234}\text{UO}_2^{238}\text{UO}_2$ pairs. A transient acquisition time of 0.5 s was chosen as it yields sufficient resolution of the $^{234}\text{UO}_2$ from its interferent, as well as suitable IR accuracy for $^{238}\text{U}^{17}\text{O}^{16}\text{O}^{238}\text{UO}_2$, at modest analysis times. Likewise, this time is beyond the crossover point where peak area-based accuracy becomes appreciably better than peak height for $^{235}\text{UO}_2^{238}\text{UO}_2$. As might be expected, the $^{235}\text{UO}_2^{238}\text{UO}_2$ IR is not significantly affected by a larger number of transients as the IR values across the entire span up to 10000 transients vary by <1% relative, overall. While this shows that the number of transients coadded does not have a significant impact on the IR, it does point to the long-term stability of the LS-APGD as the amount of time required to collect the 10000 transients (of lengths of 3 s for this comprehensive set of experiments) is ~4 h. Overall, the determined IRs are still much closer to the assay value of $^{235}\text{UO}_2^{238}\text{UO}_2 = 0.0072$ than previously shown on this Q Exactive Focus Orbitrap (0.0066–0.0068) using the standard workflow.\textsuperscript{24,25,27,28} This lack of improvement in $^{235}\text{UO}_2^{238}\text{UO}_2$ accuracy for an increasing numbers of transients coadded was also observed in previous work on the high-field Lumos Orbitrap 1 M instrument,\textsuperscript{28} which is attributed more to variability of the background ion species (and the coincident noise) than analyte signal variability. Not surprisingly, the accuracy for the lower abundance $^{234}\text{UO}_2^{238}\text{UO}_2$ is more effected by coadding more transients than the $^{235}\text{UO}_2$. The $^{234}\text{UO}_2^{238}\text{UO}_2$ values steadily increase up to ~4000 transients, as the larger
number of transients coadded results in SNR improvements leading to more accurate values. Above \(\sim 4000\) transients, the improved SNR does not affect the accuracy. The response of the \(^{238}\text{U}^{17}\text{O}^{16}\text{O} /^{238}\text{UO}_2\) IR lies between the other two species. As in the \(^{235}\text{UO}_2\) data, the peak area derived values are more accurate across all of the data set, and similar to the \(^{234}\text{UO}_2\) IR response, the values increase with increasing numbers of coadded transients. A plateau in the values is observed between 2500 and 6000 transients, though, a second step in IR values is seen above \(\sim 6000\) transients, where the value approaches the expected value (0.00076). Similar trends are seen using an AGC target of \(2 \times 10^4\) (Appendix Figure 4). When varying the number of transients, the IRs for peak areas and peak intensities follow similar trends for all of the isotopic pairs. This is different from the role of transient length. Here, for the most abundant \(^{235}\text{UO}_2 /^{238}\text{UO}_2\), the IR for the peak intensity is much lower than the peak area IR, however, the trends for both peak area and peak intensity are very similar. The lower IR values for peak intensity can be attributed to the transient length for these calculations being 0.5 s, as shown in Figure 5a, the proportional differences between the peak intensity and peak area IR values are the same. The peak intensity IR data for the lower abundance \(^{238}\text{U}^{17}\text{O}^{16}\text{O} /^{238}\text{UO}_2\) also follows similar trends to the peak area IR, however above \(\sim 1,000\) transients coadded the peak intensity IR is lower than that of the peak area and this difference increases as more transients are coadded. The different response is due to the fact that averaging more transients under higher SNR conditions (0.5 s transients), benefits peak area accuracy. The IR calculations for both peak area and peak intensity for the \(^{234}\text{UO}_2 /^{238}\text{UO}_2\) are virtually overlapped across the span of the number of transients coadded. This is most likely due to the abundance of
the peak being so low that increasing the number of scans coadded improves the signal (peak height) component along with the improved SNR that benefits area-based calculations.

\[
\bullet - T_{\text{acq}} = 0.5 \text{ s}, \quad \blacktriangle - T_{\text{acq}} = 1.4 \text{ s}
\]

AGC = $2 \times 10^6$

\(a\)

AGC = $2 \times 10^4$

\(b\)

\(c\)

\(d\)
Figure 2.6: Isotope ratio precision (%RSD) for $^{235}$UO$_2$/238UO$_2$, $^{238}$U$^{17}$O$^{16}$O/$^{238}$UO$_2$, and $^{234}$UO$_2$/238UO$_2$ as a function of SNR at different transient lengths (0.5 s = red circle and 1.4 s = grey triangle). Results for AGC = 1 $\times$ 10$^6$ using a) peak intensity and b) peak areas and results for AGC = 2$\times$10$^4$ using c) peak intensity and c) peak areas. Vertical dashed lines included to guide eye.

The IAEA ITV guidelines for natural-abundance uranium samples calls for a $^{235}$UO$_2$/238UO$_2$ measurement precision of 0.2%RSD (expanded uncertainty) in order for results to be considered valid.$^{29}$ The precision of the uranium IR measurements are plotted in Figure 6 for each of the isotopic pairs as a function of the SNR values for the most abundant isotope ($^{238}$UO$_2$). The experimental variable here is the number of coadded transients, with the SNR improving with the number of transients. Data for AGC settings of 1 $\times$ 10$^6$ and 2 $\times$ 10$^4$ are presented in Figure 2.6a,b and Figure 2.6c,d, respectively. As with the previous comparisons, both peak height- and peak area derived values are plotted for each AGC setting, with the results of transients of 0.5 and 1.4 s duration included. Across all of the data sets, the %RSD of the measurements decreases as expected with increased SNR. Conditions producing increased SNR suggest that there will be less variability in the ion signals due to the noise having less of an impact on the recovered signals of the analytes. Additionally, the random scan to scan deviation of the number of ions for different analyte peaks becomes negligible with larger accumulated ion statistics. While there were significant differences in the accuracy trends between the peak area- and peak intensity derived ratios, this difference does not appear in the measurement precision as the variability between the measurements should affect both the peak area and peak intensity proportionally.
It is interesting to note that while the IR precision increases with SNR for both of the transient lengths, the longer transient length yields slightly higher SNR for the same number of transients, yet the measurement precision is not as good. This relationship is highlighted graphically for the case of the $^{235}\text{UO}_2/^{238}\text{UO}_2$ precision for each of the experimental sets, where for the same number of coadditions the longer transients show greater SNR (as might be expected) but lower IR precision, Figure 2.6. Based on the curvature of the SNR plots as a function of transient lengths of Appendix Figure 5, this observation is not surprising. This is different from shown previously on an Orbitrap Fusion Lumos 1M, where increasing the transient length (increasing resolution) up to 2 s resulted in an increase in the $^{235}\text{UO}_2/^{238}\text{UO}_2$ measurement precision. The lack of improved precision with transient length (here) is most likely due to the (relative) inability to maintain a stable ion packet for the duration of the transients employed on this low field orbitrap cell and the employed ion optics tuning parameters. That said, it is very interesting to note that the differences in the measurement precision between the two transient lengths become smaller as the ratios decrease, where there is virtually no difference in precision performance for the transients for $^{234}\text{UO}_2/^{238}\text{UO}_2$ measurements.

Ultimately, the key metric in these efforts is the obtained $^{235}\text{UO}_2/^{238}\text{UO}_2$ precision. As noted above, in each case, optimum precision is seen in the case of the shorter (0.5 s) transient lengths, with peak height-based ratios yielding better precision than the peak area-based ratios. In the case of the higher capacity AGC setting (Figure 2.6a) a precision of 0.08% RSD was realized, a promising value toward meeting the IAEA target. This value was achieved at an SNR = 720, which required the coaddition of 2500 transients. When
looking at the lower AGC setting (Figure 2.6c) a value of 0.04% RSD was achieved at an
SNR = 700, which required the coaddition of 4000 transients. The fact that the lower
capacity accumulations yielded better measurement precision may be somewhat
counterintuitive. In fact, as discussed previously with respect to Figure 2.5, the lower trap
capacity comes with the added benefit of not introducing the influence of low abundance
nonanalyte species on the measurement noise. In addition, the lower trap load ($2 \times 10^4$)
reduces the overall space charge effects and peak interference in comparison to the higher
trap load ($1 \times 10^6$). Therefore, the variability of those low abundance species is not as much
of a contributing factor to the measurement imprecision. As expected, the IR precision
decreases slightly as a function of the minor isotope abundance where the
$^{238}U^{17}O^{16}O^{238}UO_2$ precision is slightly lower than that of the $^{235}UO_2^{238}UO_2$. Likewise, the
precision for the $^{234}UO_2^{238}UO_2$ is only an order of magnitude higher (1.5% RSD) than the
$^{235}UO_2^{238}UO_2$, but impressively it is in fact achieved for a ratio whose absolute abundance
is a factor-of-100 lower. In comparison to previous LS-APGD/Orbitrap measurements of
the $^{235}UO_2^{238}UO_2$ pair, the precision realized here is a factor of 2 better than any of the
reported efforts on the low-field Q Exactive Focus platform, and indeed, a factor of 2
better than obtained on the high-field Lumos 1 M instrument. Ultimately, the
$^{235}UO_2^{238}UO_2$ and $^{234}UO_2^{238}UO_2$ precision values compare very well with those obtained
via TIMS and multicollector (MC)-ICP-MS for single determinations for both isotope
pairs. What remains, as in the case of the MC-ICP-MS efforts, are detailed, rigorous
studies of measurement precision and error budgeting for the present system. To be clear,
the IAEA ITVs are based on the expanded uncertainty, which remains to be evaluated,
but the measurement precision bodes very well for meeting these metrics. The addition of the simultaneous $^{234}\text{UO}_2^{238}\text{UO}_2$ values at such high precision improves the potential viability of the approach further. At present, there are no ITVs related to $^{234}\text{UO}_2^{238}\text{UO}_2$, but the performance exhibited with the LS-APGD/Orbitrap coupling may indeed allow for those to be established.

These IR measurements could be improved overall by further narrowing the experimental m/z range and limiting the background species introduced into the orbitrap as shown in previous studies with the LS-APGD for uranium isotope ratio measurements.$^{26,55}$ For example, a quadrupole band-pass of 10 m/z (or less) to the C-trap would be used to limit the amount of nonuranium related species that could contribute to the nonideal ion trapping behavior (space charge effects) and noise in the spectrum.

**Quantification Characteristics of the Advanced Workflow.**

As demonstrated in Figure 2.3, one primary benefit of the advanced DAQ system-based workflow is the extended spectral dynamic range of the instrument afforded by the use of aFT processing and a practically unlimited number of coadded transients (microscans). The Q Exactive Orbitrap, utilizing the standard workflow, is limited in the low end of the concentration range because of the data reduction step and the effects of the eFT on product mass spectra. Regardless of the data system, the upper limit of the concentration range is the propensity toward space charge effects at high ion densities. The simultaneous, proportional detection of $^{234}\text{UO}_2$, $^{235}\text{UO}_2$, and $^{238}\text{UO}_2$ in a single acquisition as exhibited in Figure 3b, suggests a dynamic range of at least 4–5 orders of magnitude, which is necessary for this type of uranium IR analysis. To better quantify the advanced
DAQ-based workflow performance, an analytical response curve was generated based on the responses of the three isotopes for test solutions of total uranium content of 250 ng mL$^{-1}$, 500 ng mL$^{-1}$, 1 μg mL$^{-1}$, and 10 μg mL$^{-1}$. The corresponding isotopic concentrations range from 13.5 pg mL$^{-1}$ to 9.96 μg mL$^{-1}$. Data was acquired using 0.5 s transients and 2000 coadded transients (to reduce the experiment length and maintaining robust data). A log–log response curve for the isotopic concentrations for the four solutions is shown in Figure 2.7, with the error bars representing range of intensity values for triplicate, constant flow measurements. In this case, the total analysis time for each measurement was ~75 min, representing a sample volume of ~2.2 mL of the test solution. Good linearity ($R^2 = 0.985$) is obtained across the >6 orders of magnitude in responses, inclusive of an obvious determinant error for the second-highest concentration solution. It must be reiterated that the enhanced mass resolution realized is a key aspect in the proportional recoveries of the $^{234}$U signals. As previously described by Hoegg et al., the standard Orbitrap system shows a dynamic range of >4 orders of magnitude (non-natural uranium isotopic abundances), with ~6 orders of magnitude across five uranium concentrations realized on the Lumos 1 M platform. Thus, implementation of the advanced DAQ system on this platform (here) provides definitive quantitative performance enhancements. The limits of detection (LOD) for this method can be assessed using the standard method where the LOD is set by the precision of the lowest concentration measurement ($\sigma_{\text{low}}$) and the slope (m) of the linear response curve (LOD = $3 \sigma_{\text{low}}/ m$). Using these conditions, a value of
Figure 2.7: Log-log response curve as a function of the individual isotopic concentrations of uranium, with total elemental concentrations of 250 ng mL$^{-1}$, 500 ng mL$^{-1}$, 1 mg mL$^{-1}$, 10 mg mL$^{-1}$. The curve is segmented by the black bars into sections for each isotope’s respective responses. An AGC target of $1 \times 10^6$ was used so as not to overfill the trap at higher concentrations. Error bars not seen are within the area of the data symbols.

Conclusions

By coupling the LS-APGD/Orbitrap with an FTMS Booster X2 and allied data processing to produce aFT mass spectra, low abundance ion signals are observed concomitantly, therefore increasing the spectral dynamic range of the platform. Parallel processing of the same ion populations, but extended length, transients yields much higher resolution for this lowfield Orbitrap; indeed, 2 orders of magnitude greater than achievable
on typical sector-field atomic mass spectrometers. In addition, the use of multiple (up to several thousands) transient coadding and aFT processing yields far greater sensitivity/dynamic range than the standard operating system for this particular application. The effects of greater numbers of coadded transients and longer data acquisition transient lengths were investigated relative to the precision and accuracy of uranium IR determinations. An ability to reprocess the saved set of transients allows obtaining analytical information for diverse (truncated) transient lengths with a small time increment (e.g., 10 ms). For example, it improves the accuracy and confidence of the IR analysis by revealing the almost continuous dependences and trends rather than considering only discrete scattered data points. Finally, the simultaneous measurement of $^{234}$UO$_2$, $^{235}$UO$_2$, and $^{238}$UO$_2$ yields the ability to observe at least 4–5 orders of magnitude of linear dynamic range in a single spectral acquisition, greater than the 1:5000 dynamic range that is expected of the standard Orbitrap. Calibration functions across multiple uranium isotopes are linear across $>6$ orders of magnitude. Ultimately, the addition of the advanced external DAQ system improves the already impressive capabilities of the LS-APGD/Orbitrap coupling in terms of uranium isotopic analysis, having relevance to other IR analyses. Finally, the combination of high resolution, wide dynamic range, and high measurement precision portend well for the use of this combination in multielement trace analysis.
References


55. Williams, T. J.; Hoegg, E. D.; Bills, J. R.; Marcus, R. K. Roles of Collisional Dissociation Modalities on Spectral Composition and Isotope Ratio Measurement
CHAPTER THREE

Investigation into the Ionization of Polyaromatic Hydrocarbons Using the Liquid Sampling – Atmospheric Pressure Glow Discharge

Introduction

Polyaromatic hydrocarbons (PAH) are low-polarity, carbon based compounds created through the incomplete combustion of organic matter; or found naturally in substances like bitumen, a viscous form of petroleum. These compounds are of interest for investigation and monitoring due to their negative environmental impacts. In humans, a number of these PAHs are known or expected to be carcinogens, teratogens, or mutagens; specifically the higher molecular weight PAHs. Because these compounds are both man-made and naturally occurring, they can be easily introduced into the environment through the extraction, processing, and burning of fossil fuels. The combination of toxicity and their ubiquitous nature makes it essential to have analytical techniques capable of detecting and quantifying these PAHs.

Some of the most common ways PAHs are analyzed are via atmospheric pressure chemical ionization (APCI) – mass spectrometry and gas chromatography mass spectrometry (GCMS). While APCI forms protonated molecular species for many other solutes, the formation of radical cations here is attributed to the lack of a typical protonation site that are common in other small polar molecules. Unlike electrospray ionization (ESI) where a polar site is necessary for the protonation of the analyte, APCI forms radical cations via gas phase solvent molecule interactions which result in charge
(electron) transfer, leaving a charge on the analyte of interest. Similarly, sources like atmospheric pressure photoionization (APPI) and desorption electrospray ionization (DESI) that bombard the analyte either with energy or solvent are also capable of ionizing these species. Interestingly, while these ionization sources do result in the formation of the radical cation of PAHs, they also form a protonated species as well. This is likely due to the ability to put sufficient thermal/collisional energy into the ionization process, overcoming the aromatic stability of these molecules.

A more recent ionization source utilized in the analysis of PAHs is the liquid sampling – atmospheric pressure glow discharge (LS-APGD). The LS-APGD as an ionization source has historically centered on trace elemental analysis and isotope ratio measurements. Beyond elemental analysis, work by Zhang et al. showed the ability to analyze small polar molecules by simply altering the mobile phase composition from 2% HNO₃ to 70:30 MeOH:H₂O, predominately producing protonated molecular ions. Along with small polar molecules, the LS-APGD has been employed to analyze proteins and resulted in spectra with charge envelope reminiscent of ESI. One difference is the presence of a peak relating to the heme group which is not seen in ESI. This shows to fragmentation of the protein occurring during the ionization process which could be a result of more gas phase interactions which would be more energetic/higher temperature than in solution reactions. This fragmentation could lead to the ability to form multiply charged species. These results led to the thought that the LS-APGD has an ionization mechanism that is similar to both APCI and ESI.
Recently, work by Williams et al. has expanded the analytical versatility of the LS-APGD for the analysis of relatively non-polar molecules, the PAHs.\textsuperscript{11} This proof-of-concept work investigated the ability of the LS-APGD to analyze the aromatic compounds, and interestingly, found that both radical cations and protonated molecular ions were observed, similar to APPI and DESI.\textsuperscript{11} A preliminary investigation into the analytical capabilities of the LS-APGD showed great promise, achieving detection limits on par with EPA method 610.\textsuperscript{11} While this work showed promise in expanding the already impressive portfolio of the LS-APGD analytes, it introduced some questions about the ionization processes which are occurring.

Presented in this work are efforts to further probe the ionization mechanism of the LS-APGD to a greater extent than past works. Being able to vary the protonation/radical cationization ratio based on the type of PAH being analyzed, for example linear vs peri-fused systems, could have interesting implications relative to the analysis of isobars/stereoisomers of different PAH types. For example, chrysene and naphthacene are isobars, and are both linear but differ based on the branching of the aromatic rings, whether it be ortho-, para-, or peri-fused. Along with the look into the ionization mechanism, the plasma parameters in regard to analytical response were studied and limits of detection were found. These studies lead to a better understanding of what is taking place with the ionization of molecular species in the LS-APGD.

**EXPERIMENTAL**

The LS-APGD ionization source coupling to Orbitrap mass spectrometers has been previously shown.\textsuperscript{14, 15, 19} The microplasma apparatus consists of a solid stainless-steel
counter electrode (anode) and a hollow solution electrode (cathode). The solution electrode is comprised of a fused silica inner capillary, through which solution is introduced, and stainless-steel outer capillary, through which a helium sheath gas is delivered. Power is delivered through a custom built control box (GAA Custom Electronics, Kennewick, WA), along with the gas and liquid. The standard operating conditions, unless otherwise noted, are a solution flow rate of 30 µL min\(^{-1}\), sheath gas flow rate of 500 mL min\(^{-1}\), discharge current of 30 mA, and an electrode gap of 1 mm.

The mass spectrometer used in this work is the ThermoScientific (Waltham, MA) Q Exactive Focus. Mounting of the ionization source to this platform, detailed in previous works, requires no modification of the instrument only, the removal of the standard ESI source and replacement with the LS-APGD. For this work, the instrument operating conditions are as follows: in-source CID of 15 V, full scan mode, a digitization range of 10 centered between the respective M\(^{+}\) and M+H\(^{+}\) peaks for each analyte, and an operating resolution of \(m/\Delta m = 70k\). Sample was introduced via a six-port injector with injection volumes of 20 µL.

Samples were prepared by first dissolving in methanol (HPLC Grade; VWR; Radnor, PA) the diluting to final concentration using DI water prepared by an Elga PURELAB flex water purification system (18.2 MΩ cm\(^{-1}\)) (Veolia Water technologies, High Wycombe, England). Analytical grade toluene, DCM, and chloroform were obtained from Acros Organics (Bridgewater, NJ), VWR, and Millipore (Burlington, MA), respectively. Methanol-D\(_4\) and D\(_2\)O were obtained from Beantown Chemical (Hudson, NH) and Acros Organics, respectively. Pyrene, chrysene, acenaphthene were obtained
from Aldrich Chemical company (Milwaukee, WI). Naphthalene was purchase from JT Baker (Phillipsburg, NJ). Benzo (ghi) perylene was purchased from Acros Organics. Tetracene was purchased from TCI (Tokyo, Japan). Deuterated samples were prepared using in the same manner as those in standard solvent. Calibration solutions were prepared through serial dilution of a stock solution to concentrations ranging from 100 pg mL\(^{-1}\) – 10 \(\mu\)g mL\(^{-1}\).

**Results and Discussion**

**Solvent Effects on the Mass Spectral Characteristics of PAHs**

Recent efforts by Williams et al. sought to expand the sampling diversity of the LS-APGD through the analysis of low polarity compounds and less common solvent systems.\(^{11}\) The study of PAHs revealed simultaneous protonation and radical cationization of the introduced analytes. Operating as a glow discharge ionization source in the abnormal glow region, the radical cation formation was expected under the assumption that electron ionization and Penning ionization would be the most prominent mechanisms. As described previously, the source has the capacity to form protonated molecular ions of small polar molecules, with multiple proton additions (reminiscent of ESI charge envelopes) seen for proteins. In the case of PAHS, the lack of polar functional groups would seem to be prohibitive towards ionization via proton transfer reactions. Thus the formation of protonated PAHs by the microplasma is not intuitively obvious, though these species are common to APPI sources.\(^{2,8-10}\) Likewise, charge (electron) transfer reactions are very common in APCI of PAHs is not obvious.\(^{8}\) At this point, the mechanism of PAH protonation under is still the subject of various studies. The current consensus suggests that
these molecules are protonated by a combination of a charge/proton transfer.\textsuperscript{9} Interestingly, protonation of PAHs is seen on sources beyond APPI, including DESI, GC-ESI.\textsuperscript{6,8,9} Notably, these sources all involve bombarding the sample whether it be through solvent, or an energy source. To obtain both protonation and radical cationization of these species on the LS-APGD suggests concurrent mechanisms of ionization occurring and requires an in depth look into system processes.
Previous work had looked into the identity of the solvent system as an involved component of the ionization process for two PAHs, naphthalene and pyrene, this showed that a protonated molecular ion and a radical cation are produced. The formation of the radical cation was expected due to the thought that Penning ionization is a dominant process in the LS-APGD, however the formation of the protonated molecular ion pointed to a different ionization mechanism. To further investigate the ionization of PAHS a more in depth study was conducted. Figure 3.1 shows the mass spectra of 6 PAHs in 70:30 MeOH:H$_2$O, toluene, DCM, and chloroform solvent delivery/plasma sustaining mobile phases. One interesting thing to note here is that the dominant species in each of the solvents is not consistent across the suite of PAHs. Some species see the protonated species dominate in MeOH:H$_2$O, while others see the protonated molecular ion dominate in toluene. The ratio of protonation to radical cation formation is shown in Figure 3.2 for each PAH in each solvent. Clearly the solvents that dominate the protonation by producing M+H$^+$/M$^{++}$ >1 for many PAHs are MeOH:H$_2$O and toluene, where one or the other is the most prominent for all but one of the PAH solutes (chrysene). In fact, a dichotomy seems to exist in that for each PAH, either the alcohol or the toluene yields high M+H responses, and the other solvent is poor at producing that ionic species. Perhaps that is not a surprise as these two solvents are at opposite ends of the polarity spectrum. The chlorinated solvents, in general, have protonation ratios that fall in between the MeOH:H$_2$O and
toluene. This figure shows that while toluene results in a ratio >1 in certain cases, MeOH:H₂O provides the ability to best attain high protonated to radical cation ratios due to species resulting in a protonation ratio >8 and half of the PAH’s sampled resulting in a ratio of >1. This is most likely due to methanol providing high density of gas phase protons which is conducive to the formation of protonated molecular ions in previous studies. The energy needed to overcome the aromaticity points to the ionization mechanism relating to gas phase solvent molecule interactions, rather than solution-based proton transfer as the formation of this is unlikely in solution, a key part of the mechanism behind ESI. While it is clear that there is a formation of a protonated molecular ion peak, it is not evident the exact location of protonation as there are no polar sites for protonation. Other previously proposed mechanisms have also not suggested a site of protonation.

While toluene, chloroform, and DCM are not expected to have a proton available for donation, however, protonation is seen in all the cases here. This has been explained by the ability to act as a dopant. It has been seen in many previous works that toluene, and other organic molecules can act as a dopant for either, a charge transfer or a proton transfer. Work by Ahmed et al., originally suggested by Syage, has shown that there is potentially a combination of the two where a charge transfer happens first, followed by the proton transfer. Because of the nature of toluene and PAHs having aromatic π electrons, π stacking could be able to bring these PAHs to a position that initiates the proton transfer. The work here shows that smaller, linear PAHs are protonated best by toluene. This trend in protonation and potential π stacking is backed by work showing that π stacking is stronger
in more geometrically similar molecules.\textsuperscript{24} The larger the PAH, the less likely this $\pi$ stacking would be likely to occur.\textsuperscript{24}

While the MeOH:H\textsubscript{2}O and toluene result in almost inverse protonation for each species, the chlorinated solvents result in an M+H$^+$/M$^{++}$ ratio generally between MeOH:H\textsubscript{2}O and toluene and this has been previously shown by Syage where methanol used as a solvent in low pressure photoionization resulted in the largest amount of protonation, followed by DCM, and lastly carbon tetrachloride.\textsuperscript{25} Syage states the mechanism is thought to be the loss of an electron by photon bombardment, followed by a solvent based proton transfer.\textsuperscript{22} This is different from what is proposed by Ahmed et al., where it is stated that the solvent is responsible for both the charge and proton transfer.\textsuperscript{9} The difference in the chlorinated solvents and the other two solvents utilized in this experiment is that the chloroform and DCM proton affinities are well between the other solvents which could be a contributing factor to these species resulting in protonation between the two extremes of the solvents utilized.\textsuperscript{7, 26}
Figure 3.2: Bar graph representing the protonation to radical cation formation for each PAH in MeOH:H₂O, DCM, chloroform, and toluene.

Deuterated Solvent Study for the Source of Protonation

While it is expected that the protonation is a result of the solvent utilized based on the change in protonated molecular ion formation across the different solvents, however, it is of interest to determine exactly where this proton comes from in the 70:30 MeOH:H₂O solvent system as this is the most common solvent utilized for molecular MS with the LS-APGD. In order to probe this, a study using deuterated solvents was conducted to compare the formation of adducts in each solvent. Using one deuterated solvent and one normal solvent, it would be expected that if a deuterated molecular ion is formed, the source of the deuteron would be the deuterated solvent, similar to work done by Ahmed et al with deuterated toluene. Figure 3.3 presents the M+D⁺/M+H⁺ as a function of the solvent
system utilized, and it was found that whether the water is D₂O or H₂O determines the majority of the adduct formation. For the portion of the figure with MeOH-D₄:H₂O, there is a smaller amount of deuteration that occurs versus when the solution is MeOH:D₂O pointing to water being the source of the adducted protons/deuterons. This is expected as water has a lower proton affinity than methanol leading to the water giving up a proton more readily than methanol. It is observed that there is still a formation of a protonated molecular ion while the solvent is entirely deuterated, MeOH-D₄:D₂O. This could be explained by humidity from the atmosphere penetrating the plasma and providing a source of protons, albeit a much smaller source than is seen with any non-deuterated solvents. Another reasonable explanation is that some of the deuterated solvent had exchanged the deuteron for a proton over time.
Proton Affinity of PAHs and the formation of a Protonated Molecular Ion

While it is clear that the solvents have an effect on the formation of the protonated and radical cation species, clearly there are enthalpically-driven aspects of the solutes involving the structure and chemical properties of each specific PAH has on whether or not the protonated or radical cation form is dominant. In order to look into this, the protonation ratio \((M+H^+/M^+)\) was plotted against the proton affinities for each of the PAHs. As shown in Figure 3.4., it is clear that the proton affinity appears to play some role in degree of protonation of these PAHs. A positive-trend is observed when ignoring
the clear outliers, circled. These species that follow this trend are the linear PAHs and the number of fused rings increases with the proton affinity and in turn the protonation ratio. The two species that do not follow this trend are the PAHs that are peri-fused rings. This points to the structure also playing a role in which species is dominantly observed. While all of these species are aromatic there is an inherent difference between the linear PAHs and the peri-fused PAHs; whether or not the Hückel’s rule is followed. Hückel’s rule lays out the rules for aromaticity stating that a molecule must be cyclic, planar, and contain 4n+2 number of π electrons. While some of these PAHs do not follow Hückel’s rule as a whole, they are still deemed aromatic due to being made up of aromatic structures resulting in a cyclic, planar molecule, i.e. fused aromatic rings. The PAHs that follow Hückel’s rule follow the trend of increasing proton affinity for the protonation ratio, while the PAHs that break this rule are more easily protonated. This is because these structures that do not follow Hückel’s rule, while considered aromatic, are made overall anti-aromatic, which in general are less stable molecules. This points to the aromaticity being easier to break in those aromatic structures that do not follow Hückel’s rule. The formation of a protonated molecular ion would require the breaking of aromaticity in order to add a proton due to having to break a double bond to form this species.
Effects of Plasma Conditions on the Protonation of PAHs

Along with understanding how the solvent and structure of the PAH can affect the amount of protonation that occurs, it is also important to understand how the plasma parameters might affect the protonation of these species. Shown in Figure 3.5, the M+H⁺/M⁺ ratio is plotted against the operating parameters of the LS-APGD, a) gas flow rates (mL min⁻¹), b) current (mA), c) liquid flow rate (µL min⁻¹), and d) electrode gap (mm). Figure 5a shows that the protonation decreases as the gas flow increases. This could be explained by the fact that an increased gas flow rate reduces the residence time of the
analyte in the plasma to be able to interact with the solvent molecules in the reaction therefor causing a reduction in the amount of protonation observed. Another explanation could be that the more energetic plasma from a lower gas flow rate is more conducive to causing the proton transfer to happen from the solvent, whether it be from higher plasma energies or from a better desolvation. It has been previously shown that the rotational (kinetic) temperatures of the LS-APGD increase with decreases in the gas flow rate.\textsuperscript{16} The limiting factor in the determination of which of these is more likely is knowing the extent of desolvation at each flow rate and correlating that to the protonation. If those two were to trend together then it could be assumed that the desolvation is the limiting factor in the formation of the protonated molecular ion. However, if these were to not trend together the assumption could be made that the more energy the plasma has to effect ionization, the more protonation would occur. Another potential explanation is the formation of solvents that provide the protonation. Similarly to APCI, the more energy provided by the plasma could provide the energy needed to form H$_3$O$^+$.\textsuperscript{8} With the lower solution flow rates there is more protonation, which at first would seem to contradict that a gas phase solvent-molecule (PAH) interaction is the potential ionization mechanism, however, the more liquid present in the plasma increases the amount of energy that is going into desolvation and vaporization of the carrier solution therefore reducing the amount of energy capable of producing the protonated ions. Due to the plasma energy having an effect on the formation of the protonated molecular ion, it would appear that the biggest barrier to the formation, in regards to solvent molecule interaction would be the collision energy/frequency between the solvents and the analyte, more studies would need to be conducted to determine which
is the driving factor. Another potential reason there could be a reduction in protonation at higher flow rates is there is a potential that less efficient desolvation is occurring resulting in less gas phase solvent molecules for ionization. As shown by the gas flow rate, it appears that a hotter plasma as shown by an increase in the rotational temperature is more conducive to yielding more protonation. This concept of a more energetic plasma resulting in more protonation is also supported by increasing the current of the LS-APGD. It has previously been shown that higher currents result in a more energetic plasma conditions along with the decrease in gas flow rates. The hypothesis of a more energetic plasma being more conducive to the protonation of these PAHs is supported by the work done by Amhed et al. where a temperature dependence was found for the degree of protonation using APPI. Along with the temperature dependence, the data in this work also points to a proton transfer via a solvent molecule interaction. It has been previously shown, by comparing spectra of FITC labeled ligand tethered ligand where the spectrum from LS-APGD shows a fragment peak of the analyte where this is not seen in ESI where the spectrum just shows the pseudomolecular ion peak. This difference shows that the LS-APGD has characteristics of a more energetic system for ionization than that of ESI, again pointing more towards a similar ionization mechanism to that of APCI.
Figure 3.5: a) gas flow rate, b) liquid flow rate, c) current, d) electrode gap vs the protonation ratio of pyrene in MeOH:H₂O.
Limits of Detection and Effects of Plasma Parameters on Response

Figure 3.6: a) gas flow rate, b) liquid flow rate, c) current, d) electrode gap vs total response of pyrene in MeOH:H$_2$O.

While it is of fundamental interest to use these species to probe the ionization mechanisms of the LS-APGD, the analytical capabilities of the LS-APGD/Orbitrap pairing in regards to PAH determinations is also of interest. In order to obtain the best analytical results a one variable at a time (OVAT) studied was performed to find the best operating conditions. OVAT was used in order to study the effects that each parameter have on the analytical intensity, rather than by using a design of experiment (DoE). The trends in total response of pyrene for each of the plasma parameters studied, shown in Figure 3.6, follow
similar trends to that of the protonation ratio shown in Figure 3.5. This is not surprising, as discussed with the protonation ratio between solvents that the significant change in response is with the protonated peak, in turn affecting the ratio.\textsuperscript{11} The most significant of these is the gas flow rate where there is an \textasciitilde{}5X intensity decrease seen across the range from 0.250 mL min\textsuperscript{-1} to 1000 mL min\textsuperscript{-1}, which has previously been shown using a DoE for the parameterization of LS-APGD for small polar organic molecules by Williams et al.\textsuperscript{12} While the significance has been shown before, the effect the gas flow rate has is different than previously seen with the LS-APGD for the analysis of organic molecules where the intensity of caffeine increased over the same range of gas flow rates.\textsuperscript{20} A current of 30 mA was used for the analytical study of the PAHs although 60 mA resulted in the highest signal due to the plasma stability demonstrated at the lower current. The electrode gap was set to 0.5 mm to gain stability in the plasma over the larger electrode gaps and the liquid flow rate was set to 30 µL min\textsuperscript{-1}. The LODs obtained across the suite of PAHs using these conditions are shown in Table 3.1. These LODs were obtained using the equation LOD=3(σ_{low})/m. The variability in the lowest test concentration is used due to the inability to obtain a true blank because of the automatic noise deletion step of the processing software of the Orbitrap. These LODs are in close proximity with EPA method 610 for the analysis of polyaromatic hydrocarbons, while not all are met, they are within one order of magnitude and with a bit more optimization could be reached.\textsuperscript{30} It is clear that naphthalene stands out from the others as this LOD is much higher. This is not unexpected as it is seen that the LODs for smaller molecular weight PAHs can be higher than those of the higher molecular weight.\textsuperscript{6} Similarly to the formation of the protonated molecular ion, the PAHs
that do not follow Hückel’s rule result in sensitivities that are drastically higher than those that do. When comparing the species that form the protonated molecular ion more readily to those that do not, there appears to be a trend that is similar to that of Figure 3.4. This would follow the conclusions found previously that there is less of an effect on the formation of the radical cation when changing solvents, meaning that the sensitivity could be related to the formation of the protonated molecular ion.11

Table 1. LODs and equations for the six PAHs in this study.

<table>
<thead>
<tr>
<th>PAH</th>
<th>LOD (ng ml⁻¹)</th>
<th>Equation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Napthalene</td>
<td>28</td>
<td>y =627x +34683</td>
</tr>
<tr>
<td>Pyrene</td>
<td>0.270</td>
<td>y =375045x +373923</td>
</tr>
<tr>
<td>Acenaphthene</td>
<td>0.400</td>
<td>Y=9044x-713533</td>
</tr>
<tr>
<td>Tetracene</td>
<td>0.140</td>
<td>Y=65255x+9620</td>
</tr>
<tr>
<td>Chrysene</td>
<td>0.420</td>
<td>Y=4622x-3053</td>
</tr>
<tr>
<td>Benzo(ghi)perylen</td>
<td>0.110</td>
<td>Y=53373x-313356</td>
</tr>
</tbody>
</table>

Conclusions

The ionization mechanism of the LS-APGD is still not exactly known, but there is a further depth of knowledge about what is going on inside the plasma. Initially, the LS-APGD has been thought to operate between APCI and ESI when using MeOH:H₂O as a carrier solution, however, utilizing these PAHs it has been shown that the ionization mechanism is most likely more similar to that of APCI or APPI. This is due to the formation of protonated and radical cation PAHs being more likely to happen via gas phase solvent molecule interactions rather than in solution protonation. Along with the ionization
mechanism probed, LODs for each of the six PAHs were realized resulting in In order to 
fully understand the ionization mechanism more studies will need to be done including 
studying the deuterated solvents for the additional solvents used here, the use of different 
solvents for polar molecules. Also the ability to tune the ionization mechanism could be 
further investigated along with the ability to analyze large PAHs and perform separations 
via reversed phase HPLC.
References


CHAPTER 4

CONCLUSIONS AND OUTLOOK

The LS-APGD/orbitrap pairing in the work shown here has demonstrated vast capabilities across the field of mass spectrometry. The orbitrap employed here was able to obtain a spectrum where the $^{234}$UO$_2$, $^{235}$UO$_2$, and $^{238}$UO$_2$ are observed from a simultaneous scan. This work shows the vast dynamic range that this instrument is capable of as this represents a 50000:1 dynamic range from the most abundant to least abundant species. Along with observing this minor isotope, the resolution while utilizing the advanced DAQ was increased from the standard 70k m/Δm to nearly 1 million m/Δm at 200 m/z. The ability with this advanced DAQ shows that extended transients can be obtained beyond what is allowed by the instruments. This allowed for the study of the effect of transient length on the IR and precision. It was found that the IRs increase to a point, but due to space charge effects, this increase is not seen at the longer transient lengths. Along with the ability to measure IR, this pairing of the LS-APGD/orbitrap pairing with the advanced DAQ also allowed for an LOD of <13 pg mL$^{-1}$ to be obtained and nearly 7 orders of magnitude achieved using 4 sample solutions. This pairing demonstrates the capability of being able to separate nearly any isobar without having to use a gas phase reaction cell and in order to observe low abundant ions that would not normally be observed with other systems. The abilities shown here open the door for the ability to separate most isobars, and demonstrating this ability explicitly is of great benefit to prove that this combination could be a great addition to the realm of atomic analysis. A further look into the precision that
can be realized for the $^{234}\text{UO}_2f^{238}\text{UO}_2$ IR could open the door for potential ITVs to be established for the minor isotope.

Along with the ability to observe these low abundance ions, the LS-APGD has shown that protonated molecular ions are observed for low polarity species such as the PAHs used here. This observation of the protonated PAHs shows that the mechanism of ionization is different than initially expected. It was found that the ionization mechanism is not as similar to ESI as originally expected, but is much more similar to that of APCI or APPI. This is due to the protonation occurring at higher rotational temperature is the plasma, showing that the energy is needed to break the aromaticity of these PAHs in order to add the proton source. This points to the ionization occurring in the gas phase as opposed to solution based as it is with ESI. Most likely, this ionization mechanism revolves around a solvent molecule interaction that results in the protonation. In order to gain a better insight to these ionization processes, more work needs to be done regarding the use of deuterated solvents to determine the source of protonation in the other low-polar solvents. Along with the source of the proton, studying more in depth how the rotational temperature of the plasma has on the formation of these protonated molecular ions is also important.
APPENDIX

Appendix Figure 1: Theoretical spectrum of natural-abundance uranium dioxide species using the Peak-by-Peak software.

Appendix Figure 2: Data hierarchy in FTMS. The acquired data is time-domain ion signals (transients), which contain most of the information about the ions oscillating in the mass analyzer. Transient components are sinusoids characterized by amplitude, frequency, and initial phase. Transient length (period) is directly related to the resolution in mass spectra. Fourier processing of transients results in mass spectra that can be represented in several ways, including: full profile absorption mode FT (aFT), full profile enhanced FT (eFT), reduced profile eFT, and eFT centroided.
Appendix Figure 3: Signal-to-noise ratios (SNR) for the $^{234}$UO$_2$, $^{235}$UO$_2$, $^{238}$U$^{17}$O$^{16}$O, and $^{238}$UO$_2$ responses as a function of the number of transients coadded. Expanded mass spectra inclusive of the $^{234}$UO$_2$ and $^{238}$U$^{17}$O$^{16}$O species for representative numbers of coadds. Dashed blue line represents 5s of the spectral noise. Test solution of 1 µg mL$^{-1}$ of natural abundance uranium in 2% HNO$_3$. Acquisition transient length = 0.5 s and AGC = 2x10$^4$.

Appendix Figure 4: Mass spectra extracted for various transient lengths showing the separation of $^{234}$UO$_2$ from the interferent peak with baseline resolution occurring at 0.5 s or 109k resolution.

Appendix Figure 5: Isotope ratios of $^{235}$UO$_2$/$^{238}$UO$_2$, $^{238}$U$^{17}$O$^{16}$O/$^{238}$UO$_2$, and $^{234}$UO$_2$/$^{238}$UO$_2$ using both peak area (red circles) and peak intensity (grey triangles). a) IR values as a function of transient length (8160 coadds). b) IR values as a function of the number of transients coadded (0.5 s transients). AGC = 2x10$^4$. 

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Appendix Figure 6. Measured SNR values for target species as a function of transient detection period for a) AGC = 1 \times 10^6 and b) AGC = 2 \times 10^5. Plots correspond to different number of coadded transients, with SNR thresholds for acceptance indicated with each set of data.