Evaluation and Optimization of a Liquid-Sampling Atmospheric Pressure Glow Discharge Ionization Source for Diverse Applications in Mass Spectrometry

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ABSTRACT

Mass spectrometry is a powerful analytical tool that can be used to identify and quantify diverse classes of analytes, including atomic, small molecular, and large biological species. The limitation in this powerful technique, however, lies in the fact that these various analytes require vastly different instrumental configurations. Elemental analysis, for example, requires instrumentation capable of hard ionization, such as inductively coupled plasma mass spectrometry (ICP-MS) and thermal ionization mass spectrometry (TIMS). These instruments also require very high-resolution mass analyzers so isobaric elemental isotopes can be clearly resolved and quantified, typically employing sector field and Fourier transform ion cyclotron resonance (FT-ICR) analyzers. Conversely, large biomolecules require soft ionization methods that preserve the analytes’ native state, such as electrospray (ESI) and matrix-assisted laser desorption ionization (MALDI). The most common mass analyzers used for biological mass spectrometry are ion-traps, quadrupoles, and time-of-flight (TOF) analyzers. In this work, a novel liquid-sampling atmospheric pressure glow discharge (LS-APGD) ionization source is coupled to a high-resolution Orbitrap mass analyzer for the analysis of diverse analytical species. The potential powering modes and geometries of the LS-APGD were evaluated for the analysis of a multi-element solution as well as for the analysis of uranium isotope ratios for nuclear nonproliferation applications. The LS-APGD was then evaluated for the analysis of a seleno-mercury complex.
DEDICATION

To my family and friends, who listened to me and gave me the well-needed advice that got me through my lowest points.
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CE: capillary electrophoresis
CID: collision-induced dissociation
EI: electron ionization
ELCAD: electrolyte-as-cathode glow discharge
ESI: electrospray ionization
FT-ICR: Fourier transform-ion cyclotron resonance
HCD: higher-energy collision induced dissociation
HEU: high enriched uranium
IAEA: International Atomic Energy Agency
ICP-MS: inductively coupled plasma-mass spectrometry
IR: isotope ratio
ITC: ion transfer capillary
ITVs: International Target Values
LEU: low enriched uranium
LS-APGD: liquid-sampling atmospheric pressure glow discharge
m/z: mass to charge ratio
MALDI: matrix-assisted laser desorption ionization
MeHg: methyl-mercury complex
MS: mass spectrometry
OES: optical emission spectroscopy
SCGD: solution cathode glow discharge
SeCys: selenocysteine
HgSeCys: selenocysteine-mercury complex
SeMet: selenomethionine
SGA: solution grounded anode
SGC: solution grounded cathode
SIM: selected ion monitoring
SPA: solution grounded cathode
SPG: solution powered cathode
TIMs: thermal ionization mass spectrometry
TOF: time-of-flight
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CHAPTER ONE: INTRODUCTION

Mass spectrometry (MS) is considered one of the most powerful analytical techniques because it offers the ability to identify and quantify many diverse analytes. To perform mass spectrometry, all that is required is a method to ionize the sample, a way to separate the ions produced by mass to charge ratio (m/z), and some sort of ion detector.\(^1\) One of the difficulties associated with using this technique, however, lies in the fact that a plethora of instrumental configurations exist that satisfy these requirements, and the choice in configuration is dependent on the analyte of interest.\(^1\) MS of elemental species typically requires a high energy “hard” ionization source to completely atomize and ionize the sample, such as inductively coupled plasma mass spectrometry (ICP-MS) or thermal ionization mass spectrometry (TIMS).\(^1,2\) Atomic mass spectrometers also require high resolution mass analyzers to allow for clear determination of isobaric isotopes, such as sector field instruments.\(^2,3\)

Conversely, analysis of biological macromolecules typically requires “soft” ionization sources, which preserve the native states of analytes with minimal fragmentation.\(^1,4\) The most common ionization sources for the analysis of biomolecules to achieve this purpose are matrix-assisted laser desorption ionization (MALDI) and electrospray ionization (ESI).\(^1,4\) ESI is especially popular owing to its ability to be easily interfaced to separation methods, such as liquid chromatography and capillary electrophoresis (CE).\(^5,6\) The most common mass
analyzers for the analysis of biomolecules are current time-of-flight (TOF), quadrupole, and ion traps.\textsuperscript{1,4} In the past 20 years, however, Orbitrap mass analyzers have become ubiquitous for biological macromolecule analysis (proteomics, metabolomics) due to their exceptional resolution (>200,000 at m/z 400) and mass accuracy (<1-2 ppm with internal calibration).\textsuperscript{7,8}

Orbitrap mass analyzers work by electrostatically trapping ions between an inner spindle electrode and an outer cylindrical electrode.\textsuperscript{7,9} The ions are injected perpendicularly to the spindle electrode (which lies along the z-axis) and oscillate in stable trajectories both along the z-axis and radially around the spindle electrode (Figure 1.1).\textsuperscript{7,9} Although all ions will produce the same amplitude within the Orbitrap, different mass to charge ratios will have axial oscillations at varying frequencies.\textsuperscript{7,9} These oscillations that are detected produce an ion image current, which then undergoes a Fourier transform to create a mass spectrum.\textsuperscript{7,9} Orbitraps can achieve such high resolution, approaching that of state-of-the-art FT-ICR instruments, because the axial oscillations, dependent on mass to charge ratio, are independent of the energy and spatial spread of the ions.\textsuperscript{7,9} Because of this, the electrostatic trapping field is very well defined, reducing space charge effects of higher molecular weight ions, allowing the ions to be effectively trapped without the necessity of large, expensive magnets required in FT-ICR instruments.\textsuperscript{7,9}
An ideal MS configuration would be one that allowed for the analysis of diverse analytes with minimal manipulation to the instrument. The Orbitrap works well as a mass analyzer for biomolecules, and its high resolution is ideal for the analysis of isobaric isotopes of elemental species. The challenge lies in finding an ionization source that works for both atomic and biological species.

The liquid sampling atmospheric pressure glow discharge (LS-APGD) ionization source is a glow discharge ionization source that consists of a microplasma sustained between a solution electrode and a stainless-steel counter electrode. The solution electrode is composed of a fused silica inner capillary that delivers a sample solution into the microplasma, housed within a stainless-steel outer capillary which delivers a cooling helium sheath gas. The gas flow is maintained at a constant rate via a mass flow controller. Analyte solution can either be delivered at a constant flow rate via a syringe pump or injected by discrete
volumes into a carrier solution. The interelectrode displacement can be controlled using a micrometer, which allows the counter electrode to be displaced radially from the inlet of the mass spectrometer. Power is delivered to the microplasma via a DC power supply. This source can be easily interfaced to a commercial Orbitrap instrument by simply removing the standard ESI and plugging the LS-APGD into the housing (Figure 1.2).

![Diagram of LS-APGD Orbitrap System](image)

The LS-APGD was originally explored as an atomic excitation source for optical emission spectroscopy, and later adapted into an ionization source for atomic mass spectrometry. The first iteration of the LS-APGD ionization source interfaced to an Orbitrap instrument was an Orbitrap Exactive, which allowed for facile detection of a variety of metals dissolved in 1 M nitric acid ($^{56}$Fe, $^{58}$Ni, $^{63}$Cu, $^{115}$In, $^{114}$Cd, $^{133}$Cs, $^{208}$Pb) with low detection limits (0.67, 1.0, 2.2, 0.04, 0.60, 0.05, 0.02 µg mL$^{-1}$ respectively). Interestingly, the ions that were detected were singly charged radical cation ($M^+$) species, which showed that the LS-APGD-Orbitrap system was an effective method for detection of elemental species taking that
Initial experiments aimed at optimizing the most pertinent LS-APGD operating parameters (operating current, solution flow rate, and inter-electrode displacement) were performed to determine the optimal conditions for elemental response, but as the source developed, these experiments were repeated for optimal performance in each generation.

Different from other popular glow discharge sources, such as the electrolyte-as-cathode glow discharge (ELCAD) and the solution cathode glow discharge (SCGD), it is possible to reconfigure both the powering mode and geometry of the LS-APGD. Either a positive or negative potential can be applied to either one of the electrodes, and the electrodes can be oriented in a number of geometries (discussed in Chapter 2). When the LS-APGD was first used as an excitation source in OES, a rigorous parameterization was performed in each of the powering modes to yield the highest emission intensity as well as reproducibility, which were determined to be the solution grounded cathode (SGC) and the solution powered anode (SPA) modes. As mass spectrometry involves the detection and transport of charged species, in lieu of the detection of emitted photons, it was pertinent to reevaluate the powering modes of the LS-APGD as an ionization source. Furthermore, LS-APGD-OES is typically performed with the electrodes in line with one another and the detector oriented perpendicularly to the gap between the electrodes. LS-APGD-MS, however, is typically performed with the solution electrode in-line with the MS inlet.
This evaluation was first performed using a multielement solution to determine analytical performance for elemental analysis, and then with a standard solution with a known isotope ratio of $^{235}$U/$^{238}$U to determine isotope ratio accuracy and precision. The $^{235}$U/$^{238}$U isotope ratio is of great importance in nuclear nonproliferation applications, so much so that the International Atomic Energy Agency (IAEA) sets international target values (ITVs) for accuracy and precision for this ratio for the industry standard techniques for this analysis, ICP-MS and TIMS. The ITVs serve as a viable comparison between the isotope ratio performance of these techniques and LS-APGD-Orbitrap-MS.

The LS-APGD has shown promise as an ionization source for small organic molecules using a variety of different sampling methods and mass spectrometers. On a Thermo LCQ advantage MAX quadrupole ion trap, a variety of organic species (including caffeine, sinapinic acid, diadizin, and fluorescein) were injected into an electrolytic carrier solution of (70:30 methanol:water) and detected in the form $[\text{M+H}]^+$. The LS-APGD was later modified for ambient desorption (ADI) sampling, and a variety of small organic analytes (caffeine, nicotine, ibuprofen, and cocaine) could be easily detected.

In addition to small organic molecules, the LS-APGD has been used to detect small metal-organic complexes of both nuclear nonproliferation and biological relevance. The first iteration of this technique employed the LS-APGD interfaced to a Thermo Scientific LCQ ion trap mass spectrometer in the metal-ligand speciation of uranyl acetate, in which it was discovered that solvent pH and identity
play a pivotal role in the species that are detected by the mass spectrometer.\textsuperscript{19} Later, a biologically relevant complex between selenocysteine (SeCys) and mercury that had long been theorized was confirmed using the LS-APGD.\textsuperscript{13} Se has a much higher affinity for Hg than S, so it is currently under extensive study as a tool to sequester Hg from biological systems.\textsuperscript{31,32} However, the selenocysteine-mercury (HgSeCys) complex was identified using the traditionally “elemental” plasma operating parameters, so detailed parameterization of both plasma conditions is in order to determine the ideal conditions to guarantee the highest signal intensity and reproducibility for this complex, and other metal-organic complexes in the future.\textsuperscript{13}
References


Spectrometry (m/Δm> 1,000,000): Coupling of the Liquid Sampling-Atmospheric Pressure Glow Discharge with an Orbitrap Mass Spectrometer for Applications in Biological Chemistry and Environmental Analysis. Journal of The American Society for Mass Spectrometry, 30(7), 1163-1168.


CHAPTER TWO:
EVALUATION OF THE POWERING MODES AND GEOMETRIES OF THE
LIQUID SAMPLING—ATMOSPHERIC PRESSURE GLOW DISCHARGE—
ORBITRAP SYSTEM FOR ANALYTICAL PERFORMANCE AND ISOTOPE
RATIO ANALYSIS

Abstract
The liquid sampling atmospheric pressure glow discharge (LS-APGD) microplasma has shown promise in the fields of optical emission spectroscopy and mass spectrometry. In terms of mass spectrometry, it has allowed use of instruments normally applied in “organic” mass spectrometry, to be used for elemental/isotopic applications. The LS-APGD/Orbitrap combination is a particularly attractive alternative to traditional elemental MS systems due to its ability to perform ultra-high-resolution analyses, eliminating isobaric interferences which typically require extensive chemical separation prior to analysis. The LS-APGD is unique in its ability to operate using four different powering modes; solution grounded cathode (SGC), solution grounded anode (SGA), solution powered cathode (SPC), and solution powered anode (SPA). To investigate the utility of each powering mode, the elemental responses and isotope ratio performance were assessed for the pertinent operating parameters (discharge current, solution flow rate, gas flow rate, and inter-electrode displacement). Experiments were performed using a 500 ng mL\(^{-1}\) multielement solution containing Rb, Ag, Ba, Tl, and U. Measurements of \(^{235}\text{U}/^{238}\text{U} \) were
performed using a 200 ng mL$^{-1}$ solution of CRM-129a. Ultimately, it was determined that the SGC mode showed the best performance in terms of elemental intensity, accuracy, and precision for isotope ratio measurements. The optimal electrode configuration consists of the solution electrode in line with the ion-sampling orifice of the MS, with the counter electrode oriented perpendicular to that axis.
**Introduction**

While numerous advances have been made in developing reduced-format (and in the extreme, transportable) instruments in the areas of separation science, optical spectroscopy, sensor technologies, and organic mass spectrometry, the development of such instrumentation capable of elemental and isotope ratio analysis continues to be a challenge.\(^1\)\(^-\)\(^3\) The accurate and precise measurement of U, Pu, and their related radionuclides is of particular interest due to their use in nuclear technologies.\(^4\) Regarding U isotope ratio measurements, the ability to distinguish between \(^{235}\)U enrichment levels and their resulting applications; naturally-occurring (0.72%), civilian (0.02-0.5%), fuel (0.7-20%), and military (>20%) quickly and efficiently is especially important.\(^4\) Traditionally, radionuclide isotope ratio (IR) measurements are performed by multi-collector inductively coupled plasma mass spectrometry (MC-ICP-MS) and thermal ionization mass spectrometry (TIMS).\(^5\)\(^-\)\(^7\) Whereas TIMS is considered to be the “gold standard” in isotopic analysis, MC-ICP-MS has allowed for higher-throughput measurements while maintaining a high level of accuracy and precision.\(^5\)\(^-\)\(^7\) Though these techniques demonstrate clear advantages, they are generally limited to sophisticated laboratories due to their large instrument size and stringent operating parameters. Orbitrap instruments, on the other hand, are benchtop instruments that offer potential advantages in terms of performance relative to elemental/isotopic analysis;\(^8\)\(^-\)\(^10\) beyond which they are far more prevalent in practice. In order to benchmark the suitability of analytical techniques for IR
instrument development, the International Atomic Energy Agency (IAEA) adopted the International Target Values (ITVs) for Measurement Uncertainties in Safeguarding Nuclear Materials. While MC-ICP-MS and TIMS already routinely meet the ITVs, a commercially-accepted, a benchtop-scale instrument that could achieve these target values would be beneficial to the non-proliferation community, as well as the field in general.

The introduction of atmospheric pressure, solution electrode glow discharges has been a significant advancement towards the development of reduced-format instruments due to their compact size, low operational overhead, and versatility. These devices have shown promise as both effective excitation sources for optical emission spectroscopy (OES) and to a lesser extent ionization sources for mass spectrometry. Examples of successful sources include the electrolyte as cathode discharge (ELCAD) by Cserfalvi and Mezei and the solution cathode glow discharge (SCGD) developed by Hieftje and co-workers. Beyond these, the liquid-sampling atmospheric pressure glow discharge (LS-APGD) source developed by Marcus et al. offers additional benefits including low power consumption (<50 W) and low solution flow rates (<100 µL min⁻¹) while operating in a total consumption mode. Though initially developed as an optical emission source, the LS-APGD has demonstrated success as an ionization source for mass spectrometry. To this point, the LS-APGD has been successfully interfaced with quadrupole and Orbitrap mass spectrometers for analysis of both elemental and molecular species. Orbitrap instruments have
been of particular interest because of their ability to perform ultra-high-resolution measurements, specifically in the measurement of isotope ratios. To this point, it has been demonstrated that the LS-APGD-Orbitrap system is capable of meeting the U IAEA precision ITVs for samples with various degrees of enrichment, including natural uranium, low enriched uranium (LEU), and high enriched uranium (HEU), although longer term studies that look at reproducibility are still ongoing.  

A notable difference of the LS-APGD in comparison to other glow discharge sources is the ability to reconfigure the powering mode and geometry of the microplasma, as depicted in Figure 2.1. The plasma can be operated in solution grounded cathode (SGC), solution powered cathode (SPC), solution grounded anode (SGA), and solution powered anode (SPA) modes. Davis and Marcus have previously investigated the effect of powering modes using the LS-APGD as an excitation source for optical emission spectroscopy, determining that the SGC and SPA powering modes offered the best analytical performance in terms of both
emission intensity and reproducibility.\textsuperscript{19,32} However, the measured signal for OES is dependent only on the excitation of analyte species as the emission is an isotropic process. Mass spectrometry, on the other hand, requires the ionization and transport to the sampling orifice of the instrument for detection of charged species. Because of this, the electrical configuration of the powering mode, as well as the electrode geometry, may have a substantial effect on analytical performance. The LS-APGD microplasma operates in the abnormal glow discharge regime, where the majority of excitation and ionization takes place in the negative glow region of the plasma, which exists just outside of the cathodic electrode. As such, different powering configurations will shift this active region, and therefore affect the charged species that will enter the mass spectrometer.\textsuperscript{33}

The effects of the powering modes and geometries on analytical performance of the LS-APGD as an ionization source for mass spectrometry have yet to be investigated.

In this work, the different LS-APGD powering modes are assessed for analytical performance in terms of MS analysis. The signal intensity and reproducibility for analyte species in each powering mode were investigated as a function of operating current, gas flow rate, solution flow rate, and inter-electrode displacement. The powering modes that were deemed analytically-viable were then tested in several different sampling geometries to evaluate their performance. Ultimately, the powering modes and geometries were then investigated for accuracy and precision in U IR measurements and the results were then compared
to the IAEA ITVs. While the resulting conclusions are no different from what had been previously practiced to date, the trends observed provide a better understanding of the fundamental processes at play in the MS implementation of the device.
Experimental

For this work, the LS-APGD system was interfaced to a Thermo Scientific Orbitrap Q-Exactive Focus mass spectrometer (Thermo Fisher Scientific, Waltham, MA, USA), Fig. 2.2. The LS-APGD microplasma is sustained between a solution electrode and a stainless-steel counter electrode (SS weldable feedthrough, MDC Vacuum Products, Hayward, CA, USA). The solution electrode is composed of a fused silica inner capillary (280 µm i.d., 580 µm o.d.; Restek Corp., Bellefonte, PA, USA) that delivers a sample solution into the microplasma and is mounted within a stainless-steel outer capillary (316 SS 0.8 mm i.d., 1.6 mm o.d., IDEX Health and Science, Oak Harbor, WA, USA) which delivers a cooling helium sheath gas. The gas flow was maintained at a constant rate via a mass flow controller (MC-2SLPM-D/5M, Alicat Scientific, Tuscon, AZ, USA). Analyte solution was delivered at a constant flow rate via a Fusion 100 T syringe pump (Chemyx, Stafford, TX, USA). The interelectrode displacement was controlled using a micrometer (150-801ME, 10 µm graduations, ThorLabs, Newton, NJ, USA), which allowed the counter electrode to be displaced radially from the inlet of the mass spectrometer. Power was delivered to the microplasma via a Spellman SL60 DC power supply (0-1 kV, 0-60 mA, Spellman High Voltage Electronics Corporation, Hauppauge, NY, USA) operating in a constant-current mode. For the initial parameterization and isotope ratio experiments, the solution electrode was oriented in line with the ion transfer capillary, and the counter electrode was oriented perpendicularly to that axis (Fig. 2.2). This orientation was adopted initially
(and used to this point) because it was hypothesized that sampling of ionized species would be optimum if the most active region of the plasma was located directly in front of the ion-sampling orifice.\textsuperscript{34}

![Diagram of LS-APGD-Orbitrap System](image)

**Figure 2.2: LS-APGD-Orbitrap System operating in solution grounded cathode (SGC) mode**

A summary of the powering modes and the active regions of the plasma within them can be seen in Fig. 2.1. In the case of the microplasma, the highlighted regions of the cathode dark space and the negative glow are of greatest relevance, as the former is the acceleration region of ions towards-electrons away from the cathode, and the latter is the region wherein analyte ionization is affected. Two basic situations exist, wherein the solution electrode acts as the anode or cathode, with the mode of powering leading to four combinations. To this point, the LS-APGD ion source has been operated in the solution grounded cathode (SGC) powering mode,\textsuperscript{21} in which the solution electrode is grounded, and positive potential is applied to the counter electrode. The solution grounded anode (SGA), is configured the same way, with the solution electrode grounded and a negative
potential applied to the counter electrode. In the solution powered cathode (SPC) mode, the counter electrode is instead grounded, and a negative potential is applied to the solution electrode. In the same way, the solution powered anode (SPA) has the same set up with a positive potential applied to the solution electrode. The SGC mode has been employed most commonly in the past because it has been an eventual goal to hyphenate the LS-APGD to liquid chromatography methods,\textsuperscript{19,32} as such it is preferable from a safety standpoint to not have a high voltage in contact with the injector and chromatograph components. If indeed, the solution powered modes were to show better performance in terms of signal intensity, accuracy, and precision, they must be considerably better to justify their use with further electrically-insulating couplings employed.

After the experiments directed at understanding the powering modes of the microplasma, it is pertinent to investigate potential variations in electrode geometries of the discharge. Traditionally, when the LS-APGD is utilized as an ionization source for mass spectrometry, the solution electrode is situated directly in-line with the MS inlet with the counter electrode oriented orthogonally.\textsuperscript{13} However, alternative electrode geometries have not yet been explored. In the first experiments, the solution electrode was mounted colinearly to the ion sampling path, at an electrode displacement (tip-to-tip) of 1.5 mm from the MS inlet, and the counter electrode placed perpendicular to that axis; the geometry used throughout studies to date. Subsequently the position of the solution electrodes was placed at
angles of 45°, 135°, and 180° with respect to the counter electrode (Fig. 2.3a). Then, the experiment was repeated with the solution electrode mounted perpendicular to the MS inlet, and the solid counter electrode placed at the various angles. After those geometries were explored, a “v-shape” geometry was tested, in which the electrodes were both displaced 0.75 mm from the MS inlet and oriented at angles of 45°. The electrodes were then rotated 22.5° in either direction to have either the solution or counter electrode in line with the MS inlet (Fig 2.3b).

![Figure 2.3](image_url)  
Figure 2.3: Summary of LS-APGD powering geometries at a) varying angles and b) different v-shape geometries.

Samples were prepared by diluting elemental standards provided by High Purity Standards (North Charleston, SC, USA) in 2% nitric acid. The initial powering mode and geometry experiments were performed using a solution of Rb, Ag, Ba, Tl, and U (500 ng mL⁻¹ each). Isotope ratio experiments were performed using a 200 ng mL⁻¹ solution of CRM-129a (New Brunswick National Laboratory, Argonne, IL), with a certified $^{235}$U/$^{238}$U isotope ratio of 0.0072614.
The Thermo Q Exactive Focus instrument was operated in the positive ion mode without modification to the instrument other than replacing the standard ESI source with the LS-APGD. The instrument was controlled using the Tune software and data analysis was completed using the Xcalibur software package. Both multielement analysis and IR measurements were performed in triplicate and assessed by the average peak area over the total ion chromatogram over 100 scans of 10 microscans. Each acquisition took approximately 4.5 minutes. Reproducibility was assessed by the percent relative standard deviation (%RSD) of the peak areas for triplicate measurements.

For the multielement analysis using varying powering modes and electrode geometries, the quadrupole scan range and digitization range were restricted to 70-300 m/z. SIM was not employed in this analysis in order to evaluate multiple elements simultaneously. The trends observed with a wide scan range could however also be observed if SIM was employed, albeit with higher signal intensities. The signal intensity was taken as the average peak area over the total ion chromatogram for the most abundant elemental isotope of each analyte.

To reduce concomitant ion effects seen with uranium IR measurements detailed in previous works, in which larger digitization ranges introduce more apparent perturbations in ion dynamics and noise effects that bias measurements against low abundance ions, the quadrupole scan range was restricted to 243.5-293.5 m/z, and the digitization range was 263.5-273.5 m/z. $^{235}\text{U}/^{238}\text{U}$ values were calculated using the average peak area over the total ion chromatograms of the
\(^{235}\text{U}^{16}\text{O}_2^+\) at (m/z 267) and the \(^{238}\text{U}^{16}\text{O}_2^+\) (m/z 270) molecular ions. The molecular ion was used in the isotope ratio analysis in lieu of the elemental ion as it is the most intense U species detected. In order to eliminate additional unwanted molecular concomitant ions, usually taking the general form \((\text{H}_2\text{O})_n\text{H}^+\), collision induced dissociation (CID) and higher-energy collision induced dissociation (HCD) were both employed using values of 90 eV and 120 eV respectively.
Results and Discussion

Plasma Parameter Operation Space

Before attempting to evaluate the analytical performance of the various powering modes, it was first necessary to identify the operation space in which they were viable (i.e., temporally stable). For these experiments, the traditional SGC operating parameters (discharge current = 30 mA, solution flow rate = 30 µL min⁻¹, gas flow rate = 0.50 L min⁻¹, and inter-electrode displacement = 1.5 mm) were used as a starting point and as controls to test plasma stability. Details of the tested parameters are presented in Table 2.1. The microplasma was considered “stable” if the power supply display value remained constant (<2% variation) at a given set of conditions. The discharge maintenance voltage is employed as the determinant variable as it is a direct measure of the energy needed to operate the plasma at the desired current. The results are seen in the maintenance voltage response plots (plotted as the absolute values of the voltages) presented in Figure 2.4, in which microplasma stability in each powering mode was explored as a function of operating current, solution flow rate, gas flow rate, and inter-electrode displacement.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Powering Mode</th>
<th>Control</th>
<th>Range</th>
<th>Increment</th>
</tr>
</thead>
<tbody>
<tr>
<td>Current (mA)</td>
<td>Cathode</td>
<td>30</td>
<td>25-50</td>
<td>5</td>
</tr>
<tr>
<td></td>
<td>Anode</td>
<td>20</td>
<td>10-20</td>
<td>5</td>
</tr>
<tr>
<td>Solution Flow Rate (µL min⁻¹)</td>
<td>Cathode</td>
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<td>15-60</td>
<td>15</td>
</tr>
<tr>
<td></td>
<td>Anode</td>
<td>15</td>
<td>--</td>
<td>--</td>
</tr>
<tr>
<td>Gas Flow Rate</td>
<td>Cathode</td>
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<td>0.25-1.0</td>
<td>0.25</td>
</tr>
<tr>
<td>---------------</td>
<td>---------</td>
<td>------</td>
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</tr>
<tr>
<td>(L min(^{-1}))</td>
<td>Anode</td>
<td>0.50</td>
<td>0.50-1.0</td>
<td>0.25</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Inter-Electrode Displacement (mm)</th>
<th>Cathode</th>
<th>1.5</th>
<th>0.50-2.0</th>
<th>0.50</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Anode</td>
<td>0.50</td>
<td>--</td>
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</tr>
</tbody>
</table>

Table 2.1: Summary of parameters tested in each powering modes. The cathode modes refer to solution grounded cathode (SGC) and solution powered cathode (SPC) and the anode type modes refer to solution grounded anode (SGA) and solution powered anode (SPA).

![Graphs](image)

Figure 2.4: Discharge maintenance voltage (plotted as the absolute value) in each powering mode vs increasing a) current, b) solution flow rate, c) gas flow rate, and d) inter-electrode displacement. In the solution cathode experiments, these parameters were kept as a control throughout these experiments: discharge current = 30 mA, solution flow rate = 30 µL min\(^{-1}\), gas flow rate = 0.50 L min\(^{-1}\), and inter-electrode displacement = 1.5 mm. In the solution anode experiments, these parameters were kept as a control throughout these experiments: discharge current = 20 mA, solution flow rate = 15 µL min\(^{-1}\), gas flow rate = 0.50 L min\(^{-1}\), and inter-electrode displacement = 0.5 mm.

The initial test parameters were shown to be readily applicable for the SPC powering mode, as the plasma was stable and demonstrated the same general
response trends as in SGC. The solution-anode type powering modes (SGA and SPA), however, could not be sustained using the SGC parameters as those parameters caused the electrodes to undergo intense heating, resulting in the melting of the solution capillaries and solid electrodes. As such, the current operation range was severely restricted, indeed the plasma would not spark below 10 mA. Furthermore, the solution flow rates had to be reduced because higher flow rates would destabilize and extinguish the plasma. Sheath gas flow rates were increased to dissipate heat in the system, as gas flow rates below the 0.5 L min$^{-1}$ baseline also resulted in melting of the capillaries and electrodes. The inter-electrode displacement values for the solution-anode modes were limited to 0.5 mm as anything beyond this resulted in an inability to sustain the plasma.

Ultimately, the results of these experiments show that the respective solution powering modes (as anode or cathode) perform similarly when subject to the same conditions. The resulting voltage measurements, though opposite in sign, follow the same trend in these modes. In the solution anode powering modes, an increase in current demonstrates a linear response in voltage, which is expected of a plasma operating in the abnormal glow discharge regime. (Fig. 2.4a).$^{13,33}$ In the solution cathode powering modes, however, increases in current do not demonstrate expected increases in voltage. Instead, the voltage decreases slightly ($<5$ V) with incremental increases in current. It is important to note that these voltage measurements are of the microplasma alone, correcting for the voltage drop across the 10 kΩ resistor in the circuit. As the current increases, the
total voltage of the system also increases, as expected of an abnormal glow discharge. However, as the total current increases, the voltage drop across the resistor also increases significantly, which leads to reduced apparent total plasma voltage. Excluding aforementioned extinguishing conditions, the liquid flow rate and gas flow rates seem to have little effect on plasma voltage because changes in flow rates impart no change in power density that would be characteristic of changes in current and inter-electrode displacement for the cathode powering modes (Fig. 2.4b,c). Increases in inter-electrode displacement increase the resulting system voltage in the cathode powering modes, which can be attributed to the microplasma having a higher total resistance due to larger plasma volume (Fig. 2.4d). Because there was little variability that could be attained in the solution anode powering modes with respect to solution flow rate and inter-electrode displacement, it was not possible to discern trends given by those two parameters in those modes. However, increased gas flow rate in the anode type powering modes lead to decreased voltage, likely due to higher gas flows more efficiently dissipating plasma energy as heat. It can be expected from these results that in terms of analytical performance, the solution-as-cathode type powering modes will behave similarly to one another, and the solution-as-anode type powering modes will behave similarly to one another.

Multielement Response Dependencies to Discharge Operation Conditions

After the operating space of each of the powering modes was defined, it was next pertinent to evaluate the performance of each powering mode with the
LS-APGD/Orbitrap system regarding multi-element analysis. Unfortunately, it was quickly determined that the solution-anode powering modes were not suitable for this pairing. When the system was running in either SGA or SPA mode, the analyte signal intensity would diminish rapidly with time over the course of analysis, which was discovered to be due to internal contamination on the ion optics of the MS. Some of this contamination was clearly visible on the exit lens which follows the ion-routing S-lens (Appendix Fig. 1). In addition to visible inspection of the electrodes, which shows extensive oxidation and corrosion during operation in the solution anode modes, ICP-OES measurements of the contamination confirmed that it was neither organic in nature nor due to analyte carryover. In fact, the majority of signal from the ICP digestion was that from Ni, Fe, and Cr, which provides further evidence that the counter-electrodes themselves were degrading (Appendix Table 1). It is believed that while the LS-APGD is operating in the solution anode modes, the discharge is inefficiently dissipating heat, which leads to degradation of the electrodes, and consequently significant contamination of the ion optics. Furthermore, cathodic electrodes are used as the samples in standard glow discharge mass spectrometry, wherein sputtering of the surface is the means of sample introduction into the reduced-pressure plasma. Ionized species in the microplasma could in fact be sputtering the counter electrode, serving here as the cathode, contributing to this degradation. In past OES powering mode experiments, it was not pertinent to look for analytes that were not present in the analyte solution, so this degradation was not reported. This degradation and
contamination was not observed with the SPC or SGC powering modes wherein the counter electrode is subject to electron bombardment.

The primary plasma operating parameters known to effect analyte ionization are discharge current, the solution (analyte) feed rate, the sheath gas flow rate, and the inter-electrode spacing. Analyte response was measured as a function of operating current in the solution cathode modes (SCG as solid bars and the equivalent SPC as hashed bars of the same colors) as presented in Fig.

Figure 2.5: Multi-element intensities in both SGC and SPC as a function of a) operating current, b) solution flow rate, c) gas flow rate, and d) inter-electrode displacement. Except for the value being varied, these parameters were kept as a control throughout these experiments: discharge current = 30 mA, solution flow rate = 30 µL min⁻¹, gas flow rate = 0.50 L min⁻¹, and inter-electrode displacement = 1.5 mm.
2.5a. Clear signal intensity maxima are evident for both the SPC and SGC powering modes. While the precision of triplicate measurements across the elemental and discharge current space are quite similar between the two powering modes, there are distinct differences between the absolute responses. As a general rule, the analyte responses for the SPC mode increase with discharge current up to 30 mA, followed by an abrupt decrease at higher values, while the SGC data shows a more gradual increase to maxima at 40 mA, followed by a similarly gradual decrease. At the lowest currents tested, the microplasma exhibits low power density and therefore low ionization efficiency, while previous work indicated that water and nitrate related adducts begin to form and dominate the elemental mass spectra at higher currents. The Orbitrap Q Exactive Focus employs CID and HCD to dissociate adducted species and the resulting fragment ions fall below the instrument’s 50 Da mass cutoff, so they are not observed in the mass spectra obtained here. However, the formation of these species, noted in previous work, reduces signal intensity at higher currents as the plasma has a fixed ionization capacity. The key differences between the two modes are born out in the trends in the responses, wherein at lower currents (below the maxima) the intensities of the SGC are ~2X those of SPC, beyond that point the values for the SGC become >5X the SPC.

As the discharge current controls many aspects that effect the ultimate analyte response, the solution flow rate controls the rate of analyte introduction as well as the solvent loading. The SGC and SPC modes again show comparable
response trends with the solution flow rate variation experiments (Fig. 2.5b). In both modes, signal intensity tends to increase with solution flow rate due to more analyte being introduced and ionized within the microplasma, however the intensity begins to drop off at the highest flow rates for the SGC mode as the SPC mode yields even greater response. The signal drop at higher flow rates has been attributed by Zhang et al.\textsuperscript{22} to increased solvent loading into the microplasma, a phenomenon in which the overall analyte signal intensity drops due to the overpopulation of water-related analyte clusters, which begin to dominate the species present in the spectra. Again, due the HCD and CID employed in this experiment, these species are not observed. It worthy to note that, while operation at higher flow rates is suggested in the SPC mode, those increases in response, coincide with even greater extents of variability.

The coaxial sheath gas flow serves to cool the source components, assist in nebulization, and direct plasma species towards the MS ion inlet. Sheath gas flow rate experiments demonstrated somewhat different results for the SGC and SPC powering modes, as presented in Fig. 2.5c. Here, the SPC shows maximum response at the lowest flow rates, while in the SGC, the signal intensities pass through a maxima for all analytes at 0.5 L min\textsuperscript{-1}. This behavior has been explained in previous parameterization studies\textsuperscript{22}, and it was attributed to a counterbalance of analyte plasma residence time and transport of ionized species into the mass spectrometer. The He sheath gas confines the plasma and directs the ionized species toward the ion sampling orifice of the MS, so the signal intensity increases
as gas flow rate increases from 0.25 L min\(^{-1}\) to 0.50 L min\(^{-1}\). However, higher gas flow rates (0.75-1.0 L min\(^{-1}\)) decrease the residence time of the analyte within the plasma substantially, which decrease ionization efficiency. In contrast, the SPC powering mode shows decreasing signal intensity as the gas flow rate increases. Since the voltage of the microplasma in SGC and SPC does not change appreciably with increasing gas flow rate, this difference in trend could be attributed to a difference in ion transport. It is believed that, since the solution electrode is powered, the analytes would tend to be ionized closer to the liquid surface, so 0.25 L min\(^{-1}\) He is adequate gas flow to direct the ionized species into the plasma, and 0.50 L min\(^{-1}\) decreases the residence times of the analyte species. A notable difference between the SGC and SPC in terms of gas flow rate is that of reproducibility, where the SGC again tends to show greater robustness. In fact, while the ion signals are greater at 0.25 L min\(^{-1}\) in SPC compared to SGC, the values are much less reproducible, perhaps due to irregular transport toward the ion sampling orifice at low velocities.

The inter-electrode gap controls both the power density and the residence times of analytes within the microplasma; on the surface these are counter-indicating effects. Analyte signal intensity tends to generally increase with inter-electrode displacement (Fig. 2.5d) in both powering modes. As increasing the separation decreases the overall power density, this response is clearly due to the analyte species having longer residence times within the plasma, contributing to greater ionization. Recent monochromatic imaging experiments indeed reflect that
the negative glow region of the plasma elongates with larger electrode gaps.\textsuperscript{36} Though the counter electrode is being displaced radially, the plasma’s negative glow region, the analytically significant region is still located in-line with ion sampling orifice. At 2.0 mm, the improvement in signal intensity begins to level out and, in some cases, drops. It is believed that this is because the plasma’s negative glow is shifted out of line of sight of the MS inlet, decreasing the sampling efficiency.

After evaluating each of the operating parameters of the LS-APGD microplasma, the SGC and SPC powering modes demonstrated overall comparable performance, though the standard SGC was superior in most instances, and so they were both deemed viable toward further powering geometry experiments and isotope ratio measurements. The optimal plasma operating conditions for the SGC were: discharge current = 35 mA, solution flow rate = 45 $\mu$L min$^{-1}$, the gas flow rate = 0.5 L min$^{-1}$, and an inter-electrode displacement = 1.5 mm. The optimal conditions for SPC differed slightly: discharge current = 30 mA, solution flow rate =60 $\mu$L min$^{-1}$, gas flow rate = 0.25 L min$^{-1}$, and an inter-electrode displacement = 1.5 mm.

\textit{Electrode Geometry Dependencies}

When the LS-APGD was used in previous work as an excitation source for optical emission spectroscopy, the proper geometry of the electrodes was critical for optimum emission response.\textsuperscript{32} Far and away, the best emission responses were seen when the electrodes were oriented in a 180° geometry, with the optical
lens located perpendicular to the inter-electrode axis. In this case, the optical lens is able to capture and direct the most emission intensity to the optical spectrometer. As mass spectrometry relies on the transport and detection of ionized species, it follows that the electrode should be oriented so that the maximal amount of ionized species can be directed towards the ion sampling orifice via the sheath gas flow. This takes place when the solution electrode is in line with the ion transfer capillary, and the counter electrode is oriented perpendicularly, as seen for the SGC mode in Fig. 2.6a. Moving the solution electrode off-axis results in reduced analyte responses as might be predicted based on the needed transport of ionized species, with the 45° position being less than 135° due to physical obstruction of plasma flow by the counter electrode. When the solution electrode is instead oriented perpendicular to the ITC, the signal drops by an order of magnitude as seen in Figure 2.6b and in fact there is no dependence on the mounting angle of the counter electrode. The same experiments performed for the SPC powering mode, yield somewhat different responses. It is interesting that in SPC, however, the greatest signal intensity occurs when the solution electrode is offset by 45°, instead of in-line with the MS inlet (Fig 2.6c). In this case, different from the SGC mode, there is likely more of a directionality of the plasma striking to the counter electrode, thinning the negative glow region and perhaps allowing greater numbers of ions to exit the negative glow. In the final case, if the solution electrode is oriented perpendicularly to the ion sampling orifice in the SPC mode (Fig. 2.6d), the lowest signal intensity in any of these cases is observed as very
Few ions are being directed toward the inlet. In all, the SGC mode with the solution electrode mounted co-linearly with the ion sampling orifice yields the greatest analytical responses with the lowest amounts of variability.

Figure 2.6: Signal intensity dependence on different electrode angles. a) SGC with counter electrode orthogonal to MS inlet, b) SGC with solution electrode orthogonal to MS inlet, c) SPC with counter electrode orthogonal to MS inlet, d) SPC with solution electrode orthogonal to MS inlet. Discharge current = 30 mA, solution flow rate = 30 µL min⁻¹, gas flow rate = 0.50 L min⁻¹, and inter-electrode displacement = 1.5 mm.

The same basic trends in terms of analyte sensitivity and precision for both SGC and SPC with the electrodes mounted in a V-geometry experiments, wherein both the solution and counter electrodes are nominally pointing toward the MS inlet (Fig. 2.7). In SGC, the greatest signal intensity occurs when the solution electrode is oriented directly in line with the ITC and the counter electrode is angled 45° with...
respect to the solution electrode. However, in SPC, signal intensity is highest when
the counter-electrode is directly in-line with the ITC and the solution electrode is
offset 45°. This geometry is analogous in behavior with the geometry whose data
are presented in Fig. 2.6c. In those cases where the solution is mounted off-axis,
the SPC mode is most sensitive. However, the v-geometry experiments
yield degraded reproducibility values in each powering mode versus the orthogonal
geometries.

Figure 2.7: Signal intensity dependence on different v-geometry powering configurations in SGC
and SPC. Discharge current = 30 mA, solution flow rate = 30 µL min⁻¹, gas flow rate = 0.50 L min⁻¹,
and inter-electrode displacement = 1.5 mm.

Isotope Ratio Analysis

After investigating the viability of the four different powering modes and their
respective electrode geometries with respect to multi-element analysis, it is
another step forward to evaluate the two most optimal powering modes for their
ability to perform ²³⁵U/²³⁸U IR measurements relative to nuclear nonproliferation
applications where the random uncertainty component of the IAEA’s ITV for natural
uranium has a target value of 0.2 %. In elemental analysis, achieving the highest signal intensity (i.e. peak heights, peak areas) is the most prevalent goal, with precision being a secondary issue. However, for isotope ratio measurements, measurement accuracy and precision are IR ratio measurements differ greatly from the goals of elemental analysis. The results of the $^{235}\text{U}/^{238}\text{U}$ isotope ratio experiments are presented in Fig. 2.8 with respect to the accuracy and the corresponding %RSD. As a summary, the SGC and SPC demonstrated comparable accuracy to the true IR value of CRM-129a over most of the parameters tested. However, the $^{235}\text{U}/^{238}\text{U}$ IR values are consistently lower than the accepted values due to the background correction step of the Orbitrap’s software, as described in detail in previous works$^{24,28}$ and in greater detail in the following paragraphs.
Figure 2.8: $^{235}$U/$^{238}$U isotope ratio accuracy and precision as a function of a) plasma operating current, b) solution flow rate, c) gas flow rate, and d) inter-electrode displacement. Except for the value being varied, these parameters were kept as a control throughout these experiments: discharge current = 30 mA, solution flow rate = 30 µL min$^{-1}$, gas flow rate = 0.50 L min$^{-1}$, and inter-electrode displacement = 1.5 mm.

The response of the absolute values and measurement precision as a function of discharge current (Fig. 2.8a) are quite illustrative of the respective roles of microplasma conditions and signal processing aspects of isotope ratio measurements. As seen in Fig. 2.5a, the signal for uranium continuously increases as a function of discharge current for the SGC mode, while it passes through a maxima at 35 mA for the SPC to the point of very little signal at all. The corresponding precision in the SGC case remains relatively stable until the highest current (Fig. 2.8a), with the IR value increasing slightly. On the other hand, the SPC IR data show pronounced declines in precision and absolute value beyond 40 mA, reflective of the relative intensity. The degradation in performance on both counts are easily ascribed through the data system screen shots presented in Fig. 2.9. Seen are the peak heights and background subtraction levels (gray area) at each current for the $^{238}$UO$_2$ signal for the SPC case. As seen, the net peak
intensities (peak height minus background) increase with current to 40 mA, beyond this point the peak heights decrease, while at the same time the background levels increase. As this background subtraction effects the minor isotope to a far greater extent, the absolute IR will fall as depicted in Fig. 2.8a. At the same time, the variability of the signal, and indeed the background levels increase as reflected in the measurement precision.

![Graph showing relative abundances for different currents](image)

Figure 2.9: Tune software screenshots of the Orbitrap background deletion obscuring an increasing percentage of the $^{267}_{16}O_{2+}$ peak with increasing current in the SPC mode

In the case of variation in the solution flow rate (Fig. 2.8b), the IR accuracy values were largely comparable for both the SPC and SGC modes, even though the ion signal responses are quite different (Fig. 2.5b). In the case of the SPC powering, the signal intensity for $^{235}$U at the lowest current is so low that most of the signal is cut off by the background deletion step; lowering the isotope ratio accuracy and precision values. As in the case of discharge current, the IR value for SGC is relatively insensitive to changes in flow rate, as high signal intensities are produced. That said, there is definitely better precision seen at the moderate
flow rate of 30 µL min\(^{-1}\), which has been consistent across all previous works.\(^{28,30,31}\)

At higher liquid flow rates, there is increased solvent loading in the system, as discussed in the elemental analysis study, which results in decreased reproducibility values due to increased background species.

As would be expected based on the voltage and ion signal responses towards changes in sheath gas flow rate and inter-electrode spacings, the IR absolute values and precision are almost identical in response. In the variation in sheath gas flow rate experiment (Fig. 2.8c), both the SPC and SGC isotope ratios remain largely constant across rates tested, but the SPC demonstrates a lower IR value than SGC, as well as lower reproducibility. There is no IR value for 0.25 L min\(^{-1}\) for both SPC and SGC because the background subtraction completely obscured the \(^{235}\)U peak in both cases. In the variation of inter-electrode displacement experiment (Fig. 2.8d), the IR accuracy values were comparable in both the SPC and SGC modes. In both modes, the best reproducibility values were seen at shorter electrode gaps. This is again expected because of the Orbitrap background subtraction step. At larger electrode gaps, much higher signal intensities are observed due to longer analyte residence time. As signal intensities increase, however, there are more background ions that are also being ionized, which raise the background bar. For SGC, the ITV was reached in every case except for the greatest inter-electrode displacement tested, and the minimum RSD was at a displacement of 1 mm with a value of 0.08\%.
Though the SPC powering mode produced accuracy and precision metrics which would acceptable for many isotope ratio measurement applications, it was unable to reach the ITV at any combinations of parameters tested. SGC, however, is suitable for both elemental and isotope ratio analysis, with optimal operating conditions of 0.5 L min$^{-1}$ gas flow rate, 35 mA operating current, 30 µL solution flow rate, and a 1 mm electrode gap.
Conclusions

An evaluation of the operating parameters (liquid flow rate, gas flow rate, operating current, and inter-electrode displacement) for each of the four powering modes (solution grounded cathode “SGA”, solution powered cathode “SPC”, solution powered anode “SPA”, and solution grounded anode “SGA”) of the LS-APGD-Orbitrap system has been performed. Signal intensity and reproducibility (%RSD) were evaluated using a multi-element solution of Rb, Ag, Ba, Tl, and U. The $^{235}\text{U}/^{238}\text{U}$ isotope ratio performance was evaluated using CRM-129a.

It was determined that the solution anode-type powering modes were not viable for elemental analysis because they cause degradation of the electrodes of the LS-APGD, leading to significant contamination of the mass spectrometer ion lens elements. The reason this contamination exists is due to thermal effects and the sputtering of the counter electrode acting as the cathode. The SPC and SGC powering modes, however, were both shown to be analytically viable, and demonstrated definitive trends for signal intensity and reproducibility under the influence of each parameter; predominately paralleling each other. The optimal plasma operating conditions in SGC were determined to be a current of 35 mA, a solution flow rate of 45 µL min$^{-1}$, a sheath gas flow rate of 0.5 L min$^{-1}$, and an inter-electrode displacement of 1.5 mm. SPC performed optimally with 30 mA current, 60 µL min$^{-1}$ solution flow rate, 0.25 L min$^{-1}$ gas flow rate, and a 1.5 mm inter-electrode displacement. While the SPC mode can lead to high elemental peak intensities with slightly-altered conditions from SGC, it was ultimately decided that
the loss in flexibility of analysis due to restriction in separation hyphenation (the
desire to ground the solution input) does not warrant changing of the powering
mode for elemental analysis.

The results of the electrode geometry experiments show that SGC performs
optimally when the solution electrode is oriented directly in line with the ion transfer
capillary of the MS. This is because the most active ionization region of the plasma,
the negative glow, is directly in line with the MS inlet in this geometry. Furthermore,
the sheath gas aids in directing the ionized species toward the ion inlet. In SPC, it
is important that the solution electrode is directed at the ITC, however optimal
performance is seen when it is at a 45° angle from the counter electrode instead
of 90° seen with SGC.

The SGC and SPC powering modes were further employed to evaluate their
roles on the LS-APGD/Orbitrap system’s performance in isotope ratio analyses.
After thoroughly evaluating all potential operating parameters, it was determined
that only the SGC powering mode is viable for high accuracy and precision
$^{235}$U/$^{238}$U isotope ratio measurements using the LS-APGD-Orbitrap system.
Though for most parameters tested the SPC mode demonstrates low RSD values
(of $\sim$1%), in no case does the RSD fall below the IAEA ITV. However, in the SGC
mode the IAEA ITV is attained in most parameters tested. The optimal conditions
for $^{235}$U/$^{238}$U isotope ratio were determined to be 0.5 L min$^{-1}$ gas flow rate, 30-40
mA operating current, 30 μL min solution flow rate, and 1 mm electrode gap. These
conditions vary slightly from those optimal conditions for elemental analysis. The
isotope ratio values obtained are always somewhat lower than the accepted value due to the background deletion step that is inherent to the Orbitrap’s software, however, a correction factor derived from an isotopic standard can be used to resolve this.
References


CHAPTER THREE:
EVALUATION OF THE LS-APGD-ORBITRAP SYSTEM FOR THE
EVALUATION OF A SELENO-MERCURY COMPLEX

Abstract

The liquid sampling-atmospheric pressure glow discharge (LS-APGD) has been shown to be an effective ionization source for mass spectrometric detection of both elemental and small molecular species. Though numerous advancements have been made in those areas, only recently has the LS-APGD been employed for the detection of metal-organic complexes, including species of nuclear and biological relevance. Most recently, the combination of the LS-APGD with an Orbitrap mass spectrometer allowed for the long-theorized detection of a biologically relevant selenocysteine-mercury complex using operating conditions typically employed in the analysis of elemental species. Since the LS-APGD operates in substantially different optimal conditions when analyzing either molecular or elemental species, it is pertinent to perform a detailed parameterization of the plasma for an entirely different type of analyte, the metalloorganic selenocysteine-mercury complex. Herein this complex of selenocysteine and mercury (HgSeCys) is created by combining equimolar amounts ($3.3 \times 10^{-6}$ M) of methylmercury chloride (CH$_3$HgCl) and selenocysteine (SeCys) produced by reduction of selenocystine with dithiothreitol (DTT). The resulting HgSeCys solution was spiked with 50 µL of 5 ppm caffeine solution as an internal standard diluted 20× in 50:50 methanol water with 5% formic acid before
analysis. The plasma operating parameters of current, solution flow rate, gas flow rate, and inter-electrode displacement are assessed. The response of both reactants, SeCys and MeHg, and the HgSeCys complex were monitored and compared to a caffeine internal standard. Plasma conditions for optimal response for the metal-organic complex were less kinetically energetic than that of what is needed for elemental response, with 25 mA operating current, 60 µL min⁻¹ solution flow rate, 0.75 L min⁻¹ gas flow rate, and 0.5 mm inter-electrode displacement being the best conditions. The species that were observed in the mass spectrometer were determined by comparison to theoretical isotopic ratios.
Introduction

All sources of mercury (Hg) are known to be toxic to environmental ecosystems and human health. However, the degree of mercury toxicity is strongly dependent on its form, which can be \( \text{Hg}^0 \), oxidized \( \text{Hg}^+ \), \( \text{Hg}^{2+} \), or organic \( \text{CH}_3\text{Hg}^+, \text{CH}_3\text{HgCH}_3 \) in nature. Organic mercury exhibits the highest detriment to human health, and among organic mercury species, the methylmercury ion \( \text{CH}_3\text{Hg}^+, \text{henceforth MeHg} \) has gained the most scientific attention due to its low \( \text{LD}_{50} \) (9.3-19.6 mg kg\(^{-1}\) body weight in mice), long half-life (70-80 hr), and ability to cross the blood brain barrier. Effects of poisoning by MeHg can include deafness, vision changes, impaired coordination, numbing of the extremities, and in the most serious of cases, death.

Patients affected by MeHg poisoning are treated by a variety of methods that centralize on removing MeHg from the afflicted organism. The most commonly employed methods to achieve this are exchange transfusions, hemodialysis, and chelation therapy. Chelation therapy is a particularly effective method, which works by sequestering metals from living tissue to then excrete them from the body by way of urine and bile. Chelating agents popularly used to sequester Hg (such as N-acetylcysteine (NAC) and 2,3-dimercapto-1-propane sulfonic acid (DMPS)) are based on high affinity \( (10^{39}) \) binding between the S and Hg. However, the binding affinity between Se and Hg is a million times stronger \( (10^{45}) \) than the binding of S and Hg, due to selenium’s larger ionic radius and higher nucleophilicity. Because of the relative strength of the Se-Hg complex, Se-Hg
chelation has been an extensively researched topic as a method to reduce Hg toxicity in organisms.\textsuperscript{1-7,9}

In particular, selenium-containing amino acids (such as selenocysteine (SeCys) and selenomethionine (SeMet)) in selenoproteins have shown to be very effective at demethylating and sequestering Hg from MeHg if the two species are present in equimolar concentrations.\textsuperscript{4-7,9} It has long been postulated that a mercury complex with selenocysteine exists, but until recently it has been very difficult to characterize (Figure 3.1).\textsuperscript{4-7} Hoegg et al. recently confirmed the existence of this complex using the liquid-sampling atmospheric pressure glow discharge (LS-APGD) ionization source coupled to an Orbitrap Fusion Lumos mass spectrometer, in which the observed isotopic pattern matched that of the theoretical prediction exactly.\textsuperscript{10} In the experiment performed by Hoegg et al, the LS-APGD employed using optimal conditions for elemental analysis (30 mA operating current, 30 µL min\textsuperscript{-1} solution flow rate, 0.5 L min\textsuperscript{-1} gas flow rate), but previous work illustrates that plasma operating conditions have a substantial effect on the species that are observed.\textsuperscript{10-13}
The LS-APGD consists of a glow discharge microplasma sustained between a solution electrode (composed of an inner and outer capillary) and stainless-steel counter electrode (Figure 3.2). An electrolytic analyte solution passes through the inner capillary solution electrode by way of a syringe pump, and a helium sheath gas helps to sustain and cool the microplasma through the outer capillary. A high voltage DC power supply is connected to the counter electrode, and the solution electrode, placed in line with an atmospheric pressure mass spectrometer inlet, is grounded, effectively directing species ionized in the plasma to the mass analyzer. To this point, the LS-APGD has shown to be a versatile ionization source, which has allowed for diverse species to be easily detected with minimal modification to commercial mass spectrometers.\textsuperscript{10-17}
Extensive work has been performed interrogating the LS-APGD as an ionization source for elemental analysis, with emphasis on trace metal analysis and accurate and precise isotope ratios.\textsuperscript{10-15,17-20} Optimal conditions for these analyses employ 2\% nitric acid as the analyte solvent to aid in the dissolution of metal complexes, and rely on long analyte residence times to aid in electron ionization processes (relatively longer inter-electrode displacements, lower gas flow rates, and lower solution flow rates) to produce radical cation (\(M^+\)) species.\textsuperscript{11-14,16} Recently, the LS-APGD has shown to be an effective option for the analysis of small organic compounds as well, but instead use 70:30 methanol:water as the solvent system, which is still electrolytic, yet preserves the structure of the analytes.\textsuperscript{11-13,16} In addition, residence time of the analytes is reduced by increasing the liquid flow rates and gas flow rates, as well as decreasing the displacement between the two electrodes. Furthermore, the operating currents of the plasma are reduced to prevent excess energy contributing to analyte fragmentation.\textsuperscript{11-13,16}
Additionally, organic species are detected not as radical cations (M$^{•+}$), as seen in elemental analysis using this system as well as electron ionization sources, but instead as pseudomolecular ions ([M+H]$^+$), which rely on proton adduction to the analyte.

Preliminary work has been performed in the analysis of metal-organic complexes to investigate uranium speciation of a solution of uranyl acetate, demonstrating that plasma conditions play a pivotal role in the species detected by the mass spectrometer.\textsuperscript{11} For this reason an evaluation of pertinent plasma parameters (operating current, solution flow rate, gas flow rate, and inter-electrode displacement) were performed to achieve the greatest intensity response for the selenomercury complex, as well as to evaluate the fate of the reactants and the changes in ions detected under varying plasma conditions.
Experimental

For this work, the LS-APGD was interfaced to a Thermo Scientific Orbitrap Q-Exactive Focus mass spectrometer. The LS-APGD was set up in the same way as the powering modes experiments of Chapter 2, and it was operated in the solution grounded cathode (SGC) mode with the solution electrode oriented in-line with the ion transfer capillary and the counter electrode was oriented perpendicularly (Figure 3.2). Analyte solution was delivered by triplicate injection into a flowing carrier solution of 70:30 methanol: water solution.

The Orbitrap was operated in positive ion mode with no additional changes to the instrument besides switching out the standard ESI source with the LS-APGD. Spectra were taken over pre-filter quadrupole and digitization ranges of 160-390 m/z. No CID or HCD was used in this experiment in an attempt prevent dissociation of the metal-organic complex.

The mercury-selenocysteine (HgSeCys) complex was formed by reacting methylmercury and selenocysteine solutions of about 3.3×10^{-6} M. The selenocysteine solution was created by dissolving 50 mg of reducing agent dithiothreitol into 2 mL of 525 µg mL^{-1} (as Se) selenocystine 20 µL of the selenocysteine solution was mixed with 80 µL of a 328 µg mL^{-1} (as Hg) methylmercury (MeHg) solution to form HgSeCys. The resulting HgSeCys solution was spiked with 50 µL of 5 µg mL^{-1} caffeine solution as an internal standard diluted 20× in 50:50 methanol: water with 5% formic acid before analysis. All chemicals were obtained from Sigma Aldrich (St. Louis, MO).
Plasma parameterization was evaluated over a range of operating currents (25-50 mA), solution flow rates (15-60 µL min\(^{-1}\)), gas flow rates (0.25-1.0 L min\(^{-1}\)), and inter-electrode displacements (0.50-2.0 mm). While each parameter was varied, the control values were, for the most part, optimal parameters for elemental response: 30 mA operating current, 30 µL min\(^{-1}\) solution flow rate, 0.5 L min\(^{-1}\) gas flow rate. The control value for inter-electrode displacement was 0.50 mm because larger displacement drastically lowered HgSeCys response.

Relative intensities are evaluated by superposition of the resulting spectra for each parameter. Pertinent peaks are labeled.
Results and Discussion
Figure 3.3: Mass spectra exhibiting the relative intensities of selenocysteine (SeCys), fragment ions of HgSeCys, caffeine, and selenomercury (HgSeCys) as a function of a) current, b) solution flow rate, c) gas flow rate, and d) inter-electrode displacement. Except for the parameter being varied, these plasma conditions were set as the control: current = 30 mA, solution flow rate = 30 µL min⁻¹, gas flow rate = 0.5 L min⁻¹, inter-electrode displacement = 0.5 mm. The solvent used in this experiment was 5% formic acid.
The identity of the most dominant ions produced in the LS-APGD do not change remarkably with the plasma conditions, but their relative intensities do change. The ion seen in the highest abundance in all cases is the unreacted SeCys ion (m/z = 168.99), but a oxygenated peak can also be observed in lower abundance at m/z = 184.04, identified by isotopic pattern. Typically, the species present in the next highest abundance is that of the internal standard, caffeine, at m/z = 195.09, or the desired HgSeCys peak at m/z 371.01, depending on the operating conditions of the microplasma. Fragmented species of the HgSeCys ion are also present in lower abundance than the intact complex, with the loss of a hydroxyl (m/z = 352.99) being the next highest species, followed by the loss of an amine (m/z = 337.02). The identification of these fragmented species was performed by comparison of the isotopic abundance of the fragmented ions to the intact ion. Both peaks identified as fragments had the theoretical isotopic pattern for Se bonded to Hg with predictable losses.

Optimal plasma conditions for the analysis of metal-organic complexes are shown to be less kinetically energetic than that of what would be required for elemental analysis (Fig. 3.3), similar to what was seen in previous analysis of diverse organic species.11,13,16 The first evidence of this is that HgSeCys response tends to decrease as applied current increases (Fig 3.3a), with the maximum response shown at 25 mA. An initial sharp decline in HgSeCys response is seen as the current increases from 25 to 30 mA, and then HgSeCys response continues to gradually decline as the current increases from 30 to 50 mA, due to the following.
As seen in previous optical emission studies, the internal temperature of the microplasma increases as discharge current increases, raising the energy of the electrons and analytes within the plasma. It is believed that the energetic particles begin to perform collision-induced dissociation (CID) on the HgSeCys complex, preventing intact ions from being transported to the inlet of the mass spectrometer. Evidence of this is seen with the increase of fragmented HgSeCys peaks relative to the intact peak with increases in current. In addition, though a significant amount of MeHg is demethylated and sequestered by the SeCys during the reaction, there is still a significant amount of the reactants that can be detected, especially SeCys. The intensity of unreacted MeHg is present two orders of magnitude less than SeCys. It is believed that the ionization of radical cations, like MeHg, is less efficient than the ionization by proton adduction seen with molecular species. The highest responses of both MeHg and SeCys are observed at 30 mA, which has been shown in previous work to be the optimal operating current for the analysis of both elemental species and small organic molecules. It also follows that the MeHg cation is detected as a radical cation (M**), and not as a protonated pseudomolecular ion ([M+H]+) species, so its trend should follow that of elemental analysis.

The HgSeCys response increases drastically as solution flow rates increase, with the highest response seen at the highest solution flow rate tested (60 µL min⁻¹), as seen in Fig. 3.3b. This is due to two major phenomena. Firstly, higher solution flow rates lead to increased total amount of analyte being
introduced into the microplasma, which simply leads to more analyte being ionized and transported to the mass spectrometer’s inlet. Secondly, faster solution flow rates may lead to decreased analyte residence time. As it is believed that the HgSeCys complex is subject to fragmentation by energetic species within the microplasma, it follows that decreased analyte residence time would reduce this fragmentation. In contrast, solution flow rate has a much less drastic effect on the responses of the two reactants, SeCys and MeHg, though they both show a slight decrease as solution flow rate increases. In addition, as the solution flow rate increases, the total solvent load of the plasma also increases, thus increasing the amount of work that needs to be done to vaporize, desolvate, and ionize the molecules. This in turn leads to a decrease in not only MeHg response, but also SeCys response, as solution flow rates increase. The identity of major ions produced within the microplasma did not change throughout the current, gas flow, and inter-electrode displacement experiments, but an exception was observed in the solution flow rate experiment. Namely, a contaminant ion was observed in the 15, 60, and especially 45 uL min\(^{-1}\) solution flow rates at mass 282.278. It is believed that is a contaminant ion, and not of any analytical relevance due to the fact that its isotopic abundance pattern does not follow that of either Se or Hg. In addition, in previous iterations of this experiment, this ion was not observed. This contaminant ion is in fact one of many polysiloxane-related species, identified as \([(C_2H_6SiO)_n-H-CH_3]\). Polysiloxane contaminant ions are often observed in this m/z region, due to ambient pump oil present in the laboratory atmosphere. The the
most common species located at m/z = 371.10, corresponding to \([\text{C}_2\text{H}_5\text{SiO})_5^+\text{H}],\) which is also observed in the solution flow rate spectra. Typically, these polysiloxane species are background subtracted in order to better isolate the HgSeCys related species (present at m/z = 371.01), but occasionally these background subtractions are insufficient.

The HgSeCys response increases drastically as gas flow rate increases from 0.25 to 0.75 L min\(^{-1}\), and then drops off as gas flow rate increases from 0.75 to 1.0 L min\(^{-1}\) (Fig. 3.3c). The increase in signal intensity can once again be explained by the decrease in analyte residence time, as well as the sheath gas aiding in effectively directing the analyte species to the MS inlet. The decrease in response at the highest gas flow rates, however, is due to an increase in plasma turbulence that not only contributes to increased CID effects, but also to fewer analytes being adequately focused into the MS inlet. MeHg response follows the same trend as the HgSeCys response, which also agrees with what is seen in terms of elemental analysis. SeCys response does not follow a clear trend in terms of gas flow rate, so further experiments are warranted.

Perhaps the greatest parameter affecting the responses of all analytes is the inter-electrode displacement, as seen Fig. 3.3d. For this experiment, parameters typically employed for elemental analysis were used as control values. However, this was not possible for the inter-electrode displacement parameter, as the optimal value for elemental response (1.5 mm) severely diminished HgSeCys signal. The highest response for HgSeCys was seen at 0.5 mm displacement, with
an almost linear decrease in response seen as displacement increased from 0.5 to 2.0 mm. This drop in intensity can be explained by two phenomena. Firstly, as the inter-electrode displacement increases, the total volume of the plasma also increases, which leads to increased turbulence within the plasma, contributing to increased CID of the HgSeCys complex, as evident by the relative abundance of the fragmented HgSeCys species compared to the intact pseudomolecular ion. Secondly, as the inter-electrode displacement increases, the most active region of the plasma (the negative glow region, see Chapter 2) moves out of line with the ion transfer capillary of the mass spectrometer, leading to reduced transmission of the ionized species. These phenomena also explain why SeCys and MeHg responses increase with inter-electrode displacement up to 1.5 mm. As the inter-electrode displacement increases, the negative glow region expands and the analytes have more residence time in the plasma, allowing for higher ionization efficiencies, and therefore greater analyte intensity. As the electrode gaps increase to 2 mm and beyond, however, the negative glow region begins to shift out of line with the ion transfer capillary, reducing the ionized species that are being directed into the MS inlet.
Conclusions

An evaluation of plasma operating parameters (current, solution flow rate, gas flow rate, and inter-electrode displacement) was performed to evaluate optimal response for a biologically relevant selenomercuroy (HgSeCys) complex, as well as to identify species that were created in the plasma under those conditions. Analyte response was assessed by the superposition of the relative peak intensities under each operating parameter.

The optimal plasma operating conditions for the HgSeCys complex were shown to be less kinetically energetic than those that would be employed for elemental analysis, because the analytical objective is retaining the integrity of the intact complex. Less kinetically energetic conditions are characterized by lower currents, higher gas flow rates, higher solution flow rates, and shorter electrode gaps. In fact, the highest HgSeCys responses were seen with a current of 25 mA, a solution flow rate of 60 µL min\(^{-1}\), a gas flow rate of 0.75 L min\(^{-1}\), and an electrode gap of 0.5 mm.

As the LS-APGD has been interrogated extensively as an ionization source for elemental mass spectrometry, future work should be centralized on using the system for a combination of diverse analytes. To this point, the LS-APGD has shown to be an effective ionization source for small organic compounds and metal-organic complexes, so the next step is to evaluate the LS-APGD as an ionization source for biological macromolecules such as proteins, and then evaluate the system for a combination of diverse analytes.
In particular, it is of analytical interest to evaluate the propensity of Selenoprotein P (SelP) to demethylate and sequester Hg from the methylmercury due to the protein’s high concentration of SeCys residues. Because the LS-APGD-Orbitrap system has the ability to analyze both elemental and molecular species, this will be the next goal in the continuation of this preliminary HgSeCys analysis.
References


CHAPTER FOUR: CONCLUSIONS AND FUTURE STUDIES

Herein the LS-APGD ionization source was interfaced to a Thermo Orbitrap Q Exactive mass spectrometer. First, an evaluation of the operating parameters in each of the four powering modes (solution grounded cathode “SGA”, solution powered cathode “SPC”, solution powered anode “SPA”, and solution grounded anode “SGA”) of the LS-APGD-Orbitrap system was performed for elemental response and isotope ratio isotope and precision.

The solution anode-type powering modes were determined to be not viable due to degradation of the electrodes that they caused, which caused significant contamination of ion lenses of the mass spectrometer due to thermal effects and the sputtering of the counter electrode acting as the cathode. The SPC and SGC powering modes are analytically viable, however, and they demonstrated definitive trends for signal intensity and reproducibility for each parameter. The optimal plasma operating conditions in SGC were determined to be a current of 35 mA, a solution flow rate of 45 µL min\(^{-1}\), a sheath gas flow rate of 0.5 L min\(^{-1}\), and an inter-electrode displacement of 1.5 mm. SPC performed optimally with 30 mA current, 60 µL min\(^{-1}\) solution flow rate, 0.25 L min\(^{-1}\) gas flow rate, and a 1.5 mm inter-electrode displacement. While the SPC mode can lead to high elemental peak intensities by changing the conditions from SGC, it was ultimately decided that the restricted flexibility stemming from the change in instrumental configuration that would be required for SPC restrict the powering mode’s viability for elemental analysis.
In terms of electrode geometry, SGC performs optimally when the solution electrode is oriented directly in line with the ion transfer capillary of the MS, due to the most active ionization region of the plasma, the negative glow, being oriented in front of the MS inlet. The sheath gas also aids in confining the plasma and directing the ionized species toward the ion inlet. In SPC, it is important that the solution electrode is directed at the ITC, however optimal performance is seen when it is at a 45° angle from the counter electrode instead of 90° seen with SGC.

The LS-APGD-Orbitrap system’s performance was then evaluated in both the SPC and SGC modes for isotope ratio analyses, eventually determining that only the SGC powering mode demonstrates sufficiently high accuracy and precision for $^{235}\text{U}/^{238}\text{U}$ isotope ratio measurements using the LS-APGD-Orbitrap system. Though for most parameters tested the SPC mode demonstrates low RSD values, they do not reach the goal value set by the IAEA ITC. However, most operating parameters in SGC did reach this target value. The optimal conditions for $^{235}\text{U}/^{238}\text{U}$ isotope ratio were determined to be 0.5 L min$^{-1}$ gas flow rate, 30-40 mA operating current, 30 μL min solution flow rate, and 1 mm electrode gap, varying slightly from optimal conditions for elemental analysis. The isotope ratio values obtained are always somewhat lower than the accepted value due to the background deletion step that is inherent to the Orbitrap’s software, however, a correction factor derived from an isotopic standard can be used to resolve this in a facile manner.
After the elemental analysis and isotope ratio portion of this project, and evaluation of the plasma operating parameters (current, solution flow rate, gas flow rate, and inter-electrode displacement) was performed to evaluate optimal response for a metal-organic selenomercury (HgSeCys) complex. Analyte response was assessed by the superposition of relative signal intensities under each parameter for the analytes of interest, specifically the intact HgSeCys complex, any produced fragment ions of that complex, internal standard caffeine, and the reactants used to create the complex.

The optimal plasma operating conditions for the HgSeCys complex were shown to be less kinetically energetic than those that would be employed for elemental analysis. Less kinetically energetic conditions are characterized by lower currents, higher gas flow rates, higher solution flow rates, and shorter electrode gaps. In fact, the highest HgSeCys responses were seen with a current of 25 mA, a solution flow rate of 60 µL min\(^{-1}\), a gas flow rate of 0.75 L min\(^{-1}\), and an electrode gap of 0.50 mm.

Future work with the LS-APGD-Orbitrap system will involve continued analysis of diverse analytes. Metal-organic complexation will be a major area of study, continued from the groundwork herein. Perhaps the most interesting future study will involve the use of the LS-APGD-Orbitrap system for the analysis of biomolecules and polymers, and to compare their responses with traditional mass spectrometry methods for these classes of analytes, including MALDI and ESI. The eventual goal for the continuation of this project will be to use the LS-APGD-
Orbitrap system for both qualitative and quantitative analysis of a combined series of analytes. Examples include trace metal contamination in complex media (such as biological samples), and combined detection of both organic and elemental species in pharmaceuticals without extensive pretreatment.
Appendix Figure 1: Power density plots (W mm$^{-3}$) in each powering mode vs increasing a) current, b) solution flow rate, c) gas flow rate, and d) inter-electrode displacement. Except for the value being varied, these parameters were kept as a
control throughout these experiments: discharge current = 30 mA, solution flow rate = 30 µL min\(^{-1}\), gas flow rate = 0.50 L min\(^{-1}\), and inter-electrode displacement = 1.5 mm.

Appendix Figure 2: Contamination on Orbitrap exit lens after the ion-routing S-lens from solution-as-anode powering modes
Appendix Table 1: To determine the cause of the ion optics contamination, cotton swabs were used to scrub off the contamination. The swabs were digested in concentrated nitric acid to dissolve any metal contamination, the nitric acid was boiled away to cause the metals to leach to the sides of a clean beaker, and then the dissolved metals were collected in 2% nitric acid. The collected solution was passed through a syringe filter to collect any residual solid matter, and then it was analyzed by ICP-OES versus a blank cotton swab sample. Elements of interest were those that were present in the analyte solution (Rb, Ag, Ba, Tl, and U) as well as those that make up the electrodes (Fe, Cr, and Ni), and those of any organic species that may be present (C and N). It was concluded that the contamination was not organic, as C and N remained undetected. The high C signal in the blank is due to the organic nature of the cotton swab used in the nitric acid digest. Interestingly, it is also unlikely that the contamination is due to overloading from the analyte solution. Of the five analytes tested, three (Rb, Tl, and U) were undetected. Ba and Ag did increase slightly, but the most evident increase was by the elements that composed the electrodes (Cr, Fe, and Ni). It is possible that minor contamination stems from the analyte solution, however, the bulk of the contamination is coming from the electrodes of the LS-APGD degrading. ICP-OES experiments were performed with Thermo Scientific iCAP 7200 ICP-OES.

<table>
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</tr>
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