An electrochemical instrument for the analysis of heavy metals in water via anodic stripping coulometry for applications in remote sensing.

Kelsey Lynn Kaht
University of Louisville

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AN ELECTROCHEMICAL INSTRUMENT FOR THE ANALYSIS OF HEAVY METALS IN WATER VIA ANODIC STRIPPING COULOMETRY FOR APPLICATIONS IN REMOTE SENSING

By

Kelsey Lynn Kaht
B.A., Thomas More College, 2014
M.S., University of Louisville, 2017

A Dissertation
Submitted to the Faculty of the College of Arts and Sciences of the University of Louisville in Partial Fulfillment of the Requirements for the Degree of

Doctor of Philosophy in Chemistry

Department of Chemistry
University of Louisville
Louisville, Kentucky

December 2019
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A Dissertation Approved on

May 8, 2019

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Dr. Francis Zamborini
DEDICATION

This dissertation is dedicated to my husband

Dr. Dagan Kaht

for his endless support, guidance, care, inspiration, patience, and love.

To my parents

Mrs. Karen Sparks and Mr. Kenneth Sparks Jr.

for their unconditional love, encouragement, and motivation.

To my siblings

Ms. Leandra Sharp, Ms. Katelyn Sparks, Mr. Kenneth Sparks III, and Ms. Kristen Sparks

for filling my life with fun and enjoyable memories.
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I acknowledge my undergraduate research mentor, Dr. William Wetzel, for providing me with a fundamental background in chemistry, fueling my desire to pursue scientific research, and for his support and friendship.

I acknowledge all my family and friends that have supported me through this journey. This would not be possible without your encouragement, love, and inspiration.
ABSTRACT

AN ELECTROCHEMICAL INSTRUMENT FOR THE ANALYSIS OF HEAVY METALS IN WATER VIA ANODIC STRIPPING COULOMETRY FOR APPLICATIONS IN REMOTE SENSING

Kelsey Lynn Kaht

May 8, 2019

From the high levels of arsenic in groundwater in Bangladesh to the lead contamination of drinking water in Flint, Michigan, there are incidents across the globe that highlight the need for a reliable instrument capable of monitoring heavy metals remotely and continuously in a variety of geographical locations. Typical instrumentation for water analysis, such as ICP and AAS, must be housed in a central lab and relies on an operator traveling to the collection site, obtaining a sample, and transporting it back to the lab. This analysis provides a snapshot of the water quality that is limited to the specific time and location of collection. Portable instruments overcome delayed sample analysis time but still require a technician who must travel to the field to operate the equipment. Remote sensing overcomes these limitations as instruments are installed on-site and function autonomously to collect data continuously.

This work is focused on developing an electrochemical technique featuring in situ background correction for applications in remote sensing of heavy metals in water. The technique is based on exhaustive anodic stripping coulometry in a fixed-volume cell and the target analytes are As(III) and Pb(II).
Herein, the electrochemical device was redesigned to improve the detection limits for As(III) using double potential step-anodic stripping coulometry (DPS-ASC) to meet the WHO limit of 10 ppb. Stamp-and-stick fabrication was performed to define and control the sample volume. The gold electrode area was manipulated by fabrication of microelectrode arrays.

The DPS-ASC technique was then optimized for the detection of Pb(II) in water using gold macroelectrodes and microelectrode arrays. Furthermore, the interference of Cu(II) was explored and managed by developing an in-line pre-electrolysis device. The practicality of DPS-ASC for analysis of real samples was evaluated using Ohio River water and the stability of the sensor was evaluated over the course of two weeks by daily analysis of Pb(II) charge. Last, novel boron doped diamond electrodes were evaluated for DPS-ASC analysis of Pb(II).
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CHAPTER I
INTRODUCTION

1.1. Heavy Metals

Heavy metals are defined as those that have an atomic weight between 63.5 and 200.6 g mol\(^{-1}\) and a specific gravity greater than 5 g cm\(^{-3}\) [1]. Few heavy metals are essential elements for human life at trace levels (\(i.e.,\) Cu, Mo, Zn), some are deemed relatively harmless or have an unknown impact on humans (\(i.e.,\) Ag, Ru), and others are classified as toxic (\(i.e.,\) As, Cd, Hg, Pb) [2].

Heavy metals are found naturally within the Earth’s crust. Non-anthropogenic activities such as volcanic eruptions, weathering, and soil erosion contribute to the release of these elements into the environment. However, the main influence in heavy metal contamination is from anthropogenic sources. Processes include mining, smelting, coal burning, agricultural activities, wood preservation, and the production of plastics, textiles, pharmaceuticals, pesticides, and rubber [1,3,4]. Some of the common heavy metals emitted in these industries include Cd, Cr, Cu, Hg, Pb, Ni, Sn, and Zn [5]. Both industrial and agricultural processes generate runoff water and aerosols of heavy metals that are released into the environment and further accumulate in soil and water. Heavy metals are subsequently taken up by aquatic life, undergo biological and chemical transformations, and become stored in sediment [6].
Another method of heavy metal pollution is through accidental or intentional spills of chemical waste. These incidents can further be classified as acute (one-time) or chronic (long-term) exposures. Herein is a listing of some recent one-time occurrences. In 2018, the Hi-Crush Mine released over 10 million gallons of water containing As, Hg, and Pb into a Mississippi river tributary to rescue a trapped contractor [7]. A company owned by U.S. Steel had a pipe failure in 2017 that leached 135 kg of Cr(VI) into a Lake Michigan tributary [8]. In 2015, 3 million gallons of water which contained 22 metals, including As, Cd, Cr, Hg, and Pb, leaked out of the Gold King Mine into the Animas river in Colorado [9].

More often, chronic spills of heavy metals into the environment occur as several industries intentionally dispose of waste, theoretically, per approved waste regulations. However, waste disposal sites have been found insufficient in meeting state, federal, and/or international standards and have allowed heavy metals to accumulate in areas near sources of groundwater [10].

To address improperly managed hazardous waste sites, the United States Congress established the Comprehensive Environmental Response, Compensation, and Liability Act (CERCLA) in 1980 [11]. CERCLA, informally referred to as Superfund, allows the Environmental Protection Agency (EPA) to clean up these contaminated sites. The EPA has taken note of the polluted sites that require long-term cleanup, and these locations are listed on the National Priority List (NPL). As of February 2019, there were 1,337 sites on the NPL, 53 pending locations, and 413 areas that have been remediated and deleted from the NPL [12].
There are 23 heavy metals, including As, Cd, Cr, Hg, and Pb, that pose concern for human health [13]. The common routes of exposure to these metals are by inhalation of particulates in air or by ingestion of contaminated food or water. Several heavy metals are known to be carcinogenic, and additional health effects from exposure include increased blood pressure, increased heart rate, nausea, vomiting, diarrhea, skin rashes, skin lesions, infertility, miscarriage, death, and damage to the brain, lungs, kidneys, and liver [14–16]. The International Agency for Research on Cancer (IARC) lists As, Be, Cd, Cr(VI), and Ni as Group 1 agents (carcinogenic), Pb as a Group 2A agent (probably carcinogenic), and Co as a Group 2B agent (possibly carcinogenic) [17]. The Agency for Toxic Substances and Disease Registry (ATSDR) notes the potential threat of certain substances on human health and subsequently lists As, Pb, Hg, and Cd as #1, #2, #3, and #7, respectively, on the 2017 substance priority list [18].

Part of the impact of heavy metals on human health is due to their ability to bioaccumulate within the body, just as they bioaccumulate in wildlife tissues and the environment via soil and sediment. Some heavy metals, such as As, are cleared from the body within a few hours while others, including Cd and Pb, have a half-life of up to 30 years in the body [19]. The long biological half-lives of certain heavy metals indicate that removal from the body is a slow process, which means that there is more time for these metals to impose harm to the body.

Due to the substantial impact of heavy metals on human health, the World Health Organization (WHO) has set global guidelines for maximum contamination levels of many heavy metals in drinking water. These standards are based on studies of daily tolerable intake of contaminants and the adverse health effects. Due to the variability in
exposure and risk of these health-based targets across the world, the WHO notes that it is necessary for further national regulations to be developed that account for environmental, social, cultural, economic, and dietary conditions effecting exposure [20].

Within the United States, the EPA has developed drinking water standards and health advisory tables. Contaminants in drinking water are evaluated, and the EPA sets forth both non-enforceable health benchmark goals, referred to as maximum contaminant level goals (MCLG), and enforceable standards of the highest level of a contaminant, known as the maximum contaminant levels (MCL) [21]. The MCLG and MCL should thus be considered when evaluating contaminants in drinking water, and subsequent analytical instrumentation should be able to detect substances at these limits. The EPA drinking water regulations for inorganic chemicals (i.e., the category which includes heavy metals) are listed in Table 1.
Table 1. National primary drinking water regulations for inorganic chemicals. “MCLG” maximum contaminant level goal, “MCL” maximum contaminant level, “TT” treatment technique [21].

<table>
<thead>
<tr>
<th>Inorganic Chemical Contaminant</th>
<th>MCLG (ppm)</th>
<th>MCL or TT (ppm)</th>
</tr>
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<tr>
<td>Antimony</td>
<td>0.006</td>
<td>0.006</td>
</tr>
<tr>
<td>Arsenic</td>
<td>0</td>
<td>0.010 as of 01/23/06</td>
</tr>
<tr>
<td>Asbestos (fiber &gt; 10 µm)</td>
<td>7 million fibers per liter (MFL)</td>
<td>7 MFL</td>
</tr>
<tr>
<td>Barium</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>Beryllium</td>
<td>0.004</td>
<td>0.004</td>
</tr>
<tr>
<td>Cadmium</td>
<td>0.005</td>
<td>0.005</td>
</tr>
<tr>
<td>Chromium (total)</td>
<td>0.1</td>
<td>0.1</td>
</tr>
<tr>
<td>Copper</td>
<td>1.3</td>
<td>TT; Action Level=1.3</td>
</tr>
<tr>
<td>Cyanide (as free cyanide)</td>
<td>0.2</td>
<td>0.2</td>
</tr>
<tr>
<td>Fluoride</td>
<td>4.0</td>
<td>4.0</td>
</tr>
<tr>
<td>Lead</td>
<td>0</td>
<td>TT; Action Level=0.015</td>
</tr>
<tr>
<td>Mercury (inorganic)</td>
<td>0.002</td>
<td>0.002</td>
</tr>
<tr>
<td>Nitrate (measured as Nitrogen)</td>
<td>10</td>
<td>10</td>
</tr>
<tr>
<td>Nitrite (measured as Nitrogen)</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Selenium</td>
<td>0.05</td>
<td>0.05</td>
</tr>
<tr>
<td>Thallium</td>
<td>0.0005</td>
<td>0.002</td>
</tr>
</tbody>
</table>

1.2. Instrumentation

The WHO-recommended instrumentation for the detection of heavy metals includes atomic absorption spectroscopy (AAS), inductively coupled plasma (ICP), and high performance liquid chromatography (HPLC) [20]. These instruments are typically housed in a central laboratory and are effective in the determination of heavy metal levels down to the safety standard guidelines. While these instruments offer advantages, such as low detection limits and high selectivity, they rely on having an operator travel to the collection site, obtain a sample, and transport it back to a laboratory for analysis. This delay between sample collection and analysis provides a limited snapshot of the water quality as it is only representative of the particular time and location associated with collection.
Portable instrumentation has been utilized to decrease the delay between sample collection and in-lab analysis. These portable instruments are transported to the site of interest so that an operator can perform the sample collection and analysis on-site, in real time. This allows rapid data collection and decreases risk of sample contamination during collection and transportation. There are several commercial portable instruments for monitoring heavy metals. For example, Labmate sells a portable atomic absorption spectrometer that features a tungsten coil electrothermal atomizer and miniature CCD spectrometer, all battery powered, that is intended for the analysis of As, Cd, Cr, Cu, Pb, and Se [22], and Skyray Instruments features the HM-3000P Portable Water Quality Analyzer which utilizes anodic stripping voltammetry to detect As, Cd, Cr, Cu, Hg, Mn, Ni, Tl, and Zn [23].

Although portable instruments allow for analysis of samples in real-time, which is an advantage over traditional instrumentation, these devices still require a skilled operator to travel to the field and perform the analysis. For areas that require frequent sample analysis or locations that are remote and hard to access, portable instrumentation is not an ideal solution. Instead, the consideration should fall to remote sensors, which are devices that are installed on-site and function autonomously. These instruments have the capability to collect data 24/7 without the need for operator intervention. The experimental findings are sent wirelessly back to the central laboratory so that field analysis is possible without the need for a field technician.

With remote sensing there is also the possibility of developing large sensor networks which would entail multiple sensors installed across numerous sites of interest. These sensors would operate independently but work together as one unit to give real-
time results about a variety of sites. In the case of an unexpected environmental spill, the origin of the contamination source and the downstream impact could be quickly and continuously assessed using such a network, much faster than assessment completed by a field technician returning gathered samples back to a central lab.

Furthermore, a common requirement for analytical instrumentation is the need for calibration and recalibration as the sensor signal may drift with time after exposure to environmental (i.e., real-world) samples. However, when considering remote sensors, a (re)calibration-free method should be explored because it would not be ideal to store calibration standards out in the field since a technician would have to regularly replenish these stocks, defeating the benefit of operator-free analysis. To date, the most plausible methods for developing such a (re)calibration-free system are based in electrochemistry [24].

1.3. Electrochemical Methods

Compared to conventional analytical instrumentation, electrochemistry offers unique advantages and potential. The cost is inexpensive compared to traditional analytical instrumentation, such as AAS, ICP, and HPLC. Electrochemical instrumentation is small, has little power demand, and does not require special installation compared to more sophisticated instruments [25]. The key limitation is that electrochemical measurements are only viable for analysis of redox active species. This includes metals such as Ag, As, Au, Ba, Bi, Cd, Co, Cs, Cu, Ga, Ge, Hg, In, K, Mg, Mn, Na, Ni, Pb, Pt, Rb, Rh, Sb, Se, Sn, Sr, Tc, Te, and Zn [25]. However, this limitation is not
an issue in the analysis of heavy metals as the most toxic metals – As, Cd, Hg, and Pb – are present within this list.

Electrochemistry is described simply as “the science of the application of electricity to chemistry” [26]. There are many ways to execute electrochemical analyses, but the overall goal is to relate electrical energy and chemical change. Analytical electrochemical methods are divided into two categories: potentiometric techniques and amperometric techniques. These methods differ in the property measured, and they offer their own advantages and disadvantages.

The first of the electrochemical methods is potentiometry. Potentiometry is the electroanalytical technique of measuring potential during an experiment in which there is minimal to no current flow [27]. The system is without flow of current, and the potential difference can be related to concentration via the Nernst equation (Equation 1). The main use of potentiometry is focused on ion selective electrodes (ISEs). For example, the pH electrode, which is sensitive to the hydrogen ion, is the most common potentiometric ISE device [28]. pH electrodes measure the potential difference, and the magnitude of the difference is linearly related to the hydrogen ion concentration via the Nernst equation, which is further solved to determine pH as seen in Equation 2. In these equations, E is potential, E⁰ is the standard reduction potential, z is the number of transferred electrons, and C_O and C_R are concentrations of the oxidized and reduced species, respectively.

\[
\text{Equation 1. } E = E^0 + \frac{0.059V}{z} \log \left( \frac{C_O}{C_R} \right)
\]

\[
\text{Equation 2. } E = E^0 + 0.059V(pH)
\]
The second category of electrochemical techniques is amperometry. Amperometry is the method of measuring current in an experiment. Amperometry can further be divided into subdivisions when the current is measured as a function of an independent variable, such as potential (voltammetry) or time (chronoamperometry). These two methods are discussed in detail below.

Within the technique of amperometry, voltammetry is the subset in which current is monitored as potential is changed. The data is recorded in a graph of current versus potential and is referred to as a voltammogram. Figure 1 shows an example voltammogram, specifically a cyclic voltammogram, of a common analyte ferri-/ferro-cyanide, Fe(CN)$_6$$^{3-/4-}$. The solution contained 0.50 mM Fe(CN)$_6$$^{3-/4-}$ in 100 mM KNO$_3$. The potential was scanned from -100 mV to 500 mV and then returned to -100 mV. The change in current is indicative of the electrochemical processes occurring as a result of potential change. In this case, the curves are due to the oxidation and reduction of Fe(CN)$_6$$^{3-/4-}$. Voltammograms are useful to determine the redox potentials for analytes in solution and can also give insight as to which species are likely present in solution and the relative amounts of those species in the solution.
Figure 1. Example voltammogram of Fe(CN)$_6^{3-/4-}$. Scan rate of 0.1 V sec$^{-1}$ from -0.1 V to 0.5 V to -0.1 V.

There are several different types of voltammetry. These include linear sweep, cyclic, square wave, staircase, normal pulse, differential pulse, anodic stripping, and cathodic stripping. These methods differ in the how the potential is applied and the potential waveform. For example, linear sweep voltammetry gradually changes the potential linearly via a sweep from one value to another. The potential is scanned at a selected rate in units of voltage per time between the two values. This is particularly useful for determination of E$_{1/2}$ and in the analysis of irreversible reactions. Anodic stripping voltammetry, on the other hand, is focused on the quantitative determination of specific analytes. In this technique, a potential is applied to reduce analytes onto the working electrode surface, and then a stripping step is performed during which the current is monitored. Whereas linear sweep voltammetry scans potential linearly, anodic stripping voltammetry changes potential either by linear, pulse, square wave, step, or staircase methods. Anodic stripping voltammetry differs from linear sweep voltammetry
in how the potential is applied, thus leading to different results and applications for these methods. The type of voltammetry performed is typically based on the desired application noting sample characteristics such as concentration and mass transport.

Chronoamperometry is the subclass of amperometry wherein current is measured as a function of time. In constant potential chronoamperometry, a potential is applied via a potential step method to cause an analyte in solution to undergo oxidation or reduction, and the current is monitored over time at that potential. This is referred to as single potential step chronoamperometry. Double potential step chronoamperometry is associated with the application of one potential to perform an oxidation/reduction followed by a step to another potential for the complimentary reduction/oxidation, all while monitoring current. In both cases, the current and time are monitored at the applied potential, and the current eventually decays toward zero as the reaction proceeds to completion. The resulting graph of current versus time is called an amperogram. An example amperogram from a single potential step is shown in Figure 2. This shows an instantaneous decay in current due primarily to charging current (i.e., non-Faradaic current) which decays exponentially as any RC circuit. The amperogram is also composed of Faradaic current which decays according to the Cottrell equation (Equation 3) for reversible processes at planar electrodes that are limited by diffusion. This equation defines the relationship between this current (i), number of electrons (n), Faraday’s constant (F), electrode area (A), concentration (C), diffusion coefficient (D), and time (t) [29]. In chronoamperometry, this equation is relevant in predicting current as a function of t^{-1/2}. Accordingly, a plot of i(t) versus t^{-1/2} will be linear. Any deviations from linearity
indicate that the electrochemical reaction is not exclusively diffusion-controlled or that the electrode geometry is non-planar.

**Figure 2.** Example amperogram of 2 ppm Pb(II) in 10 mM HNO₃/10 mM NaCl. At t=0, the potential was stepped from 500 mV to -400 mV and then held at -400 mV for 2 minutes.

**Equation 3.**  
\[ i = \frac{nFAC\sqrt{D}}{\sqrt{\pi t}} \]

As mentioned, an amperogram is a graph of current (y-axis) versus time (x-axis). The amperogram curve can be integrated and the area under the curve corresponds to the total charge in units of coulombs. The technique in which charge is measured is referred to as coulometry. The most common subset of coulometry is controlled potential coulometry in which the potential is kept constant as current versus time is measured (akin to chronoamperometry), but then the amperogram is integrated to determine the total charge [30]. The charge is associated with the species in solution that underwent an oxidation or reduction at the specific applied potential.
In the nineteenth century, Michael Faraday proposed two laws of electrolysis that relate charge to the amount of substance oxidized or reduced [31]. Faraday’s first law (Equation 4) states that the mass of electrolyzed material \( m \) is proportional to the total quantity of passed electricity or charge \( Q \). The electrochemical equivalent of the substance \( k \) is also included in Equation 4 and is a constant that is proportional to the mass of the substance deposited during electrolysis with the passing of 1 Coulomb of charge. Faraday’s second law (Equation 5) states that the mass of deposited material \( m \) is proportional to its equivalent weight, which is the molecular mass \( M \) divided by the number of transferred electrons \( z \) [30]. Charge \( Q \) and Faraday’s constant \( F \) are also included in this equation. Faraday’s second law of electrolysis can be solved for concentration \( C \) knowing the sample volume \( V \), as shown in Equation 6. These principles are fundamental in using coulometry for the absolute determination of concentration of analyte in solution and will be referred to throughout this dissertation.

Equation 4. \[ m = kQ \]

Equation 5. \[ m = \left( \frac{Q}{F} \right) \left( \frac{M}{z} \right) \]

Equation 6. \[ C = \frac{Q}{FzV} \]

1.4. Background

Our research group has been focused on the electrochemical detection of species in thin-layer volumes for the past decade [33–37]. These microfluidic devices have been created using microfabrication techniques such as stamp-and-stick and photolithography. Several on-chip variations of electrochemical sensors have been developed and
subsequently utilized for the determination of pH [33] and for the electrochemical determination of Fe(CN)$_6^{3/-4}$ [34] and Cu(II) [35]. In the past few years, the research focus has shifted to the development of electrochemical sensors for possible applications in remote sensing.

Most recently, Marei et al. developed a novel method termed double potential step-anodic stripping coulometry (DPS-ASC) [36]. DPS-ASC is a specific coulometric technique in which a series of potentials is applied to perform *in situ* background correction. DPS-ASC allows for a (re)calibration-free experiment in which the analyte solution serves as both the blank and the analyte. This is ideal for a remote sensor which would be installed on-site in the field and left to operate autonomously. The overall method is much simpler as (re)calibration (*i.e.* calibration after the initial volume calibration of the device) is unnecessary and device maintenance on-site would be reduced as there is no need for replenishment of calibration standards.

DPS-ASC entails a four-pulse sequence based on chronoamperometry. Figure 3 shows an example of the four amperograms that are produced from this four-pulse series. There is no applied potential prior to this pulse sequence. DPS-ASC begins with a short-time deposition (Figure 3 pulse 1) which is indicative of non-Faradaic processes, such as charging current. This time is short enough to prevent significant analyte deposition but long enough to charge the electrode surface. Subsequently, a stripping step occurs (Figure 3 pulse 2), and the resulting amperogram can be integrated (Figure 4 A) to determine the charge, primarily due to non-Faradaic current, that results from the previous short-time deposition. Then, a long-time deposition (Figure 3 pulse 3) allows for both exhaustive deposition of analytes and charging of the electrode surface. Therefore, this pulse is
representative of Faradaic + non-Faradaic charges. Finally, a stripping step (Figure 3 pulse 4) occurs to determine the charge related to the long-time deposition. This stripping step is integrated as before (Figure 4 B).

![Graph showing pulse sequences](image)

Figure 3. Example sequence of double potential step-anodic stripping coulometry.

![Graph showing integrations and subtractions](image)

Figure 4. Visual representation of A) integrating amperograms for stripping pulse 2 (gray), B) integrating amperograms for stripping pulse 4 (black), and C) subtraction of pulse 2 charge from pulse 4 charge (maroon).
Ideally, the charge from the two stripping amperograms would be the same as the charge from the two deposition amperograms. However, the deposition steps rely on diffusion and thus require a longer experiment time. Integration of amperograms from the deposition steps includes more noise because the experiment time is increased, and this noise is not favorable. Therefore, the two stripping amperograms are integrated, as opposed to the two deposition amperograms, because the stripping processes do not rely on mass transport like the deposition steps.

The stripping amperograms (Figure 3 pulses 2 and 4) are overlaid on top of each other, and the resulting figure produces a crescent shape defined by the blue and yellow traces in Figure 4. The area inside of that crescent, marked in maroon in Figure 4 C, is the charge that corresponds only to Faradaic charge. By performing this potential step sequence, the analyte sample is used in correcting for the background/charging current. Because DPS-ASC allows for \textit{in situ} background correction, it is considered for applications in remote sensing as this method offers simplicity which is ideal for autonomous operation.

The use of DPS-ASC for the analysis of As(III) in a thin-layer cell was explored by Marei \textit{et al.} [36]. This work evaluated the DPS-ASC method for the \textit{in situ} background-corrected detection of As(III) in standard solutions, solutions containing interferent metals, and samples of Ohio River water. However, in this work, the achieved detection limit of 75 ppb did not meet the EPA maximum contamination level of 10 ppb for As. This was the point at which this work began, with the goal being \textit{in situ} background-corrected analysis of heavy metals with detection limits below the EPA guidelines.
As mentioned previously, heavy metals pose a threat to the environment and human health. Therefore, the focus of this work will be on the detection of heavy metals – specifically As(III) and Pb(II) – as these metals are ranked highest on the ATSDR substance priority list. The electrochemical detection of these metals has been explored previously by others via voltammetry and amperometry, but the application of DPS-ASC is unique in achieving in situ background-corrected results. As such, this method is well suited for novel remote sensors that do not require (re)calibration.

### 1.5. Chapter Overview

The overall focus of this dissertation is in developing a (re)calibration-free electrochemical method for the detection of heavy metals in water. The ideal application for this work would be in remote sensing and in the development of sensor networks that could be installed across a location to monitor real-time environmental spills.

Chapter II emphasizes changes in the microfabrication sequence of gold electrode sensors for use in DPS-ASC of As(III) in water. A room-temperature bonding technique, stamp-and-stick, is evaluated to create devices of known thicknesses and volumes. According to Faraday’s second law of electrolysis, a larger volume correlates to a larger charge (\textit{i.e.}, signal) which would present an opportunity to reach lower limits of detection by improving the signal-to-noise ratio.

Another technique to reach lower detection limits is to decrease the electrode area, thus reducing noise due to charging current. This work is explored in Chapter III. The gold electrode sensors are modified into arrays of microelectrodes of varied
diameters. The performance of these microelectrode arrays is evaluated by DPS-ASC analysis of As(III) in water, and the impact of interfering metals is explored.

Chapter IV focuses on a new analyte, Pb(II). Considering the recent water crisis in Flint, Michigan, this analyte is explored further to determine if DPS-ASC would prove useful for remote sensing of Pb(II). Both the gold macroelectrode and gold microelectrode arrays are evaluated in the detection of Pb(II). The interference of Cu(II) is explored, the practicality of DPS-ASC for Pb(II) analysis in Ohio River water is reviewed, and the stability of Pb(II) charge over time is evaluated.

Then, a novel electrode material, boron doped diamond, will be utilized for the detection of Pb(II) by DPS-ASC in Chapter V. Finally, further considerations and conclusions will be discussed in Chapter VI.
CHAPTER II

STAMP-AND-STICK FABRICATION OF ELECTRODE SENSORS AND APPLICATION IN DPS-ASC ANALYSIS OF ARSENIC

2.1. Introduction

Previously, our research group developed an electrochemical device for the coulometric detection of Cu(II) and Hg(II) in water [35]. This apparatus is comprised of two fluidic chambers that are separated by a membrane to isolate a gold working electrode in the bottom chamber from the graphite counter electrode and miniaturized Ag/AgCl reference electrode located in the top chamber (Figure 5). Fluidic valves are located at the inlet and outlet of each chamber for stopped-flow analysis.

Figure 5. Simplified schematic of the electrochemical detector.
Under stopped-flow conditions, DPS-ASC is performed to determine charge which is then related to concentration through Faraday’s second law of electrolysis (Equation 6). To successfully determine the concentration using Equation 6, all other variables (F, z, and V) must be known. While F is a constant and z is known for each metal redox process, V is dependent on the geometry (i.e., the width, length, and height) of the working electrode chamber.

Our electrochemical device was designed to have a volume of 1-2 μL contained in the working electrode compartment. While the cell volume is set at a specific value once the coulometry device is assembled, reassembly of the apparatus can change the volume due to the nature of the materials used in construction. Specifically, the component that defines the working electrode chamber walls is flexible silicone gasket rubber with a specified thickness of 125 μm ± 75 μm (as purchased). This gasket is sandwiched between the working electrode and the membrane, as seen in Figure 6. Due to the extensive compressibility and variability of the original thickness of this material, the height of the gasket can change unpredictably from one assembly to another, which decreases reproducibility of the volume between assemblies. In addition, it is not possible to specify an exact volume in the working electrode chamber, meaning that experiments cannot compare devices of different sizes to explore relationships between volume and detection limit.
Accordingly, the focus of this chapter is to redesign the coulometry device in order to find a substitute for the silicone gasket layer and thereby make possible an exact and reproducible volume in the working electrode chamber. There are many requirements that the replacement layer must meet. Most important, the layer must not be compressible in order to provide and maintain a fixed cell height between device assemblies. The material used must be chemically compatible with solutions containing heavy metals in aqueous solutions of pH 2 and should not degrade or swell over time. It is important that the material makes a proper seal between the working electrode and the membrane to avoid fluidic leaks between layers. The material must be able to be cut or etched to allow for the elliptical hole to be created through the piece to define the chamber walls. Finally, the height of the chosen material must be near 100 μm with the capability of changing the height to 200 μm or 300 μm as desired.

The coulometry device volume is being manipulated not only to enhance reproducibility between device assemblies of the same electrode, but also to facilitate adjustment of the cell volume when necessary. Increasing the volume is desirable.
because larger sample volumes correlate to a higher signal in the form of charge, per Faraday’s second law of electrolysis (Equation 6). This larger signal paired with the same noise, due to a controlled electrode area, should improve the overall signal-to-noise ratio and thus allow lower detection limits. However, a larger volume, and thus a taller working electrode chamber with a longer diffusion distance, will require a longer time for complete, exhaustive deposition. This is predicted by the relationship between time (t), diffusion distance (x), and diffusion coefficient (D), as indicated in Equation 7 [27].

Equation 7. \[ t \approx \frac{x^2}{2D} \]

Techniques that will be evaluated to alter the volume of the working electrode chamber include 3D printing, microfabrication, and stamp-and-stick fabrication. The use of 3D printing is becoming more common in microfluidics and offers potential for unique geometries, microfabrication is more routinely used in the creation of microdevices, and stamp-and-stick fabrication is considered due to the previous success of this method in our group for other applications [32]. The most successful of these three methods will be utilized further for the proof-of-concept analysis of As(III) in water using DPS-ASC.

The study of As is of interest because it is ranked as the #1 substance on the 2017 Agency for Toxic Substances and Disease Registry (ATSDR) substance priority list and is classified by the International Agency for Research on Cancer (IARC) as a Group 1 known human carcinogen [18,37]. The World Health Organization (WHO) estimates that over 200 million people are exposed to As in drinking water at levels above the 10 ppb safety guideline [38]. Greater than 10 ppb As has been reported in drinking water in large
regions of Bangladesh, China, and India, and in portions of Argentina, Australia, Chile, England, Ghana, Mexico, Taiwan, the United States, and Vietnam [39–41].

The primary route of As exposure is by ingestion of contaminated food and water with an average daily intake of 20-300 µg per day for the general population, making As the 12th most common element in the human body [42]. Health effects from overexposure to As have been associated with the development of cancers, skin lesions, diabetes, cardiovascular disease, respiratory symptoms, and peripheral neuropathy [43–45].

As is the 20th most common element in the Earth’s crust and has been found in more than 200 mineral species, of which arsenopyrite is most prevalent [42]. In addition, As is the 14th most abundant element in seawater [46]. Sources of As contamination of air, water, and soil include mining, metal smelting, volcanic activity, pesticide production, and wood preservation [47].

As primarily exists in four oxidation states, including -III (arsine), 0 (arsenic), III (arsenite), and V (arsenate) [48]. As(V) species dominate at basic pH values and/or under oxidizing conditions. As(V) is typically present as the triprotic arsenic acid (H₃AsO₄) or one of its conjugate bases (H₂AsO₄⁻, HAsO₄²⁻, and AsO₄³⁻). As(III), on the other hand, occurs as arsenous acid (As(OH)₃) in reducing conditions and/or at acidic pH values. The occurrence of each the species is related to applied potential and pH, as outlined in the As-O₂-H₂O Pourbaix (Eh-pH) diagram [49]. The chemical structures of the common As compounds are shown in Figure 7 [50]. Trivalent forms of As, including As(-III) and As(III), are 60 times more toxic than As(0), As(V), and organic As compounds [51]. Due to its high toxicity and wide prevalence, this work focuses specifically on the detection of As(III).
Figure 7. Chemical structures of common As compounds.

The electrochemical detection of As(III) has been investigated by others. Forsberg et al. reported on the determination of As(III) via anodic stripping voltammetry and differential pulse anodic stripping voltammetry in 1975 [52]. They found that a gold electrode was better suited than a platinum, silver, or hanging mercury drop electrode for As(III) analyses because gold has a higher hydrogen overpotential than platinum. Several others have since used gold electrodes for As(III) analysis via anodic stripping voltammetry [53–56]. Simultaneous detection of As(III) in the presence of other metals, such as Cu(II), Hg(II), and Pb(II), has been reported using gold electrodes with sub ppb
detection limits [57–61]. Furthermore, devices have been fabricated for the on-site electrochemical analysis of As(III) [62,63]. Huang and Dasgupta developed a portable device for the on-site determination of As(III) in potable water by anodic stripping voltammetry on a gold film electrode with a 0.5 ppb detection limit. Then they determined total As by adding an oxidant to solution to convert all As(III) to As(V) and then analyzed As(V) by anodic stripping voltammetry in highly acidic conditions at a more negative potential [62]. Feeney and Kounaves, on the other hand, created a portable sensor featuring a gold ultramicroelectrode array for As(III) analysis in groundwater via square wave anodic stripping voltammetry with a 0.05 ppb limit of detection [63].

Review of the literature gave insight into the appropriate choice of electrode material (gold) and experimental conditions (addition of chloride) for As(III) detection. However, despite the accomplishments thus far in the literature, there is still a need for an electrochemical method that does not require (re)calibration and is ultimately suited for remote, operator-free analysis of As(III). Therefore, this work will focus on developing DPS-ASC for As(III) detection as this method entails in situ background correction which is compatible with remote sensing and thus fills the current void in environmental analysis of As(III).

In the determination of As(III) using DPS-ASC, the electrochemical reaction of arsenous acid (As(OH)$_3$), seen below, was studied. The $E^0$ for this reaction is 0.035 V vs. Ag/AgCl [64]. Due to the acidic conditions and the potentials applied herein, other reactions involving As(III) did not occur in this work but are listed in Appendix I for review. As(V), not studied in this work, is known to be electrochemically inactive in the absence of certain complexing agents and is therefore less studied compared to As(III). If
electrochemical determination of total As is desired, it is most common to first chemically convert As(V) to As(III) and then perform anodic stripping voltammetry analysis of As(III). Detailed information on the underpotential deposition potential of As(III) on gold was not available, but monolayer coverage of As(0) was avoided herein, as discussed later.

\[ \text{H}_3\text{AsO}_3 + 3\text{H}^+ + 3\text{e}^- \leftrightarrow \text{As(s)} + 3\text{H}_2\text{O} \]

2.2. Materials and Methods

2.2.1. Chemicals, Reagents, and Solution Preparation

All chemicals and reagents were purchased at the highest purity and used without further purification. Nitric acid and 1,000 ppm single element atomic absorption standards (AAS) for As(III) (from As$_2$O$_3$) and Cu(II) (from Cu(NO$_3$)$_2$) were purchased from Sigma Aldrich (St. Louis, MO). Buffered oxide etch (BOE) (6:1), acetone, sodium chloride, and sulfuric acid were purchased from VWR (Radnor, PA).

All solutions were prepared fresh each day using deionized water. 10 mM HNO$_3$/10 mM NaCl was prepared in a 1 L volumetric flask by addition of concentrated HNO$_3$ via pipet and the appropriate mass of NaCl via analytical balance to deionized water in the flask. 10 ppm As(III) standard was prepared by pipetting 0.50 mL of 1,000 ppm As(III) AAS into a 50 mL volumetric flask and using 10 mM HNO$_3$/10 mM NaCl as diluent. Subsequent As(III) standards were prepared by pipetting the appropriate amount of 10 ppm As(III) into 50 mL volumetric flasks and diluting to the mark with 10 mM HNO$_3$/10 mM NaCl.
Safety is of utmost concern due to the toxic nature of As(III). Skin contact with As(III) was avoided by wearing protective safety goggles, long-sleeve shirts, long pants, closed-toe shoes, and nitrile examination gloves from VWR (Radnor, PA) during all solution preparations. Spills of As(III) solutions were cleaned with paper towels that were disposed of in appropriate solid waste containers.

Handling of BOE involved additional precautions due to the hazards of aqueous hydrofluoric acid. Hydrofluoric acid is a contact poison that induces painless burns and can ultimately interfere with calcium metabolism and cause cardiac arrest and/or death. Therefore, specific personal protective equipment for handling BOE was always worn in addition to the eyewear, clothing, shoes, and gloves listed before. The additional specialized equipment for handling BOE included a full-face shield, an acid-resistant long-sleeve apron, and elbow-length neoprene gloves. The BOE was kept contained within a single fume hood designated for use of hydrofluoric acid, and solutions were stored in plastic containers. Calcium gluconate gel and eyewash kits were kept readily available in the lab in case of hydrofluoric acid spills and exposures.

All solution waste was collected in containers provided by the Department of Environmental Health and Safety (DEHS) at the University of Louisville. Waste BOE was collected in a separate container, whereas all other acidic solutions were collected in general acidic waste containers. Solid waste was collected in a solid waste disposal bucket. Once the containers were full, DEHS was notified to collect the containers and undergo proper disposal per federal regulations.
2.2.2. Gold Working Electrode

A thin-film gold macroelectrode was prepared using equipment in the Micro/Nano Technology Center (MNTC) at the University of Louisville (Louisville, KY). The details of electrode fabrication are listed in Appendix II. Briefly, a 4-inch oxidized silicon wafer was placed into a Lesker PVD-75 thin film deposition system to deposit 20 nm of titanium followed by 120 nm of gold onto the wafer via sputtering. Then, the wafer was diced into individual 30 mm by 13 mm chips using a Disco programmable dicing saw.

2.2.3. Working Electrode Compartment

The working electrode compartment was built directly on top of the rectangular 30 mm by 13 mm gold electrode chips via three different techniques – 3D printing, microfabrication, and stamp-and-stick fabrication – discussed individually.

2.2.3.1. 3D Printing

Fused deposition modeling, an extrusion-based 3D printing method, was utilized to print the bottom working electrode chamber directly onto the working electrode chip to create one part. A MakerBot Replicator 2X 3D Printer at FirstBuild (Louisville, KY) was used to extrude a thermoplastic filament, acrylonitrile butadiene styrene (ABS), onto the electrode surface to encapsulate the chip while leaving an open, elliptical reservoir in the center to define the cell volume. The electrode chip was heated at 200 ºC on the printing platform before extrusion to promote better adhesion between the chip and acrylonitrile butadiene styrene. Once printing was complete, the acrylonitrile butadiene styrene
solidified at room temperature, and a subsequent treatment with acetone annealed any printing imperfections.

2.2.3.2. Microfabrication

A permanent photoresist, known as SU-8, was utilized to build the cell compartment directly on top of the microfabricated electrode. SU-8 is an epoxy-based negative photoresist commonly used in microfluidics and in the creation of MEMS devices [65]. Two methods using SU-8 were evaluated: SU-8 on gold and SU-8 on silicon nitride (Si₃N₄). Figure 8 and Figure 9 show the fabrication sequences for the SU-8 on gold and on silicon nitride, respectively. The gold wafer was created using the method discussed in section 2.2.2. The wafer with the silicon nitride surface was created by starting with an oxidized silicon wafer and subsequently layering 20 nm titanium, 120 nm gold, and 300 nm silicon nitride. This wafer was ordered from the Minnesota Nano Center at the University of Minnesota (Minneapolis, MN). Processing of the SU-8 was completed using equipment within the University of Louisville MNTC.
Figure 8. Microfabrication sequence of SU-8 photolithography and processing on a gold substrate. A) Spinning of SU-8 onto the gold, B) exposure and patterning of the ellipse and channels into the SU-8.

Figure 9. Microfabrication sequence of SU-8 photolithography and processing on a silicon nitride substrate. A) Spinning of SU-8 onto the silicon nitride, B) exposure and patterning of ellipse and channels into SU-8, C) etching of silicon nitride using BOE.
Photolithography using SU-8 50, SU-8 100, SU-8 2100, and SU-8 3000 was explored. Each version of SU-8 offered unique benefits in processing, adhesion, and film thickness. The most successful SU-8 recipe, described in detail in Appendix II, is discussed here briefly. SU-8 was spun onto the wafer via Headway Spinners at 500 rpm for 10 sec followed by 3,000 rpm for 30 sec to produce a 100 μm thick layer of the SU-8. A soft bake procedure in a YES Polyimide Oven ramped the temperature of the wafer from 50 ºC to 115 ºC to 50 ºC over a span of two hours. Then, a Süss Mask Aligner was used to pattern and expose the SU-8 for 65 sec. The wafer then endured a post exposure bake via the same conditions as the soft bake. The SU-8 was developed in a solution of SU-8 developer to remove unexposed SU-8 from the chamber ellipse and inlet/outlet channels. A hard bake was completed on a hot plate at 200 ºC for 10 min to strengthen the photoresist before dicing into 30 mm by 13 mm individual chips. Finally, for the wafer with a layer of silicon nitride, the exposed silicon nitride was etched away using the vapor from 6:1 BOE over 16 hours to expose the gold electrode.

OmniCoat, an adhesion promoter for SU-8, was explored to ensure proper adhesion of SU-8 to the gold and silicon nitride substrates. This adhesion layer was spun onto the substrate before the addition of the SU-8. The processing of OmniCoat is explained in Appendix II and was investigated for SU-8 50, SU-8 100, SU-8 2100, and SU-8 3000 photoresists.

2.2.3.3. Stamp-and-Stick Fabrication

A room temperature bonding technique, called stamp-and-stick, involves the use of an optical adhesive to anneal two materials together to create one part. This method
was explored to create electrode chips that had a defined volume. Norland optical adhesive (NOA 68) from Norland Products (Cranbury, NJ) was spun via Headway Spinners to a 10-20 nm thickness on a bare silicon wafer in the MNTC at the University of Louisville (Louisville, KY). An AB-M Inc Aligner was used to transfer NOA 68 from the silicon wafer onto one of many substrates (i.e., glass) via a stamping process, as seen in Figure 10. Then, the coated substrate was aligned and put into contact with the gold electrode and set to cure for 300 sec using UV light.

![Diagram](image.png)

**Figure 10.** Stamp-and-stick manufacturing process using a glass substrate as an example. A) Silicon wafer coated with NOA 68 brought into contact with glass, B) silicon wafer removed, leaving a thin layer of NOA 68 on the glass, C) glass with thin layer of NOA 68 is brought into contact with a gold electrode, D) UV light is passed through the glass for 300 sec to cure the NOA 68.

In addition to the requirements for volume-defining materials listed previously, such as rigidity and chemical compatibility, the substrates used in stamp-and-stick must also be UV transparent to allow the NOA 68 to optically cure. Materials that were explored include transparency paper from Staples (Framingham, MA), 0.005 inch thick acetate rolls, polystyrene, 0.005 inch thick polyvinyl chloride, polyethylene terephthalate, and 0.005 inch thick polycarbonate sheets from TAP Plastics (Stockton, CA), crosslinked
SU-8 photoresist fabricated at the University of Louisville (Louisville, KY), and borosilicate microscope cover glass of size #0 (thickness 0.085-0.13 mm), #1 (thickness 0.13-0.17 mm), and #2 (thickness 0.19-0.23 mm) from Ted Pella, Inc. (Redding, CA).

Each of the materials explored for stamp-and-stick were first laser cut to create the 8 mm by 4 mm ellipse with 5 mm by 1 mm channels on each end. This design was embedded into a rectangular piece that was 30 mm by 10 mm. Then, each piece was coated with NOA 68, aligned, put into contact with a 30 mm by 13 mm gold working electrode, and exposed to UV light for 300 sec to cure and create electrode sensor chips.

2.2.4. Coulometry Device

The previously described coulometry device [36] was modified with a restructuring of the working electrode chip and membrane configuration. The device features a working electrode compartment and a counter/reference electrode compartment that are separated by a membrane and have external fluidic flow shut-off valves. Starting from the bottom, as depicted in Figure 11, the polycarbonate base is recessed to hold the 30 mm by 13 mm working electrode chip. The membrane was redesigned so that the 200 molecular weight cutoff (MWCO) membrane from Koch Membrane Systems (Wilmington, MA) was laser cut into an 8 mm by 4 mm ellipse and then embedded in Kapton tape to create a 30 mm by 13 mm piece that was affixed on top of the working electrode chip to define the top of the working electrode compartment. The membranes were replaced after 10-15 days of use. Three laser-cut silicone gaskets were then added on top of the membrane piece to define the height of the counter/reference electrode compartment. The Panasonic pyrolytic graphite sheet counter electrode (Newark, NJ) was
positioned atop the gaskets. One final gasket to protect the counter electrode was added, followed by the polycarbonate top. A custom miniature Ag/AgCl reference electrode was inserted through an access hole in the polycarbonate top into the counter/reference electrode compartment. The polycarbonate top and bottom pieces were screwed together using four screws, and the torque on each screw was set to 0.51 kg-cm using a torque-measuring screwdriver. The device was assembled at the beginning of each day and disassembled at the end of each day. All gaskets were washed twice a week with soap and water to prevent grease build-up and contamination.

Figure 11. Schematic of the coulometry device assembly.
2.2.5. Electrochemical Measurements

The three-electrode system encompassed a fabricated gold macroelectrode for the working electrode, custom miniature Ag/AgCl reference electrode fabricated in-house described previously [35], and a pyrolytic graphite sheet as the counter electrode. The various gold macroelectrodes utilized in this work are summarized in Appendix III. The miniature Ag/AgCl reference electrode was inserted into the coulometry device through an opening in the polycarbonate top and was sealed in-place using chemically compatible silicone grease. Reference electrodes were replaced every 5-10 days to ensure proper reference conditions (i.e., no potential drift). The pyrolytic graphite sheet was replaced after one month of use. A BASi Epsilon potentiostat (West Lafayette, IN) was used for all electrochemical measurements.

The following procedure was utilized for DPS-ASC experiments. First, the counter/reference electrode chamber was filled with 10 mM HNO₃/10 mM NaCl and fluid valves were closed. This solution was not replaced throughout the following series of experiments. Then, the working electrode chamber was filled with 50 mM H₂SO₄ and the gold working electrode was electrochemically cleaned by slowly flushing the 50 mM H₂SO₄ through the chamber as the potential was cycled from 0 mV to 1,400 mV at 100 mV sec⁻¹ for 4-6 scans until cyclic voltammetry traces were reproducible. This was necessary to eliminate errors due to electrode fouling. Then, the working electrode chamber was filled with analyte sample (blank or As(III) solution) and fluid valves were closed before applying the DPS-ASC potential sequence. The DPS-ASC experiments were repeated at least three times for each analyte sample. Between each experiment, the fluid valves were opened, the working electrode chamber was filled with fresh analyte.
sample, and fluid valves were closed. Once all analyte samples were evaluated, the working electrode was cleaned at the end of the day by flushing 50 mM H$_2$SO$_4$ through the chamber as the potential was cycled from 0 mV to 1,400 mV until traces were reproducible.

DPS-ASC employs a four-pulse sequence encompassing a quick reduction/oxidation, representative of non-Faradaic charge, and a long, exhaustive reduction/oxidation, indicative of non-Faradaic + Faradaic charge. The Faradaic charge, corresponding to As(III) background-corrected charge, is determined by subtracting the stripping charge from the quick step (pulse 2) from the long, exhaustive step stripping charge (pulse 4). For As(III) standards, the DPS-ASC pulse sequence, as described in Chapter 1 and outlined in Figure 3, is listed below. There was no applied potential prior to this pulse sequence.

Pulse 1: -600 mV for 0.1 sec
Pulse 2: 500 mV for 1 sec
Pulse 3: -600 mV for 130 sec to 480 sec (time varied based on volume)
Pulse 4: 500 mV for 1 sec

By overlaying the two oxidation traces (pulse 2 and pulse 4), the data appears in the shape of a crescent along an x-axis of time and y-axis of current as shown previously in Figure 4. These current-time curves (amperograms) are integrated to obtain charge and the difference between the curves provides the Faradaic charge. The integration of the current-time curves was completed by summing the area under the curves until the traces met one another. The area represents charge with the units of coulombs (ampere
multiplied by second). All integrations and data processing were done by extracting text files from the BAS Epsilon software and using Microsoft Excel to perform calculations.

2.3. Results and Discussion

2.3.1. 3D Printing

Fused deposition modeling was successful in encapsulating a gold electrode with the thermoplastic filament (acrylonitrile butadiene styrene) and is shown in Figure 12. There were some challenges with getting acrylonitrile butadiene styrene to create a proper seal to the gold electrode, but these were overcome by post-treatment of the device with acetone. Acetone temporarily dissolved acrylonitrile butadiene styrene so that the gap between the gold electrode and acrylonitrile butadiene styrene was filled. Then, the acetone was evaporated away to allow the acrylonitrile butadiene styrene to reharden. However, due to the printing resolution of the fused deposition modeling 3D printer, the smallest achievable cell height was 700 μm which was not acceptable for our work as we desired 100 μm height. Therefore, this method was ceased.

Figure 12. Gold electrode encapsulated in acrylonitrile butadiene styrene via fused deposition modeling, untreated (left) versus acetone-treated (right).
2.3.2. Microfabrication

A multitude of microfabrication recipes were evaluated to pattern a 100 µm thick layer of SU-8 photoresist atop gold or silicon nitride. While the SU-8 was successfully patterned to create the desired ellipse and channel geometry, the adhesion of the SU-8 to the underlying gold or silicon nitride was poor, and the developed SU-8 would inevitably peel away from the substrate. The adhesion issues were obvious under microscope inspection wherein the SU-8 was seen peeling away from the substrate as indicated by the green regions in Figure 13. OmniCoat, a SU-8 adhesion promoter, was tested to overcome the adhesion issues. However, this was also unsuccessful. After several months of working with SU-8 with no successes, this process was dismissed.

Figure 13. Microscope images showing poor adhesion (*green*) of the SU-8 (*pink*) to the underlying substrate (*gray*).

2.3.3. Stamp-and-Stick Fabrication

Transparency paper, acetate, crosslinked SU-8 photoresist, polystyrene, polycarbonate, polyvinyl chloride, polyethylene terephthalate, and borosilicate glass were all tested for their adhesion to gold substrates using NOA 68 optical adhesive. Transparency paper and acetate did not adhere to gold; so they were eliminated. Due to
the complex fabrication of crosslinked SU-8, use of this material was excluded. Polystyrene was not available in sheets of 100 µm thickness; so it was also removed as an option. Therefore, devices made from polycarbonate, polyvinyl chloride, polyethylene terephthalate, and borosilicate glass using stamp-and-stick were left for further evaluation of volume reproducibility and reliability.

Ultimately, borosilicate glass was determined to be the most successful material for creating devices of different heights that were non-compressible and chemically compatible. The chemical composition of this glass is 64.1% SiO₂, 8.4% B₂O₃, 6.9% K₂O, 6.4% Na₂O, 5.9% ZnO, 4.2% Al₂O₃, 4.0% TiO₂, and 0.1% Sb₂O₃. The borosilicate glass has excellent resistance to chemical attack and is therefore well suited for exposure to acidic solutions containing heavy metals.

An example of laser-cut borosilicate glass bound to a gold electrode by stamp-and-stick fabrication is shown in Figure 14. By varying the thickness of the borosilicate glass used from 80 µm to 230 µm in the fabrication of the gold macroelectrode chips, the volume of the working electrode compartment was able to be manipulated. Devices of different working electrode chamber volumes were created and electrode chips of volumes 4.3 µL, 5.6 µL, and 6.8 µL were subsequently analyzed using DPS-ASC for the detection of As(III). The volume was calculated using device geometry which was confirmed by measurements using a micrometer. Exhaustive electrolysis of Cu(II) was performed to verify the volume by using the experimental charge from Cu(II) in Faraday’s second law of electrolysis to calculate volume.
Figure 14. Borosilicate glass bound to a gold electrode by stamp-and-stick fabrication. The working electrode compartment is defined within the ellipse and channels. Quarter shown for size reference.

2.3.4. DPS-ASC of As(III) Using Controlled Volumes

Stamp-and-stick fabrication was performed to create devices of different working electrode chamber volumes by using varied heights of borosilicate glass. Devices of volumes 4.3 µL, 5.6 µL, and 6.8 µL were utilized for the analysis of As(III) via DPS-ASC. Experiments were replicated over the course of several weeks to ensure reliable results. Analysis of As(III) standards was conducted four times using the 4.3 µL device, four times using the 5.6 µL device, and three times using the 6.8 µL device. Several other devices were produced and evaluated with volumes ranging from 4.3 µL to 6.9 µL. Although not discussed in detail, the usage of these devices is listed in Appendix III.

A reduction potential of -600 mV vs. Ag/AgCl was found necessary over the -500 mV vs. Ag/AgCl employed previously [36] to ensure 100% deposition of As(III), which differs from the $E^{0}$ of 35 mV vs. Ag/AgCl. Previously, a large overpotential has been necessary for As(III) to ensure complete deposition on a gold electrode as a more negative potential is needed to achieve higher As(0) coverage because of As(0) metalloid properties and increased resistance [66,67]. Therefore, sub monolayer coverage was preferred in this work. Monolayer coverage herein was calculated to occur at a concentration of 4.4 ppm As(III) assuming an electrode geometric area of 35.1 mm² and
volume of 6.8 µL (Appendix IV). It is noted that this calculation of monolayer coverage includes assumptions of packing efficiency and As(0) radius that could explain some differences between this concentration and the experimental findings.

DPS-ASC was utilized for the analysis of As(III). The DPS-ASC sequence allows for in situ background correction and produced two stripping amperograms which were overlaid to create a crescent. As seen in Figure 15, Figure 16, and Figure 17, the crescent size grew as the As(III) concentration increased. Also, the crescents for the device of volume 4.3 µL (Figure 15) were smaller than those of the 5.6 µL device (Figure 16) which were subsequently smaller than the crescents of the 6.8 µL device (Figure 17) for the same As(III) concentration. This agrees with Faraday’s second law of electrolysis that predicts larger charges with increased volume.

Figure 15. DPS-ASC crescents from analysis of 0 ppb to 1,000 ppb As(III) for a device of volume 4.3 µL and a gold macroelectrode.
Figure 16. DPS-ASC crescents from analysis of 0 ppb to 1,000 ppb As(III) for a device of volume 5.6 µL and a gold macroelectrode.

Figure 17. DPS-ASC crescents from analysis of 0 ppb to 1,000 ppb As(III) for a device of volume 6.8 µL and a gold macroelectrode.
For each concentration from 50 ppb to 1,000 ppb As(III), the charge was greater as the device volume increased, as shown in Tables 2-4 and Figure 18. Each device had an electrode area near 35.1 mm²; and correspondingly, the noise due to charging current remained unchanged. However, since the Faradaic charge (i.e., signal) increased with larger volume, this enhanced the detection limit by improving the signal-to-noise ratio.

The detection limits for As(III), as calculated using 3σ₁ (Appendix IV), were 55 ppb, 32 ppb, and 20 ppb for the 4.3 µL, 5.6 µL and 6.8 µL devices, respectively.

Table 2. DPS-ASC numerical results from analysis of As(III) standards from 0 ppb to 1,000 ppb using a device of volume 4.3 µL (# trials = 3). Pulse 2 charge was obtained after deposition at -600 mV for 0.1 sec. Pulse 4 charge was obtained after deposition at -600 mV for 130 sec. Background-corrected (BgC) charge is pulse 4 minus pulse 2 charge. Intercept-corrected (IcC) charge is the BgC charge minus the intercept charge (i.e. the BgC charge for 0 ppb As(III)). Calculated (calc) charge based on Faraday’s law where n is 3 for As(III)/As(0), F is the constant 96,485 C/mol, C is As(III) concentration (mol/L), and V is 4.3 x 10⁻⁶ L.

<table>
<thead>
<tr>
<th>As(III) ppb</th>
<th>Pulse 2 Charge µC</th>
<th>Pulse 4 Charge µC</th>
<th>BgC Charge µC (RSD)</th>
<th>IcC Charge µC</th>
<th>Calc Charge µC</th>
<th>% Error</th>
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<td>0</td>
<td>19.60</td>
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<td>0.00</td>
<td>–</td>
</tr>
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<td>1.66</td>
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<td>250</td>
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<td>5.79 (1.9%)</td>
<td>4.30</td>
<td>4.15</td>
<td>3.5%</td>
</tr>
<tr>
<td>500</td>
<td>20.01</td>
<td>29.88</td>
<td>9.87 (1.6%)</td>
<td>8.38</td>
<td>8.31</td>
<td>0.8%</td>
</tr>
<tr>
<td>750</td>
<td>20.21</td>
<td>34.15</td>
<td>13.94 (1.1%)</td>
<td>12.45</td>
<td>12.46</td>
<td>-0.1%</td>
</tr>
<tr>
<td>1,000</td>
<td>20.46</td>
<td>38.89</td>
<td>18.43 (1.0%)</td>
<td>16.94</td>
<td>16.61</td>
<td>2.0%</td>
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Table 3. DPS-ASC numerical results from analysis of As(III) standards from 0 ppb to 1,000 ppb using a device of volume 5.6 µL (# trials = 3). Pulse 2 charge was obtained after deposition at -600 mV for 0.1 sec. Pulse 4 charge was obtained after deposition at -600 mV for 240 sec. Background-corrected (BgC) charge is pulse 4 minus pulse 2 charge. Intercept-corrected (IcC) charge is the BgC charge minus the intercept charge (i.e. the BgC charge for 0 ppb As(III)). Calculated (calc) charge based on Faraday’s law where n is 3 for As(III)/As(0), F is the constant 96,485 C/mol, C is As(III) concentration (mol/L), and V is 5.6 x 10^{-6} L.

<table>
<thead>
<tr>
<th>As(III) ppb</th>
<th>Pulse 2 Charge µC</th>
<th>Pulse 4 Charge µC</th>
<th>BgC Charge µC (RSD)</th>
<th>IcC Charge µC</th>
<th>Calc Charge µC</th>
<th>% Error</th>
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<tr>
<td>50</td>
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<td>1.08</td>
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<td>-7.5%</td>
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Table 4. DPS-ASC numerical results from analysis of As(III) standards from 0 ppb to 1,000 ppb using a device of volume 6.8 µL (# trials = 3). Pulse 2 charge was obtained after deposition at -600 mV for 0.1 sec. Pulse 4 charge was obtained after deposition at -600 mV for 480 sec. Background-corrected (BgC) charge is pulse 4 minus pulse 2 charge. Intercept-corrected (IcC) charge is the BgC charge minus the intercept charge (i.e. the BgC charge for 0 ppb As(III)). Calculated (calc) charge based on Faraday’s law where n is 3 for As(III)/As(0), F is the constant 96,485 C/mol, C is As(III) concentration (mol/L), and V is 6.8 x 10^{-6} L.

<table>
<thead>
<tr>
<th>As(III) ppb</th>
<th>Pulse 2 Charge µC</th>
<th>Pulse 4 Charge µC</th>
<th>BgC Charge µC (RSD)</th>
<th>IcC Charge µC</th>
<th>Calc Charge µC</th>
<th>% Error</th>
</tr>
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<td>-</td>
</tr>
<tr>
<td>50</td>
<td>21.10</td>
<td>25.28</td>
<td>4.18 (3.7%)</td>
<td>1.37</td>
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<td>4.0%</td>
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<td>100</td>
<td>20.48</td>
<td>25.94</td>
<td>5.46 (1.7%)</td>
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<td>250</td>
<td>20.31</td>
<td>29.88</td>
<td>9.57 (3.3%)</td>
<td>6.76</td>
<td>6.57</td>
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<tr>
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<td>36.78</td>
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<td>27.59 (1.5%)</td>
<td>24.78</td>
<td>26.27</td>
<td>-5.7%</td>
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</table>
Figure 18. As(III) concentration versus intercept-corrected charge (IcC) values for devices of volume 4.3 µL (dotted), 5.6 µL (solid), and 6.8 µL (striped).

As shown in Tables 2-4, RSD values remained under 7.0% except for the 4.3 µL device at 0 ppb As(III) which had a 16% RSD. The R^2 value was 0.9997 for the 4.3 µL device, 0.9968 for the 5.6 µL device, and 0.9993 for the 6.8 µL device from 50 ppb to 1,000 ppb As(III), indicating linearity as seen in Figure 19. As the device volume grew, there was an increase in slope due to the larger signals. The intercept of the graph (i.e. signal for 0 ppb As(III)) was nearly the same at 1.49 µC and 1.12 µC for devices of 4.3 µL and 5.6 µL, respectively, but the device of 6.8 µL had a larger intercept value of 2.81 µC. It was hypothesized that the 6.8 µL device had a larger electrode area due to a laser cutting error in which there was a wider ellipse in the borosilicate glass than expected. This larger area would, in turn, produce a larger signal for 0 ppb As(III) due to increased charging current.
Figure 19. As(III) concentration versus background-corrected charge (BgC) values for devices of volume 4.3 µL (dashed), 5.6 µL (solid), and 6.8 µL (dotted).

Furthermore, the experimental charge values (IcC Charge) were compared to the expected charge values (Calc Charge). These values, along with the relative errors, are listed in Tables 2-4. The relative error for the 50 ppb As(III) solution in the 4.3 µL device was the highest observed at 24%. Because 50 ppb is technically below the 55 ppb detection limit for the 4.3 µL device, the high error is not surprising. Otherwise, the relative error values were found to be less than 8.0% for all other concentrations and volumes.

It is noted that the signal for a 0 ppb As(III) solution is non-zero for macroelectrodes. This is due to the inherent differences of the two stripping steps as pulse 2 was performed after only 0.1 sec at -600 mV while pulse 4 was performed after a much longer time (130 sec to 480 sec, depending on device volume) at -600 mV. As these two steps are not identical, there is a resulting charge for 0 ppb As(III) solutions. The 0 ppb
As(III) charge for the 4.3 µL, 5.6 µL, and 6.8 µL volume devices was 1.49 µC, 1.12 µC, and 2.81 µC, respectively, as seen in Tables 2-4. The differences in these charges were attributed to variations in electrode area and differences in pulse 3 deposition times. The corresponding 0 ppb As(III) charge was subtracted from the DPS-ASC experimental charges obtained for As(III) standards (BgC Charge) to obtain the intercept-corrected charge (IcC Charge) values listed in Tables 2-4.

A longer time for pulse 1 decreases this charge for 0 ppb As(III), as the time begins to resemble that of pulse 3. However, the longer pulse 1 time is not acceptable as a longer pulse 1 would allow more As(III) to deposit, leading to an incorrect subtraction of background charge; and subsequent As(III) analysis would be adversely affected.

Overall, while an increase in volume resulted in a lower detection limit, there was one disadvantage. As the volume of the device increased, the working electrode compartment height grew taller. This made the diffusion distance to the electrode longer, resulting in more time needed to exhaustively reduce electroactive species. Therefore, while experiments showed that it only took 2 min to exhaust the 4.3 µL volume device, devices with 5.6 µL and 6.8 µL volumes took 4 min and 8 min, respectively, for complete deposition. Thus, while a lower detection limit would be expected for devices of even larger volumes, this will come at the cost of a longer experiment time.

The lowest detection limit of 20 ppb for As(III) did not meet the WHO guidelines of 10 ppb. Therefore, an even larger volume device would be required to reach lower detection limits. The volume to reach a detection limit of 10 ppb (and meet the WHO standard) was calculated using Faraday’s second law of electrolysis to be 8.0 µL. While it would be feasible to create this device by using thicker borosilicate glass in the stamp-
and-stick fabrication, this was not done experimentally because the exhaustive deposition time would be 16 minutes per experiment for such a large volume. In noting the application of DPS-ASC for remote sensing, a deposition time of 16 minutes is unfavorable in the field because it would require an increased power supply and more reagents on-site for pretreatment of these larger sample volumes. This is a serious concern as the goal of remote sensing is in the operator-free analysis, which would be jeopardized by these demands. Therefore, it would be beneficial to explore other methods to reduce the detection limit for As(III) in lieu of using larger volumes.

2.4. Conclusion

The compressible gasket previously used to define the working electrode compartment was replaced to create a more reliable device with the ability to control and manipulate the volume. Methods including 3D printing and microfabrication using SU-8 were explored, but ultimately stamp-and-stick fabrication was selected to create the working electrode devices. Stamp-and-stick is simple to perform experimentally; and the compartment height, and thus volume, were easily altered by adhering different thicknesses of borosilicate glass to gold electrode chips. The resulting devices were chemically compatible with solutions at pH 2 containing heavy metals and the borosilicate glass was non-compressible, ensuring that the volume repeatability between device assemblies had a variance of only ± 7.5%.

Stamp-and-stick fabrication was used to create devices of volumes 4.3 µL, 5.6 µL and 6.8 µL that were subsequently used to analyze As(III) standards using DPS-ASC. Charge values were higher for the devices with larger volumes, as predicted by Faraday’s
second law of electrolysis. As the volume of analyte increased, the detection limit for As(III) improved down to 20 ppb. However, this was at the cost of increasing the experiment time from 2 to 8 minutes as the diffusion distance was enlarged.

To reach the WHO safety standard of 10 ppb for As, a volume of 8.0 µL would be required. This device would require an exhaustive deposition time of 16 minutes. A longer experiment time is not practical in the field as it would demand a larger power supply and the larger volume would require more reagents stored on-site for pretreatment of sample. Therefore, this led to the consideration of other approaches to meet the 10 ppb target detection limit for As(III).
CHAPTER III
FABRICATION OF GOLD MICROELECTRODE ARRAYS FOR DETECTION OF ARSENIC USING DPS-ASC

3.1. Introduction

DPS-ASC was used to detect As(III) at 100 ppb levels in the electrochemical device equipped with a gold macroelectrode previously by our group [36], and the work was further expanded in Chapter II by increasing the volume up to 6.8 µL to reach a 20 ppb detection limit [68]. However, the WHO safety guideline is 10 ppb for As [20]. Thus, another tactic to improve the LOD was explored.

This approach consisted of decreasing the overall electrode area in order to decrease the noise. By keeping the same volume, the signal should remain unchanged. The constant signal with decreased noise should improve the overall signal-to-noise ratio. The reduction of electrode area decreases the noise because the charging current \( i_c \) decreases with the capacitance of the double layer \( C_{dl} \) which is proportional to electrode area \( A \) via the parallel plate capacitor equation (Equation 8) where \( \varepsilon_0 \) is the dielectric constant and \( d \) is plate separation. The relationship of \( i_c \) and \( C_{dl} \) is shown in Equation 9, where \( E \) is potential, \( R_u \) is uncompensated resistance, and \( t \) is time.

\[
\text{Equation 8.} \quad C = \varepsilon_0 \frac{A}{d}
\]

\[
\text{Equation 9.} \quad i_c = \frac{E}{R_u} e^{-\frac{t}{R_u C_{dl}}}
\]
An effective method to decrease electrode area is by the fabrication of microelectrodes, which are defined as electrodes that have at least one dimension under 50 µm [69]. Microelectrodes can be classified as individual microelectrodes or interdigitated arrays; have planar, recessed, spherical, 3D, linear, micro-disk, or micro-band geometries; and have either equal spacing or irregular spacing between the individual array elements [70]. Microelectrodes are advantageous in that they offer lower background charging currents, high current density, small size, reduced double-layer capacitance resulting in small RC time constants, and enhanced rate of mass transport from radial diffusion [71].

Microelectrodes have key differences in their diffusion profile compared to macroelectrodes. In conditions of diffusion-controlled currents, large planar electrodes primarily experience linear diffusion perpendicular to the electrode surface. In this case, for an ideal reversible redox system, the current-time response is described by the Cottrell equation (Equation 3). Disk microelectrodes, on the other hand, encounter diffusion-controlled currents due to both planar diffusion and radial diffusion. Equation 10 shows that the total current ($i_{\text{total}}$) at microelectrodes is the sum of the planar diffusion current ($i_{\text{planar}}$) and radial diffusion current ($i_{\text{radial}}$). For disk microelectrodes, the radial diffusion current (i.e. steady state current) is related to the electrode radius ($r$), the number of electrons ($n$), Faraday’s constant ($F$), the diffusion coefficient ($D$), and concentration ($C$) via Equation 11 [28].

Equation 10. $i_{\text{total}} = i_{\text{planar}} + i_{\text{radial}}$

Equation 11. $i_{\text{radial}} = 4rnFDC$
The extent to which the planar or radial diffusion component dominates is dependent on electrode dimensions, diffusion layer, and electrolysis time [72]. At short times, the linear diffusion is dominant, whereas the impact of radial diffusion is more prevalent at long times and higher electrode coverage. The evolution of the diffusion profile with time at an array of disk microelectrodes is outlined in Figure 20.

![Figure 20](image)

Figure 20. Diffusion, represented by arrows, to disk microelectrodes (blue) in an insulating layer (grey) at A) short times, B) intermediate times, and C) long times.
Fabrication of microelectrodes is feasible by a variety of methods including physical assembly of components, self-assembly of molecules onto a surface, photolithography, screen printing, and electrodeposition [70]. To date, there have been numerous papers focused on the fabrication of and analysis using microelectrode arrays for applications in heavy metal monitoring [63,73–76]. For example, Uhlig et al. manufactured microelectrode arrays of Au and Pt on Si substrates using photolithography for the detection of Cd(II), Cu(II), Hg(II), and Pb(II) by square wave anodic stripping voltammetry [75].

Microelectrodes are most commonly fabricated on silicon wafers using photolithography techniques [77]. Silicon-based microelectrode arrays have been fabricated using Au, Ag, Ir, Pt, Ir(Hg), and Pt(Hg), among others, and have been used in the detection of various species such as As(III), Cd(II), Cu(II), H$_2$O$_2$, Hg(II), Mn(II), NH$_4$Cl, Ni(II), Pb(II), Se(IV), Zn(II) [78].

Because microelectrodes present key advantages that should improve the signal-to-noise ratio, this chapter explores the use of microelectrodes and continues its focus on the analysis of As(III). As(III) will be evaluated using the same chemical reactions and solution criteria as in Chapter II. DPS-ASC is investigated further because of its potential in the (re)calibration-free detection of As(III), which is ideal for applications in remote sensing.

Early work completed by our research group [79] demonstrated promise for the use of microelectrode arrays. Preliminary data compared the performance of various sized gold disk microelectrode arrays of individual diameters of 5 µm, 20 µm, and 50 µm to that of a planar gold macroelectrode for the analysis of As(III) via DPS-ASC. The plot
of As(III) concentration versus charge showed differences in the slope of the calibration lines for each array (Figure 21) despite the expectation that, as array diameter size changes, the slope should remain the same. It was thought that the differences in the slopes of the reported data thus far were due to volume inconsistency from the reassembly of the coulometry device between array sizes. Having devices of different volumes led to different slopes. Now that the stamp-and-stick fabrication method has been developed (Chapter II), this approach can be used to create devices of the same volume in an attempt to achieve the anticipated results that feature the same slope, as shown in Figure 22.

![Graph showing As(III) concentration versus charge for different array sizes.](image)

Figure 21. Preliminary results of As(III) concentration versus intercept-corrected charge (IcC) for a 5 µm array (red), 20 µm array (green), 50 µm array (blue), and macroelectrode (black) [79].
In the earlier work (Figure 21), the smallest array (5 µm) was not used for concentrations above 100 ppb As(III) due to underpotential deposition effects. Due to the small size of these arrays, the electrode became saturated with As(0) faster than with larger electrodes. The rate of deposition decreased as the thickness and coverage of As(0) increased because As(0) films have a large resistance [66]. Tammann and Warrentrup reported that the electrical resistance of electrodeposited As(0) is $10^{13}$ times greater than that of crystalline As(0) [80]. Therefore, while the smallest diameter array is expected to have the lowest detection limit, there will be a compromise with the range of detection and overall linearity because 100% of As(III) deposition may not occur at large concentrations on small microelectrode arrays.

In addition to using microelectrode arrays to reach the 10 ppb detection limit for As(III), another objective is to address concerns of selectively to demonstrate the
practicality of using DPS-ASC for detection of As(III) in real environmental samples. Specifically, Cd(II), Hg(II), and Pb(II) are considered as these interfering metals deposit more positively than As(III) and are also ranked in the top ten on the ATSDR substance priority list. To investigate these interferences in the presence of As(III), the method explored was a subtraction method in which the charge measured from metals that deposit at -200 mV (Cd(II), Hg(II), Pb(II)) is subtracted from the charge at -600 mV (corresponding to Cd(II), Hg(II), Pb(II), and As(III)), so that the resulting charge is attributed solely to As(III). This subtraction method has been previously evaluated for the targeted detection of As(III) among Cd(II), Cu(II), Pb(II), and Zn(II) interferences [36].

3.2. Materials and Methods

3.2.1. Chemicals, Reagents, and Solution Preparation

All reagents were purchased at the highest purity and used without further purification. Nitric acid and AAS 1,000 ppm single element standards were purchased from Sigma Aldrich (St. Louis, MO) for As(III) (As$_2$O$_3$), Cd(II) (Cd(NO$_3$)$_2$), Cu(II) (Cu(NO$_3$)$_2$), Hg(II) (Hg(NO$_3$)$_2$), and Pb(II) (Pb(NO$_3$)$_2$). Buffered oxide etch (BOE) (6:1), acetone, sodium chloride, and sulfuric acid were purchased from VWR (Radnor, PA).

All solutions were prepared fresh each day using deionized water. 10 mM HNO$_3$/10 mM NaCl was prepared in a 1 L volumetric flask by addition of concentrated HNO$_3$ via pipet and the appropriate mass of NaCl via analytical balance to deionized water in the flask. As(III), Cd(II), Hg(II), and Pb(II) standards (10 ppm) were prepared by pipetting 0.50 mL of the appropriate 1,000 ppm AAS into a 50 mL volumetric flask and using the 10 mM HNO$_3$/10 mM NaCl as diluent. Subsequent standards were prepared by
pipetting the appropriate amount of 10 ppm standard into 50 mL volumetric flasks and diluting to the mark with 10 mM HNO$_3$/10 mM NaCl.

Safety is of utmost concern due to the toxic nature of these heavy metals. Skin contact with As(III), Cd(II), Hg(II), and Pb(II) was avoided by wearing protective safety goggles, long-sleeve shirts, long pants, closed-toe shoes, and nitrile examination gloves from VWR (Radnor, PA) during all solution preparations. Spills of heavy metal solutions were cleaned with paper towels that were disposed of in appropriate solid waste containers.

Handling of BOE involved additional precautions due to the hazards of aqueous hydrofluoric acid. Specific personal protective equipment for handling BOE was always worn in addition to the eyewear, clothing, shoes, and gloves listed before. The additional specialized equipment for handling BOE included a full-face shield, an acid-resistant long-sleeve apron, and elbow-length neoprene gloves. The BOE was kept contained within a single fume hood designated for use of hydrofluoric acid, and solutions were stored in plastic containers. Calcium gluconate gel and eyewash kits were kept readily available in the lab in case of hydrofluoric acid spills and exposures.

All solution waste was collected in containers provided by the Department of Environmental Health and Safety (DEHS) at the University of Louisville. Waste BOE was collected into a separate container, whereas all other acidic solutions were collected in acidic waste containers. Solid waste was collected in a solid waste disposal bucket. Once the containers were full, DEHS was notified to collect the containers and ensure proper disposal per federal regulations.
3.2.2. Gold Microelectrode Array

The detailed fabrication process to create gold microelectrode arrays is listed in Appendix II. Briefly, a 4-inch oxidized silicon wafer with subsequent layering of 20 nm titanium, 200 nm gold, and 300 nm silicon nitride was ordered from the Lurie Nanofabrication Facility (LNF) at the University of Michigan (Ann Arbor, MI). Using the MNTEC facilities, Shipley 1827 photoresist was used to pattern the silicon nitride to create the microelectrode arrays, and then the wafer was diced into 30 mm by 13 mm chips. The exposed silicon nitride was etched, one chip at a time, for 15 hours using vapor from 6:1 BOE. After the etching was complete, the Shipley 1827 protective layer was removed by sonication in acetone, and then the arrays were inspected visually using a Zeiss Axiotron Microscope. This fabrication sequence is shown in Figure 23. The individual gold microelectrode diameters were either 50 µm, 20 µm, or 10 µm, and the edge-to-edge distance for each individual microelectrode was always 50 µm.
Figure 23. Microfabrication sequence depicting A) patterning of the microelectrode arrays using Shipley 1827, B) etching of the silicon nitride using BOE, C) removal of the Shipley 1827 photoresist using acetone.

3.2.3. Working Electrode Compartment

The working electrode compartment was built directly on top of the 30 mm by 13 mm gold microelectrode array chips via stamp-and-stick fabrication using borosilicate glass, as described in Chapter II. Briefly, NOA 68 was spun via Headway Spinners to a 10-20 nm thickness on a bare silicon wafer. An AB-M Inc Aligner was used to transfer a thin layer of NOA 68 onto 80 µm thick borosilicate glass that had been previously laser cut to reveal the 8 mm by 4 mm ellipse with 5 mm by 1 mm channels within the 30 mm by 10 mm piece of glass. Then, the coated borosilicate glass was aligned and put into contact with a 30 mm by 13 mm chip featuring patterned gold microelectrode arrays. Finally, the borosilicate glass/electrode combination was exposed to UV light for 300 sec to cure the NOA 68 and create electrode sensor chips with permanent, defined volumes.
3.2.4. Coulometry Device

The coulometry device was described previously in Chapter II. Briefly, the device features a working electrode compartment and a counter/reference compartment that are separated by a membrane and have external fluidic flow/shut-off valves. Starting from the bottom, the polycarbonate base is recessed to hold the 30 mm by 13 mm working electrode chip. A 200 MWCO membrane was affixed on top of the working electrode chip to define the top of the working electrode compartment (membrane pieces were replaced after 10-15 days of use). Then, three laser-cut silicone gaskets, the Panasonic pyrolytic graphite sheet counter electrode, thin laser-cut silicone gasket, and polycarbonate top were added to define the counter/reference electrode compartment. A custom Ag/AgCl reference electrode was inserted through an access hole in the polycarbonate top into the counter/reference electrode compartment and sealed in place using chemically compatible silicone grease. The polycarbonate pieces were then screwed together using four screws, and the torque on each screw was set to a value of 0.51 kg-cm using a torque screwdriver. The device was assembled at the beginning of each day and disassembled at the end of each day. All gaskets were washed twice a week with soap and water to prevent grease build-up and contamination.

3.2.5. Electrochemical Measurements

The three-electrode system encompassed a fabricated gold microelectrode array for the working electrode, a custom miniature Ag/AgCl reference electrode fabricated in-house [35], and a pyrolytic graphite sheet as the counter electrode. A listing of the various gold microelectrode chips created and utilized is included in Appendix III. The
miniature Ag/AgCl reference electrodes were replaced every 5-10 days to ensure proper reference conditions (i.e., no potential drift). The pyrolytic graphite sheet was replaced after one month of use. A BASi Epsilon potentiostat (West Lafayette, IN) was used for all electrochemical measurements.

The following procedure was utilized for DPS-ASC experiments. First, the counter/reference chamber was filled with 10 mM HNO₃/10 mM NaCl and fluid valves were closed. This solution was not replaced throughout the following series of experiments. Then, the working electrode chamber was filled with 50 mM H₂SO₄ and the gold microelectrode array was electrochemically cleaned by slowly flushing the 50 mM H₂SO₄ through the chamber as the potential was cycled from 0 mV to 1,400 mV at 100 mV sec⁻¹ for 4-6 scans until cyclic voltammetry traces were reproducible. This was necessary to eliminate errors due to electrode fouling. Then, the working electrode chamber was filled with analyte sample (blank or As(III) solution) and fluid valves were closed before applying the DPS-ASC potential sequence. The DPS-ASC experiments were repeated at least three times for each analyte sample. Between each experiment, the fluid valves were opened, the working electrode chamber was filled with fresh analyte sample, and fluid valves were then closed. Once all analyte samples were evaluated, the working electrode was cleaned at the end of the day by flushing 50 mM H₂SO₄ through the chamber as the potential was cycled from 0 mV to 1,400 mV until traces were reproducible.

For As(III) standards, the DPS-ASC pulse sequence, as described in Chapter 1 and outlined in Figure 3, is listed below. There was no applied potential before this sequence.
Pulse 1: -600 mV for 0.1 sec
Pulse 2: 500 mV for 1 sec
Pulse 3: -600 mV for 130 sec
Pulse 4: 500 mV for 1 sec

Subtractive methods were then used to target As(III) in the presence of interferents by subtracting the charge obtained at -200 mV (Cd(II), Hg(II), Pb(II)) from the charge at -600 mV (Cd(II), Hg(II), Pb(II), As(III)). For analysis of Cd(II), Hg(II), and Pb(II), the DPS-ASC parameters for this method were:

Pulse 1: -200 mV for 0.1 sec
Pulse 2: 500 mV for 1 sec
Pulse 3: -200 mV for 130 sec
Pulse 4: 500 mV for 1 sec

By overlaying the two oxidation traces from DPS-ASC, the data appears in the shape of a crescent along an x-axis of time and y-axis of current. These current-time curves are integrated to obtain charge, and the difference between the curves indicates Faradaic charge due to metal stripping. The integration of the current-time curves was completed by summing the area under the curves until the traces met one another. The area corresponds to charge with the units of coulombs. All integrations and data processing were done by extracting text files from the BAS Epsilon software and using Microsoft Excel to perform all calculations.
3.3. Results and Discussion

3.3.1. Gold Microelectrode Array Characterization

Gold microelectrode array electrodes were fabricated in-house and characterized. Each electrode chip contained only one microelectrode diameter size that was duplicated to create an array of thousands of individual microelectrodes in the shape of an 8 mm by 5 mm ellipse in which all electrodes measured 50 µm from edge-to-edge. The 50 µm edge-to-edge distance was selected as it is lower than the 80 µm height of the working electrode compartment and therefore should not increase the diffusion time needed to deplete the coulometry device. The individual microelectrode sizes explored in this work have a diameter of 50 µm, 20 µm, or 10 µm. Table 5 compares the number of individual electrodes, electrode area, and deposition time of each array size. It was found that the background charge (i.e., noise due to charging current) decreased from 0.64 µC to 0.05 µC as the electrode area was decreased from 6.6 mm² to 2.0 mm².

Table 5. Comparison of individual (ind.) electrode diameter size, number of ind. electrodes, electrode area, deposition time, and background-corrected charge (BgC) for a 0 ppb As (III) solution.

<table>
<thead>
<tr>
<th>Ind. Electrode Diameter</th>
<th># of Ind. Electrodes</th>
<th>Electrode Area</th>
<th>Deposition Time</th>
<th>BgC for 0 ppb As(III)</th>
</tr>
</thead>
<tbody>
<tr>
<td>50 µm</td>
<td>3,351</td>
<td>6.6 mm²</td>
<td>2 min</td>
<td>0.64 ± 0.04 µC</td>
</tr>
<tr>
<td>20 µm</td>
<td>9,972</td>
<td>3.1 mm²</td>
<td>2 min</td>
<td>0.12 ± 0.02 µC</td>
</tr>
<tr>
<td>10 µm</td>
<td>25,642</td>
<td>2.0 mm²</td>
<td>2 min</td>
<td>0.05 ± 0.01 µC</td>
</tr>
</tbody>
</table>

A Zeiss Axiotron Microscope was used to visually inspect and measure the various microelectrode arrays before and after etching the silicon nitride using 6:1 BOE. Examples of microscope images from the 50 µm, 20 µm, and 10 µm arrays are shown in Figure 24 wherein the pale-yellow circles are the individual gold microelectrodes and the
surrounding orange layer is the insulating silicon nitride film. The expected array diameters and edge-to-edge distances were frequently achieved with few exceptions.

Figure 24. Microscope images of the 50 µm, 20 µm, and 10 µm diameter gold microelectrode arrays.
In addition, electrochemical deposition of Cu(II) was performed to confirm the electrochemical activity of the gold microelectrodes. Electrodes were placed into a solution of 10 ppm Cu(II), and the potential was held at -200 mV vs. Ag/AgCl for 5 minutes in order to deposit Cu(0) onto the gold surfaces. The appearance of the individual gold electrodes changed from a pale-yellow color to a red-brown color (Figure 25), indicating that Cu(0) deposits were present which confirms that the gold microelectrode arrays were electrochemically active. Inactive individual microelectrodes were not discovered for any electrode tested (n=7). In addition, Cu(0) deposits were not present across the silicon nitride which indicates that this material is not conductive, as expected.

Figure 25. Microscope images of a 10 µm diameter gold microelectrode array before (left) and after (right) deposition of Cu(0) to demonstrate electroactive behavior of the gold microelectrode arrays.
3.3.2. DPS-ASC of As(III) Standards

As(III) standards from 0 ppb to 1,000 ppb were analyzed using the 50 μm, 20 μm, and 10 μm gold microelectrode arrays. DPS-ASC experimental analysis of As(III) standards was repeated 3 times using the 50 μm array electrode, 5 times using the 20 μm array electrode, and 8 times using the 10 μm array electrode. Other microelectrode array devices were produced and evaluated. Although not discussed in detail, the usage of these devices is listed in Appendix III.

The sequence of DPS-ASC allowed for *in situ* background correction and produced two stripping amperograms which were overlaid to create a crescent. Figure 26 shows that the crescent size (*i.e.*, charge) grew with increasing As(III) concentration from 0 ppb to 1,000 ppb using the 50 μm gold microelectrode array.

![Figure 26. DPS-ASC crescents from analysis of 0 ppb to 1,000 ppb As(III) using a 50 μm gold microelectrode array.](image)
DPS-ASC using the 20 µm and 10 µm gold microelectrode arrays was also performed. As(III) standards of 0 ppb to 100 ppb were evaluated, and the crescents are shown for the 20 µm (Figure 27) and 10 µm (Figure 28) microelectrode arrays. As seen before, the crescent size increased as the concentration increased.

![Figure 27. DPS-ASC crescents from analysis of 0 ppb to 100 ppb As(III) for a 20 µm gold microelectrode array.](image_url)
Figure 28. DPS-ASC crescents from analysis of 0 ppb to 100 ppb As(III) for a 10 µm gold microelectrode array.

The two stripping amperogram traces from DPS-ASC using the various microelectrode arrays were found to meet one another much faster compared to a macroelectrode. Pulse 2 and pulse 4 stripping curves met in 10-50 msec for microelectrode arrays compared to more than 250 msec for macroelectrodes. This agrees with Equation 9 which indicates that charging time is faster when the electrode area (i.e., double-layer capacitance) is smaller.

The charge values for As(III) standards for the 50 µm, 20 µm, and 10 µm array electrodes are listed in Table 6, Table 7, and Table 8, respectively. The charges from pulse 2 and pulse 4 were recorded experimentally. The background-corrected charge (BgC) results from subtraction of pulse 2 charge from pulse 4 charge. The RSD values for the BgC results were 7.1% or less with only four exceptions, indicating that these
results were reproducible. The intercept-corrected charge (IcC) was determined by subtracting the 0 ppb As(III) BgC from each BgC value. Clearly, the 20 µm and 10 µm microelectrode arrays do not perform linearly above 100 ppb As(III) as the IcC values level off, indicating that concentrations above 100 ppb are outside of the linear range. By comparison, the 50 µm microelectrode array excels in analysis of As(III) up to 1,000 ppb but could not accurately detect concentrations of As(III) less than 50 ppb. The linear ranges are shown in Figure 29 wherein IcC values and As(III) concentration are graphed for each array electrode. The IcC values were then compared to the calculated charge values per Faraday’s law, and relative error was calculated (Tables 6-8). For the 50 µm array electrode, relative error was highest at -17% for 50 ppb As(III) and -10% for 1,000 ppb As(III) indicating that these values near the end limits of linearity. Otherwise, error was less than 4.0% for all other concentrations analyzed using the 50 µm array. For both the 20 µm and 10 µm array electrodes, the relative error increased for As(III) concentrations above 100 ppb As(III) as these values exceed the linear range for these electrodes. Error remained under 4.0% for As(III) standards from 10 ppb to 100 ppb for the 20 µm array electrode and 13% or less for As(III) standards from 5 ppb to 100 ppb for the 10 µm array electrode.
Table 6. DPS-ASC numerical results from analysis of As(III) standards from 0 ppb to 1,000 ppb using a 50 µm array electrode in a device of volume 2.5 µL (# trials = 3). Pulse 2 charge obtained after deposition at -600 mV for 0.1 sec. Pulse 4 charge obtained after deposition at -600 mV for 130 sec. Background-corrected (BgC) charge is pulse 4 minus pulse 2 charge. Intercept-corrected (IcC) charge is the BgC charge minus the intercept charge (*i.e.* the BgC charge for 0 ppb As(III)). Calculated (calc) charge based on Faraday’s law where n is 3 for As(III)/As(0), F is the constant 96,485 C/mol, C is As(III) concentration (mol/L), and V is 2.5 x 10⁶ L.

<table>
<thead>
<tr>
<th>As(III) ppb</th>
<th>Pulse 2 Charge µC</th>
<th>Pulse 4 Charge µC</th>
<th>BgC Charge µC (RSD)</th>
<th>IcC Charge µC</th>
<th>Calc Charge µC</th>
<th>% Error</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>5.43</td>
<td>6.07</td>
<td>0.64 (5.7%)</td>
<td>0.00</td>
<td>0.00</td>
<td>–</td>
</tr>
<tr>
<td>50</td>
<td>5.45</td>
<td>6.49</td>
<td>1.04 (1.3%)</td>
<td>0.40</td>
<td>0.48</td>
<td>-17%</td>
</tr>
<tr>
<td>100</td>
<td>5.38</td>
<td>6.99</td>
<td>1.61 (3.0%)</td>
<td>0.97</td>
<td>0.97</td>
<td>0.0%</td>
</tr>
<tr>
<td>250</td>
<td>5.47</td>
<td>8.51</td>
<td>3.04 (4.2%)</td>
<td>2.40</td>
<td>2.41</td>
<td>-0.4%</td>
</tr>
<tr>
<td>500</td>
<td>5.71</td>
<td>11.37</td>
<td>5.66 (0.3%)</td>
<td>5.02</td>
<td>4.83</td>
<td>3.9%</td>
</tr>
<tr>
<td>750</td>
<td>5.94</td>
<td>13.86</td>
<td>7.92 (2.3%)</td>
<td>7.28</td>
<td>7.24</td>
<td>0.6%</td>
</tr>
<tr>
<td>1,000</td>
<td>6.08</td>
<td>15.38</td>
<td>9.30 (3.1%)</td>
<td>8.66</td>
<td>9.66</td>
<td>-10%</td>
</tr>
</tbody>
</table>

Table 7. DPS-ASC numerical results from analysis of As(III) standards from 0 ppb to 1,000 ppb using a 20 µm array electrode in a device of volume 2.3 µL (# trials = 3). Pulse 2 charge obtained after deposition at -600 mV for 0.1 sec. Pulse 4 charge obtained after deposition at -600 mV for 130 sec. Background-corrected (BgC) charge is pulse 4 minus pulse 2 charge. Intercept-corrected (IcC) charge is the BgC charge minus the intercept charge (*i.e.* the BgC charge for 0 ppb As(III)). Calculated (calc) charge based on Faraday’s law where n is 3 for As(III)/As(0), F is the constant 96,485 C/mol, C is As(III) concentration (mol/L), and V is 2.3 x 10⁶ L.

<table>
<thead>
<tr>
<th>As(III) ppb</th>
<th>Pulse 2 Charge µC</th>
<th>Pulse 4 Charge µC</th>
<th>BgC Charge µC (RSD)</th>
<th>IcC Charge µC</th>
<th>Calc Charge µC</th>
<th>% Error</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>1.91</td>
<td>2.03</td>
<td>0.12 (15%)</td>
<td>0.00</td>
<td>0.00</td>
<td>–</td>
</tr>
<tr>
<td>5</td>
<td>1.97</td>
<td>2.12</td>
<td>0.15 (5.4%)</td>
<td>0.03</td>
<td>0.04</td>
<td>-25%</td>
</tr>
<tr>
<td>10</td>
<td>1.99</td>
<td>2.20</td>
<td>0.21 (3.0%)</td>
<td>0.09</td>
<td>0.09</td>
<td>0.0%</td>
</tr>
<tr>
<td>25</td>
<td>1.89</td>
<td>2.23</td>
<td>0.34 (1.6%)</td>
<td>0.22</td>
<td>0.22</td>
<td>0.0%</td>
</tr>
<tr>
<td>50</td>
<td>1.99</td>
<td>2.56</td>
<td>0.57 (2.0%)</td>
<td>0.45</td>
<td>0.44</td>
<td>2.3%</td>
</tr>
<tr>
<td>100</td>
<td>2.15</td>
<td>3.13</td>
<td>0.98 (7.1%)</td>
<td>0.86</td>
<td>0.89</td>
<td>-3.4%</td>
</tr>
<tr>
<td>250</td>
<td>2.26</td>
<td>4.17</td>
<td>1.91 (2.1%)</td>
<td>1.79</td>
<td>2.22</td>
<td>-19%</td>
</tr>
<tr>
<td>500</td>
<td>2.32</td>
<td>4.85</td>
<td>2.53 (1.2%)</td>
<td>2.41</td>
<td>4.44</td>
<td>-46%</td>
</tr>
<tr>
<td>750</td>
<td>2.45</td>
<td>5.36</td>
<td>2.91 (1.8%)</td>
<td>2.79</td>
<td>6.66</td>
<td>-58%</td>
</tr>
<tr>
<td>1,000</td>
<td>2.48</td>
<td>5.68</td>
<td>3.20 (1.2%)</td>
<td>3.08</td>
<td>8.89</td>
<td>-65%</td>
</tr>
</tbody>
</table>
Table 8. DPS-ASC numerical results from analysis of As(III) standards from 0 ppb to 1,000 ppb using a 10 μm array electrode in a device of volume 2.2 μL (# trials = 3). Pulse 2 charge obtained after deposition at -600 mV for 0.1 sec. Pulse 4 charge obtained after deposition at -600 mV for 130 sec. Background-corrected (BgC) charge is pulse 4 minus pulse 2 charge. Intercept-corrected (IcC) charge is the BgC charge minus the intercept charge (i.e. the BgC charge for 0 ppb As(III)). Calculated (calc) charge based on Faraday’s law where n is 3 for As(III)/As(0), F is the constant 96,485 C/mol, C is As(III) concentration (mol/L), and V is 2.2 x 10^{-6} L.

<table>
<thead>
<tr>
<th>As(III) ppb</th>
<th>Pulse 2 Charge μC</th>
<th>Pulse 4 Charge μC</th>
<th>BgC Charge μC (RSD)</th>
<th>IcC Charge μC</th>
<th>Calc Charge μC</th>
<th>% Error</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>2.62</td>
<td>2.67</td>
<td>0.05 (24%)</td>
<td>0.00</td>
<td>0.00</td>
<td>–</td>
</tr>
<tr>
<td>5</td>
<td>2.53</td>
<td>2.62</td>
<td>0.09 (16%)</td>
<td>0.04</td>
<td>0.04</td>
<td>0.0%</td>
</tr>
<tr>
<td>10</td>
<td>2.55</td>
<td>2.69</td>
<td>0.14 (4.5%)</td>
<td>0.09</td>
<td>0.08</td>
<td>13%</td>
</tr>
<tr>
<td>25</td>
<td>2.60</td>
<td>2.88</td>
<td>0.28 (3.2%)</td>
<td>0.23</td>
<td>0.21</td>
<td>10%</td>
</tr>
<tr>
<td>50</td>
<td>2.62</td>
<td>3.10</td>
<td>0.48 (9.2%)</td>
<td>0.43</td>
<td>0.42</td>
<td>2.4%</td>
</tr>
<tr>
<td>100</td>
<td>2.66</td>
<td>3.56</td>
<td>0.90 (1.1%)</td>
<td>0.85</td>
<td>0.85</td>
<td>0.0%</td>
</tr>
<tr>
<td>250</td>
<td>2.71</td>
<td>4.69</td>
<td>1.98 (1.2%)</td>
<td>1.93</td>
<td>2.12</td>
<td>-9.0%</td>
</tr>
<tr>
<td>500</td>
<td>2.76</td>
<td>5.71</td>
<td>2.95 (3.6%)</td>
<td>2.90</td>
<td>4.25</td>
<td>-32%</td>
</tr>
<tr>
<td>750</td>
<td>2.85</td>
<td>6.21</td>
<td>3.36 (2.9%)</td>
<td>3.31</td>
<td>6.37</td>
<td>-48%</td>
</tr>
<tr>
<td>1,000</td>
<td>2.88</td>
<td>6.45</td>
<td>3.57 (2.4%)</td>
<td>3.52</td>
<td>8.50</td>
<td>-59%</td>
</tr>
</tbody>
</table>
Figure 29. As(III) concentration versus intercept-corrected charge (IcC) values for devices of array diameter size of 50 µm (striped), 20 µm (solid black), and 10 µm (dotted). All compared to calculated values per Faraday’s law (solid white) where n is 3 for As(II)/As(0), F is the constant 96,485 C/mol, C is As(III) concentration (mol/L), and V is 2.3 x 10^{-6} L.

The decrease in charging current, as realized by the decrease in the charge for a 0 ppb As(III) solution, subsequently resulted in lower detection limits because the signal-to-noise ratio was improved as the noise was decreased. The limits of detection, calculated using 3σ_{bl} (Appendix IV), were 25 ppb, 6 ppb, and 4 ppb for the 50 µm, 20 µm, and 10 µm microelectrode arrays, respectively. This demonstrates that the 20 µm and 10 µm microelectrode arrays have suitable detection limits to meet the requirements of the 10 ppb limit set by the WHO.

As mentioned before, preliminary work in our group struggled to keep consistent volumes between device assemblies; and the resulting calibration curves therefore
produced lines of differing slope. Now, by using stamp-and-stick fabrication techniques, the volume was accurately manipulated so that device-to-device volumes were consistent. As seen in Figure 30, the slopes for the 50 µm, 20 µm, and 10 µm microelectrode arrays are now much more comparable at 0.0090, 0.0090, and 0.0083 µC ppb⁻¹, respectively, compared to the preliminary results. Also, the R² values were 0.9920 for the 50 µm array, 0.9942 for the 20 µm array, and 0.9986 for the 10 µm array from 0 ppb to 100 ppb, indicating linearity in this range. Figure 31 demonstrates the lack of linearity above 100 ppb for the 20 µm and 10 µm microelectrode arrays.

Figure 30. As(III) concentration from 0 ppb to 100 ppb versus intercept-corrected charge (IcC) for a 50 µm array (dotted), 20 µm array (solid), and 10 µm array (dashed).
Figure 31. As(III) concentration from 0 ppb to 1,000 ppb versus intercept-corrected charge (IcC) for a 50 µm array (dotted), 20 µm array (solid), and 10 µm array (dashed).

Linearity was limited to a range of 0 ppb to 100 ppb for the 20 µm and 10 µm arrays, yet monolayer coverage of As(0) was not expected until concentrations of 1,100 ppb and 770 ppb, respectively, were reached (Appendix IV). While the decrease in linearity above 100 ppb suggested that the electrode area was small enough that it was becoming saturated with As(0) at higher concentrations of As(III), that theory does not match the monolayer coverage calculation. This suggests that an assumption made in the monolayer coverage calculation may be incorrect or that another factor is limiting further deposition.

If not attributed to monolayer coverage, there are two hypotheses that could explain the plateau of charge seen at high As(III) concentrations. First, partial electrode coverage could increase the resistance on each microelectrode to prevent significant amounts of further As(III) deposition at that applied potential. For example, coverage at the microelectrode edges with As(0) would reduce radial diffusion and then linear
diffusion would be limited by the increased resistance seen at the microelectrode edges. Second, it is possible that As(0) atoms occupy hole positions between gold atoms on the electrode surface [53]. This would only permit As(0) coverage across 25% of the total electrode area, thus altering the calculation for monolayer coverage such that a lower As(III) concentration would cause monolayer coverage, thus supporting the results obtained herein. These hypotheses are based on findings from the literature and were not tested in this work.

The intended application for this electrochemical sensor is in a remote device that operates 24/7 as an early warning detection system of heavy metals. Upon sensing and reporting of high levels of As(III), a technician would be sent to investigate and remedy the situation. Therefore, although the 20 µm and 10 µm microelectrode arrays have a small linear range, it is noted that concentrations above 100 ppb As(III) exceed the goal of the WHO safety standard of 10 ppb, and accordingly, any linear range above 100 ppb is likely unnecessary in our remote sensor.

3.3.3. Interferents

The deposition of As(III) onto a gold electrode will occur simultaneously with other metal ions in solution that also deposit at -600 mV [57,60,63]. To address the feasibility of using DPS-ASC for more complex samples, a solution of metal interferents was prepared. Cd, Hg, and Pb were chosen as they accompany As on the ATSDR substance priority list as #7, #3, and #2, respectively [18]. The concentration of the metals in the interfering solution was based on the maximum contaminant level (MCL) set by the US EPA. These values were 5 ppb for Cd(II), 2 ppb for Hg(II), and 15 ppb for
Pb(II) [81]. The interfering metal concentrations were kept constant while As(III) concentration was varied from 0 ppb to 100 ppb. This analysis was completed in triplicate using the 20 µm diameter gold microelectrode array and compared to results acquired using As(III) standard solutions (i.e., without interferences). It is noted that previous work in our group explored the effects of higher concentrations of interferents using a gold macroelectrode for applications in waste water analysis [36], but herein As(III) was monitored in the presence of interferences all near the EPA maximum contaminant levels which is most realistic of drinking water samples.

Figure 32 compares the DPS-ASC crescents for a step to -200 mV to those from a step to -600 mV for both As(III) standards and interferent solutions. The crescents from a step to -200 mV do not increase with As(III) concentration, indicating that As(III) deposition is not occurring. However, crescents from a step to -600 mV do increase with As(III) concentration for both the standard solutions and interferent solutions. This indicates that this potential is satisfactory for As(III) deposition, even in the presence of Cd(II), Hg(II), and Pb(II).
Using the DPS-ASC sequence with a deposition potential of -200 mV, only Cd(II), Hg(II), and Pb(II) were expected to be reduced. The DPS-ASC sequence with a deposition potential of -600 mV, however, was predicted to reduce these three metals plus As(III). By subtracting the charge obtained by a step to -200 mV from the charge by a step to -600 mV, the charge due only to As(III) was determined. These charge values are listed in Table 9.
Table 9. DPS-ASC background-corrected charge (BgC) values from a step to -200 mV (Cd(II) + Hg(II) + Pb(II)) and a step to -600 mV (Cd(II) + Hg(II) + Pb(II) + As(III)). Subtraction of -200 mV BgC from -600 mV BgC results in BgC due to As(III) only. Intercept-corrected charge (IcC) results from subtraction of 0 ppb As(III) BgC from each As(III)-only BgC value. As(III) in standard solutions was compared to As(III) in interferent solutions containing Cd(II), Hg(II), and Pb(II) (# trials = 3).

### As(III) Standard Solutions

<table>
<thead>
<tr>
<th>As(III) Concentration</th>
<th>-200 mV BgC</th>
<th>-600 mV BgC</th>
<th>-600 mV minus -200 mV BgC</th>
<th>-600 mV minus -200 mV IcC</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 ppb</td>
<td>0.01 ± 0.01 µC</td>
<td>0.11 ± 0.03 µC</td>
<td>0.10 µC</td>
<td>0.00 µC</td>
</tr>
<tr>
<td>10 ppb</td>
<td>0.02 ± 0.01 µC</td>
<td>0.18 ± 0.02 µC</td>
<td>0.16 µC</td>
<td>0.06 µC</td>
</tr>
<tr>
<td>25 ppb</td>
<td>0.02 ± 0.02 µC</td>
<td>0.32 ± 0.01 µC</td>
<td>0.30 µC</td>
<td>0.20 µC</td>
</tr>
<tr>
<td>50 ppb</td>
<td>0.01 ± 0.00 µC</td>
<td>0.54 ± 0.01 µC</td>
<td>0.53 µC</td>
<td>0.43 µC</td>
</tr>
<tr>
<td>75 ppb</td>
<td>0.03 ± 0.01 µC</td>
<td>0.74 ± 0.03 µC</td>
<td>0.71 µC</td>
<td>0.61 µC</td>
</tr>
<tr>
<td>100 ppb</td>
<td>0.04 ± 0.02 µC</td>
<td>0.86 ± 0.03 µC</td>
<td>0.82 µC</td>
<td>0.72 µC</td>
</tr>
</tbody>
</table>

### As(III) Interferent Solutions

<table>
<thead>
<tr>
<th>As(III) Concentration</th>
<th>-200 mV BgC</th>
<th>-600 mV BgC</th>
<th>-600 mV minus -200 mV BgC</th>
<th>600 mV minus -200 mV IcC</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 ppb</td>
<td>0.02 ± 0.02 µC</td>
<td>0.11 ± 0.02 µC</td>
<td>0.09 µC</td>
<td>0.00 µC</td>
</tr>
<tr>
<td>10 ppb</td>
<td>0.01 ± 0.01 µC</td>
<td>0.17 ± 0.03 µC</td>
<td>0.16 µC</td>
<td>0.07 µC</td>
</tr>
<tr>
<td>25 ppb</td>
<td>0.01 ± 0.02 µC</td>
<td>0.27 ± 0.02 µC</td>
<td>0.26 µC</td>
<td>0.17 µC</td>
</tr>
<tr>
<td>50 ppb</td>
<td>0.03 ± 0.01 µC</td>
<td>0.49 ± 0.01 µC</td>
<td>0.46 µC</td>
<td>0.37 µC</td>
</tr>
<tr>
<td>75 ppb</td>
<td>0.05 ± 0.02 µC</td>
<td>0.63 ± 0.03 µC</td>
<td>0.58 µC</td>
<td>0.49 µC</td>
</tr>
<tr>
<td>100 ppb</td>
<td>0.05 ± 0.03 µC</td>
<td>0.76 ± 0.05 µC</td>
<td>0.71 µC</td>
<td>0.62 µC</td>
</tr>
</tbody>
</table>

As seen in Table 9, the charge remained between 0.01 µC and 0.05 µC after a step to -200 mV despite an increase in As(III) concentration for both the As(III) standard solution and interferent solution. This indicates that As(III) was not depositing at this potential but also that these concentrations of Cd(II), Hg(II), and Pb(II) were too low to detect. Higher concentrations of interfering metals were not explored in this work as As(III) was simply evaluated in a sample based on drinking water for which these low-level interferent concentrations were appropriate. The charge for a step to -600 mV, however, increased with As(III) concentration for both standard As(III) solutions and
interferent solutions. This was expected, as the application of -600 mV should induce As(III) deposition.

Figure 33 compares the intercept-corrected subtracted charge values (-600 mV minus -200 mV charge) for As(III) standards to the subtracted charge values of As(III) solutions containing metal interferents. The subtracted charges increased linearly with As(III) concentration for both the standard As(III) solutions and interferent solutions, as noted by the 0.9889 and 0.9942 correlation coefficients, respectively. The metal interferents caused slightly lower charge values for As(III), indicating that the presence of low levels of Cd(II), Hg(II), and/or Pb(II) does alter the response for As(III), particularly at higher As(III) concentrations. This was found to occur consistently. Despite this small decrease in charge, however, the detection limit for As(III) in the standard solutions, calculated using $3\sigma_{bi}$ (Appendix IV), was 5 ppb, while the As(III) limit of detection in the presence of Cd(II), Hg(II), and Pb(II) was 8 ppb, which remains below the WHO safety guideline of 10 ppb for As(III).

Although the DPS-ASC method was shown suitable for As(III) detection in the presence of Cd(II), Hg(II), and Pb(II) in drinking water, serious interferent issues may arise as concentrations of interfering metals increase. Detection of As(III) may be compromised in the presence of high-level interferents as experienced in polluted water and wastewater samples. This consideration is essential in the study of As(III) in samples that are more complex than drinking water.
Figure 33. As(III) concentration versus intercept-corrected charge (IcC) for a standard As(III) solution (solid line) and an interferent solution containing Cd(II), Hg(II), and Pb(II) (dashed line).

3.4. Conclusion

The gold macroelectrode was replaced with arrays of either 50 µm, 20 µm, or 10 µm diameter gold microelectrodes to lower the noise due to charging current to improve the detection limit to meet the WHO safety standard guideline of 10 ppb for As(III). Microelectrode arrays were fabricated by patterning silicon nitride using photolithography techniques and subsequently etching the silicon nitride using 6:1 BOE to reveal individual gold microelectrodes. Stamp-and-stick fabrication was performed to maintain a constant, small volume between devices of differing microelectrode array size.

As(III) standards from 0 ppb to 1,000 ppb were analyzed using each array size. Whereas the linear range for the 50 µm array included As(III) concentrations from 0 ppb up to 1,000 ppb As(III), the 20 µm and 10 µm arrays had a smaller linear range up to 100 ppb As(III). The limit of detection for As(III) was 6 ppb and 4 ppb when using the 20 µm
and 10 µm diameter arrays, respectively. This indicates that either of these arrays would be acceptable for analysis of As(III) in drinking water following the WHO safety standard guideline of 10 ppb. Furthermore, it was noted that the time for stripping amperograms to meet was 10-50 msec for the microelectrodes compared to 250 msec for the macroelectrodes.

Last, the effect of three metal interferents on As(III) detection was evaluated. Subtractive DPS-ASC was performed to target the analysis of only As(III). Even in the presence of low levels of Cd(II), Hg(II), and Pb(II), the limit of detection for As(III) was 8 ppb, which still met the WHO safety standard criteria of 10 ppb.
CHAPTER IV
DPS-ASC FOR THE ANALYSIS OF LEAD USING GOLD MACROELECTRODES AND MICROELECTRODE ARRAYS

4.1. Introduction

In this chapter, the attention turns away from As and instead focuses on Pb. Whereas As is a metalloid with an atomic weight of 74.92 g mol$^{-1}$ and the As(III)/As(0) reaction studied here involves 3 transferred electrons, Pb is a post-transition metal with a higher atomic weight of 207.2 g mol$^{-1}$ and the Pb(II)/Pb(0) reaction evaluated here entails only 2 transferred electrons. These differences between As and Pb are highlighted because Faraday’s laws of electrolysis factor in both the molecular weight and the number of transferred electrons, as outlined previously in Equation 5 and Equation 6.

Pb is a significant heavy metal to study as IARC lists Pb as a Group 2A agent (probably carcinogenic) [17] and the ATSDR ranks Pb as #2 priority on the 2017 substance priority list [18]. Pb is a neurotoxin which causes an array of health complications [19]. Some of the most studied impacts include renal disease, hypertension, interference with enzymes in the biosynthesis of haem (heme), inhibition of ferrochelatase, interference with calcium metabolism, and central and peripheral nervous system damage [82]. Fetuses, infants, children under six years of age, and pregnant women are most susceptible to Pb toxicity [82]. In the United States, blood Pb levels of children have decreased since the 1970s when Pb-based paints and Pb-containing gasoline were
phased out, but Pb still remains prevalent in certain regions across the globe where Pb-based products are not under regulation [83,84].

Noting the previously listed impacts of Pb exposure on human health, the WHO set a provisional guideline for Pb in drinking water at 10 ppb, whereas the EPA maximum contaminant action level for Pb in drinking water is 15 ppb [20,81]. The WHO notes that the presence of Pb in drinking water is primarily from corrosive water effects on plumbing systems that contain Pb in the pipes, solder, or fittings. Temperature, pH, water hardness, and standing time of water all contribute to the amount of Pb that can dissolve from the plumbing system into the water supply [20].

The concern of Pb in drinking water has been highlighted through a variety of incidents within the last few years. For example, the Flint, Michigan water crisis began in April 2014 when the city switched water sources without the addition of corrosion inhibitors, which increased Pb leaching from the water pipes [85]. Furthermore, elevated Pb levels have been reported in water at schools in Richmond, Virginia; Phoenix, Arizona; New Brunswick, New Jersey; Mountain View, California; and Berkeley, California [86]. A rise in Pb levels in water in Washington DC has been reported since 2003, attributed to a change in the water disinfectant treatment method [87]. And in 2019, over 20% of homes sampled in Newark, New Jersey exceeded the 15 ppb EPA limit [88].

Clearly, implementation of a better detection and warning system could rapidly report any spikes in Pb levels that may occur as in the cases listed previously. Such a system, described in Chapter I, would entail a network of remote sensors that could be installed around a variety of locations and serve as a 24/7 monitor of heavy metals in water. As mentioned before, the utilization of electrochemical methods has great promise
for (re)calibration-free analysis of heavy metals, eliminating the need for blank samples and complex sample treatment procedures performed on-site.

However, prior to the analysis of Pb via a remote sensor, some background on the chemistry of Pb must be reviewed. In aqueous solutions, Pb exists primarily in two oxidation states, (II) and (IV). However, few inorganic Pb(IV) compounds are known. Therefore, Pb(II) compounds are most common in traditional inorganic Pb analyses. In non-complexing solutions, Pb(II) ions prevail at pH values less than 7 per the Pourbaix diagram [89]. As solutions of pH 2 are studied in this work, Pb(II) is the target. In the electrochemical detection of Pb(II), the Pb(II) is reduced to Pb(0) via the reaction shown below for the solution conditions employed here. The $E^0$ for the reduction of Pb(II) to Pb(0) is -0.330 V vs. Ag/AgCl. For reference, other reactions involving Pb(II) are shown in Appendix I but did not occur under the solution conditions used in this work.

$$\text{Pb}^{2+} + 2e^- \leftrightarrow \text{Pb}_{(s)}$$

To date, there have been many experimental studies of Pb(II) electrochemistry. Anodic stripping voltammetry is most commonly used for electrochemical detection of Pb(II) and is thus the basis for comparison in this work. Anodic stripping voltammetry has been performed for Pb(II) determination using gold [58,60,90–95], mercury [96–98], carbon [99–101], boron-doped diamond [102–104], and modified [105–108] working electrodes. The use of mercury electrodes is becoming less common due to the hazards and environmental impact of mercury, whereas the use of boron doped diamond and modified electrodes is increasing as alternatives to mercury. Pb(II) analyses by anodic
stripping voltammetry show desirable detection limits (sub 10 ppb) in a variety of samples, including freshwater, saltwater, wastewater, soil, fuel, and human blood which are commonly adjusted to a pH of 2-5 using nitric acid, hydrochloric acid, or acetate buffer. In addition, analysis of Pb(II) in the presence of common heavy metal interferences, such as As(III), Cd(II), Cu(II), and Hg(II), has been possible using anodic stripping voltammetry with gold, mercury, carbon, and boron doped diamond electrodes [60,97,100,109–111]. Furthermore, Wang et al. developed a portable electrochemical sensor featuring a gold electrode for on-site analysis of Pb(II) in the presence of Cu(II), Hg(II), and Se(IV) with a detection limit for Pb(II) of 1.1 ppb in ground water [112].

Because prior work with gold electrodes in our group has been positive due to the simplicity of electrode fabrication and ease of electrode cleaning, gold electrodes were first considered for DPS-ASC analysis of Pb(II). From literature on the electrochemical detection of Pb(II), it was determined that gold electrodes would be suitable for Pb(II) analysis at sub ppb detection limits in acidic media with chloride. The low pH reduces the formation of Pb(II) complexes and the addition of chloride has been found to increase current response [60,92]. For example, Noh and Tothill found that the addition of only 0.05 mM HCl improved the Pb(0) current response by $6 \times 10^{-8}$ A [92]. It is theorized that chloride ions act as electron bridges and facilitate metal redox reactions on gold electrodes, indicating a benefit for Pb(II) reduction [113].

Although reports from the literature have already shown low detection limits for Pb(II) using a gold electrode with decent selectivity, the current methods lack the ability for operator-free analysis and are therefore incapable of truly remote detection of Pb(II). Therefore, this work is focused on developing DPS-ASC for the analysis of Pb(II) for
applications in remote sensing and creating an alert system to detect changes in Pb(II) levels to provide early warning of contamination events, such as what occurred in Flint, Michigan. Gold electrodes were utilized due to simple fabrication processes in creating specific designs and for their ability to be electrochemically cleansed, as reported previously [68]. The issue of selectivity was addressed as Pb(II) was evaluated in the presence of a common interferent, Cu(II), and the applicability of DPS-ASC for field analysis of Pb(II) was explored using water samples collected from the Ohio River.

Furthermore, the longevity of the electrochemical sensor was explored. It would be ideal if the device could be installed in a remote location and used reliably for extended periods of time before human intervention must occur. However, if parts of the device become fatally inactive after a certain amount of time, it is critical to know 1) which components have the shortest lifespan, 2) if there is anything that can be altered to extend their shelf life, and 3) how frequently devices will need to undergo maintenance. These considerations were addressed by analysis of Pb(II) using DPS-ASC over the span of two weeks.

4.2. Materials and Methods

4.2.1. Chemicals, Reagents, and Solution Preparation

All reagents were purchased at the highest purity and used without further purification. Nitric acid and AAS 1,000 ppm single element standards were purchased from Sigma Aldrich (St. Louis, MO) for Cu(II) (from Cu(NO₃)₂) and Pb(II) (from Pb(NO₃)₂). Buffered oxide etch (BOE) (6:1), acetone, sodium chloride, and sulfuric acid were purchased from VWR (Radnor, PA).
All solutions were prepared fresh each day using deionized water. Nitric acid solutions were made by pipetting the needed volumes of concentrated HNO₃ into volumetric flasks filled halfway with deionized water and subsequently diluting to the mark with deionized water. Cu(II) and Pb(II) standards (10 ppm) were prepared by pipetting 0.50 mL of the appropriate 1,000 ppm AAS into a 50 mL volumetric flask and diluting to volume with diluent. Subsequent standards were prepared by pipetting the appropriate amount of 10 ppm standard into 50 mL volumetric flasks and diluting to the mark with blank solution.

Safety is of utmost concern due to the toxic nature of these heavy metals. Skin contact with Cu(II) and Pb(II) was avoided by wearing protective safety goggles, long-sleeve shirts, long pants, closed-toe shoes, and nitrile examination gloves from VWR (Radnor, PA) during solution preparations. Spills of heavy metal solutions were cleaned with paper towels that were disposed of in appropriate solid waste containers.

Handling of BOE involved additional precautions due to the hazards of aqueous hydrofluoric acid. Specific personal protective equipment for handling BOE was always worn in addition to the eye ware, clothing, shoes, and gloves listed before. The additional specialized equipment for handling BOE included a full-face shield, an acid-resistant long-sleeve apron, and elbow-length neoprene gloves. The BOE was kept contained within a single fume hood designated for use of hydrofluoric acid, and solutions were stored in plastic containers. Calcium gluconate gel and eyewash kits were kept readily available in the lab in case of hydrofluoric acid spills and exposures.

All solution waste was collected in containers provided by the Department of Environmental Health and Safety (DEHS) at the University of Louisville. Waste BOE
was collected into a separate container, whereas all other acidic solutions were collected in an acidic waste container. Solid waste was collected within a solid waste disposal bucket. Once the containers were full, DEHS was notified to collect the containers and undergo proper disposal per federal regulations.

4.2.2. Ohio River Water

Ohio River water was collected into 500 mL polypropylene containers from the Kentucky-side of the riverbank at GPS coordinates 38°15’38”N, 85°44’44”W in Louisville, Kentucky. Water was collected from at least 6 inches below the water surface. Samples were immediately refrigerated after collection until use. For analysis, samples were brought to room temperature by sitting on the lab bench for at least 2 hours. Then, concentrated HNO₃ was added to the river water until the pH was ~2, and NaCl was added to a final concentration of 10 mM. The samples were not filtered.

4.2.3. Gold Working Electrodes

Both thin-film gold macroelectrodes and gold microelectrode arrays were prepared as described previously in Chapter II and Chapter III, respectively. Briefly, the thin-film gold macroelectrode was prepared by depositing 20 nm titanium followed by 120 nm gold onto an oxidized silicon wafer. The wafer was then diced into 30 mm by 13 mm chips. The gold microelectrode arrays began with an oxidized silicon wafer with subsequent layering of 20 nm titanium, 200 nm gold, and 300 nm silicon nitride. Shipley 1827 photoresist was used to pattern the silicon nitride to create the microelectrode arrays, and then the wafer was diced into 30 mm by 13 mm chips. The silicon nitride was
etched using vapor from 6:1 BOE to expose the gold arrays, and then the Shipley 1827 protective layer was removed by sonication in acetone.

4.2.4. Working Electrode Compartment

The working electrode compartment was built directly on top of the 30 mm by 13 mm gold electrode chips via stamp-and-stick fabrication using borosilicate glass, as described in Chapter II. Briefly, NOA 68 was spun via Headway Spinners to a 10-20 nm thickness on a bare silicon wafer. An AB-M Inc Aligner was used to transfer a thin layer of NOA 68 onto 80 µm thick borosilicate glass (30 mm long by 10 mm wide) that had previously been laser cut to reveal the 8 mm by 4 mm ellipse with 5 mm by 1 mm channels. Then, the coated borosilicate glass was aligned and put into contact with the 30 mm by 13 mm gold macroelectrode or gold microelectrode array chips. Finally, the borosilicate glass/electrode combination was exposed to UV light for 300 sec to cure and create electrode sensor chips with permanent, defined volumes.

4.2.5. Coulometry Device

The coulometry device was described previously in Chapter II. It features a working electrode compartment and a counter/reference electrode compartment that are separated by a membrane and have independent sample inlets and outlets with external fluidic flow shut-off valves. A polycarbonate base was recessed to hold the 30 mm by 13 mm working electrode chip. A 200 MWCO membrane was affixed on top of the working electrode. Membrane pieces were replaced after 10-15 days of use. Then, three laser-cut silicone gaskets, the Panasonic pyrolytic graphite sheet counter, thin laser-cut silicone
gasket, and polycarbonate top were added to define the counter/reference electrode compartment. A custom Ag/AgCl reference electrode was inserted through an access hole in the polycarbonate top and sealed in place using silicone grease. The polycarbonate pieces were screwed together using four screws, and the torque on each screw was set to a value of 0.51 kg-cm using a torque screwdriver. The device was assembled at the beginning of each day and disassembled at the end of each day. All gaskets were washed twice a week with soap and water to prevent grease build-up and contamination.

4.2.6. Electrochemical Measurements

The three-electrode system encompassed either a gold macroelectrode or a gold microelectrode array for the working electrode, a custom miniature Ag/AgCl reference electrode, and a pyrolytic graphite sheet as the counter electrode. Details describing the usage of each of the various gold macroelectrodes and gold microelectrode array chips utilized in this work are listed in Appendix III. Reference electrodes were replaced every 5-10 days to ensure proper reference conditions (i.e., no potential drift) and the pyrolytic graphite sheet was replaced after one month of use. A BASi Epsilon potentiostat (West Lafayette, IN) was used for all electrochemical measurements.

The following procedure was utilized for DPS-ASC experiments. First, the counter/reference chamber was filled with 10 mM HNO₃/10 mM NaCl and the fluid valves were closed. This solution was not replaced throughout the experiment. Then, the working electrode chamber was filled with 50 mM H₂SO₄ and the gold working electrode was electrochemically cleaned by slowly flushing the 50 mM H₂SO₄ through the chamber as the potential was cycled from 0 mV to 1,400 mV at 100 mV sec⁻¹ for 4-6 scans until
cyclic voltammetry traces were reproducible. Then, the working electrode chamber was filled with analyte sample (blank or Pb(II) solution) and fluid valves were closed before applying the DPS-ASC potential sequence described below. The DPS-ASC experiments were repeated at least three times for each analyte sample. Between each experiment, the fluid valves were opened, the working electrode chamber was filled with fresh analyte sample, and then the fluid valves were closed. Once all analyte samples were evaluated, the working electrode was cleaned at the end of the day by flushing 50 mM H₂SO₄ through the chamber as the potential was cycled from 0 mV to 1,400 mV until traces were reproducible.

The DPS-ASC sequence, as described in Chapter 1 and outlined in Figure 3, used in the detection of Pb(II) is outlined below. There was no applied potential before this pulse sequence was applied.

Pulse 1: -400 mV for 0.1 sec
Pulse 2: 500 mV for 1 sec
Pulse 3: -400 mV for 130 sec
Pulse 4: 500 mV for 1 sec

By overlaying the two oxidation traces (pulse 2 and pulse 4) from DPS-ASC, the data took the shape of a crescent with an x-axis of time and y-axis of current. These current-time curves (amperograms) were integrated to obtain charge, and the difference between the two curves gave Faradaic charge. The integration of the current-time curves was accomplished by summing the area under the curves until the traces met one another. The area is indicative of charge with the units of coulombs. All integrations and data
processing were done by extracting text files from the BASi Epsilon software and using Microsoft Excel to perform all calculations.

4.2.7. Pre-Electrolysis Device

A device was constructed for the removal of Cu(II). The pre-electrolysis unit, depicted in Figure 34, consisted of a three-electrode system housed in-line before the coulometry device. The working electrode was a cylindrical piece (10 mm long, 13 mm diameter) of 100 pores-per-inch (PPI) reticulated vitreous carbon (RVC) from ERG Duocel (Oakland, CA) that was wrapped in carbon cloth and contained inside of the barrel of a 5 mL plastic syringe from the BD Company (Franklin Lakes, NJ). The syringe barrel had one-way stopcocks installed on both ends connected by Luer-lock fittings. The carbon cloth was used to keep the brittle RVC intact, and the carbon cloth protruded through an opening in the syringe barrel that provided a means for electrical connection by alligator clip to the potentiostat. The reference electrode was a commercial Ag/AgCl electrode from BASi (West Lafayette, IN), and a platinum wire from Alfa Aesar (Ward Hill, MA) was used as the counter electrode. The reference and counter electrodes were inserted into the device through holes in the syringe barrel and were sealed in place with PTFE thread tape from Grainger, Inc. (Miami, FL) or Amazing Goop all-purpose adhesive from The Home Depot (Atlanta, GA), respectively. A BASi Epsilon potentiostat (West Lafayette, IN) was used for all electrochemical measurements. Samples were pumped through the device at a flow rate of 0.15 mL min\(^{-1}\) via a Harvard Apparatus 22 dual syringe infusion pump (Holliston, MA), and the fluid continued from the pre-electrolysis unit into the coulometry device through Tygon tubing (roughly 9 inches long
and 0.25 inch internal diameter). The deposition potential used for Cu(II) removal was -500 mV, and a potential of 700 mV was applied to oxidize the Cu(0) to Cu(II) when cleaning the pre-electrolysis device.

Figure 34. Schematic of the pre-electrolysis device for capture of Cu(II). A) platinum wire counter electrode, B) Ag/AgCl reference electrode, C) reticulated vitreous carbon wrapped in carbon cloth for a working electrode, D) fluidic flow direction through the device, E) sample inlet valve, F) sample outlet valve. All electrodes were housed within a plastic syringe barrel as surrounded by the fluid valves, E and F.

4.2.8. Longevity Study

A study of the reliability and consistency of charge measured by DPS-ASC over the course of two weeks was performed. A new coulometry device, referred to as the 3D-printed device, was utilized in lieu of the coulometry device discussed in 4.2.5. The 3D-printed device, akin to the previous coulometry device, features a working electrode compartment and a counter/reference electrode compartment separated by a membrane (Figure 35). The 3D-printed device is circular to disperse force equally between the top and bottom pieces. The base was constructed from polycarbonate by CNC milling at Protolabs (Maple Plain, MN) and included a recessed groove to hold the 13 mm by 30
mm gold working electrode, four holes for alignment pins, and six threaded holes for connection to the top piece. A 200 MWCO membrane was affixed on top of the working electrode followed by a silicone gasket, pyrolytic graphite sheet counter electrode from Panasonic (Newark, NJ), and the top piece which was 3D-printed using stereolithography at the Additive Manufacturing Competency Center (Louisville, KY). The top piece featured built-in Luer-lock connectors and serpentine fluidic channels. Furthermore, an Ag/AgCl reference electrode (3M NaCl) from BASi (West Lafayette, IN) was inserted through an access hole in the top piece and was sealed in place using Teflon tape. The 3D-printed top piece and polycarbonate base were screwed together using six screws, and the torque on each screw was set to a value of 0.51 kg-cm using a torque screwdriver. The device was assembled at the beginning of this study and was not disassembled until the end of the study (\textit{i.e.} two weeks later).
Each set of experiments during the study began with cleaning the working electrode by flushing 50 mM H$_2$SO$_4$ through the working electrode chamber and cycling the potential between 0 mV and 1,400 mV until cyclic voltammetry traces were reproducible. After cleaning, three DPS-ASC measurements were taken of a 2 ppm Pb(II) solution using the DPS-ASC potential sequence described in section 4.2.6. The cleaning
step and Pb(II) analyses were performed at three different times each day – morning, afternoon, and evening – resulting in nine measurements of Pb(II) each day that were then averaged to give one daily charge for 2 ppm Pb(II). This was repeated each day for two weeks.

Between the morning, afternoon, and evening measurements, pumping valves were left closed so that both fluidic chambers were filled with stagnant fluid. The counter/reference electrode compartment was filled with 3 M NaCl to ensure that the reference electrode was stored properly, whereas the working electrode compartment was filled with a blank solution of 10 mM HNO₃/10 mM NaCl.

4.3. Results and Discussion

4.3.1. Optimizing Parameters for Analysis of Pb(II) Standards

The appropriate parameters, such as solution conditions and deposition/stripping potentials, were optimized for standard Pb(II) solutions using a gold macroelectrode in a beaker. These parameters were then confirmed through analysis in the coulometry device using both a gold macroelectrode and gold microelectrode array. Findings for the beaker experiments agreed with the coulometry device, as expected. These parameters were subsequently used for all DPS-ASC experiments.

First, the effects of oxygen on the deposition of Pb(II) and stripping of Pb(0) were evaluated. Linear sweep stripping voltammetry of a blank solution (10 mM HNO₃/10 mM NaCl) and 2 ppm Pb(II) in 10 mM HNO₃/10 mM NaCl was performed using a gold macroelectrode in the coulometry device. A deposition potential of -500 mV was applied for 120 sec, and then the potential was swept at 100 mV sec⁻¹ to 500 mV. The peak
currents were evaluated for both ambient and deoxygenated solutions (Figure 36), and it was determined that the presence of oxygen did not impact the deposition of Pb(II) or stripping of Pb(0) as the oxidation peak current near -60 mV was not altered. Therefore, solutions were further analyzed as is, without deoxygenation.

**Figure 36.** Linear sweep stripping voltammetry of 10 mM HNO$_3$/10mM NaCl (*black*) compared to 2 ppm Pb(II) in 10 mM HNO$_3$/10mM NaCl (*red*), in ambient (*solid*) versus deoxygenated (*dashed*) conditions. Deposition potential held at -500 mV for 120 sec and scanned to 500 mV at a scan rate of 100 mV sec$^{-1}$.

Cyclic voltammetry and linear sweep stripping voltammetry of Pb(II) in 10 mM HNO$_3$/10 mM NaCl were performed both in a beaker and in the coulometry device using a gold macroelectrode to determine the appropriate deposition and stripping potentials. The peak deposition potential for Pb(II) was determined to be -360 mV vs. Ag/AgCl by cyclic voltammetry, which agrees closely with the E$^0$ of -330 mV vs. Ag/AgCl. As seen
in Figure 37, the Pb(0) stripping peak current did not increase with application of potentials more negative than -400 mV. Therefore, -400 mV was selected to ensure 100% deposition in further experiments. Application of -400 mV overlaps with the onset of hydrogen evolution on a bare gold electrode, but as Pb(II) deposits on gold and forms Pb(0) adatoms, the hydrogen evolution reaction is inhibited as Pb(0) requires a high overpotential for hydrogen evolution [114]. This was confirmed in this work as hydrogen evolution was not observed visually at -400 mV.

Figure 37. Linear sweep stripping voltammetry of 2 ppm Pb(II) on a gold electrode. Potential was held for 120 seconds at -0.1 V (blue), -0.2 V (yellow), -0.3 V (green), -0.4 V (black), or -0.5 V (red) and then scanned at 0.1 V sec\(^{-1}\) to 0.3 V.

Although the stripping of Pb(0) was found to be complete at 200 mV, the potential of 500 mV was selected for all oxidations to both completely rid Pb(0) from the
gold surface and to maintain consistency for later as other metal interferents may require a more positive stripping potential.

4.3.2. Consideration of Monolayer Coverage

Underpotential deposition occurs when a species is reduced onto a material other than itself at a potential less negative than the equilibrium potential. In the analysis of metal ions, such as Pb(II), underpotential deposition results in the deposition of a metal monolayer onto the electrode surface at a less negative potential compared to the potential required for subsequent deposition of that metal onto the metal monolayer.

Before the analysis of Pb(II) using DPS-ASC in the coulometry device, underpotential deposition and electrode surface coverage must be considered as deposition potentials and rates may differ for Pb(II) on gold compared to Pb(II) on Pb(0). It was predicted that the reduction of Pb(II) to Pb(0) on the gold electrode would occur at a less negative potential than the reduction of Pb(II) to Pb(0) on the Pb(0) monolayer. This would suggest that a more negative potential would be required for reduction of Pb(II) above Pb(0) monolayer coverage on the gold electrode. Failure to apply the appropriate potential would result in incomplete deposition of Pb(II) above Pb(0) monolayer coverage which would contribute to an inaccurate measurement of total Pb(II) concentration. Therefore, knowing underpotential deposition characteristics of Pb(II)/Pb(0) is critical in choosing appropriate concentrations of Pb(II) on macroelectrodes and microelectrode arrays so that monolayer coverage is not exceeded.

Pb(0) underpotential deposition and coverage has been extensively studied [114–118]. On a gold electrode, Pb(0) favors the formation of incommensurate adlayers
because the atomic size of Pb(0) is approximately 20% larger than that of gold. This
means that the Pb(0) forms a structural monolayer which is not based on (i.e., is out of
registry with) the structure of the gold electrode surface. A hexagonal incommensurate
structure has been confirmed via x-ray diffraction, scanning tunneling microscopy, and
atomic force microscopy [114].

There are many assumptions in the calculation of Pb(0) coverage. Hexagonal
closed packing is assumed based on the hexagonal character of the incommensurate
structure, which entails 74% packing efficiency (i.e. atoms occupy 74% of packing
volume), and the Van der Waals radii for Pb are utilized. The Pb(II) concentration at
which monolayer coverage is expected to occur has been calculated using these
assumptions (Appendix IV), and results are listed in Table 10. Monolayer coverage is
expected at 8.0 ppm for the gold macroelectrode. The 50 µm, 20 µm, and 10 µm
microelectrode arrays are predicted to reach monolayer coverage at 2.2 ppm, 0.93 ppm,
and 1.0 ppm, respectively. These concentrations are lower than the macroelectrode
because the overall electrode area decreases.

Table 10. Comparison of calculated surface coverage parameters for gold electrodes.

<table>
<thead>
<tr>
<th>Electrode Type</th>
<th>Pb(II) Volume</th>
<th>Pb(II) Charge at Surface Coverage</th>
<th>Pb(II) Conc. at Surface Coverage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Macroelectrode</td>
<td>5.5 µL</td>
<td>41.0 µC</td>
<td>8.0 ppm</td>
</tr>
<tr>
<td>50 µm Array</td>
<td>3.7 µL</td>
<td>7.6 µC</td>
<td>2.2 ppm</td>
</tr>
<tr>
<td>20 µm Array</td>
<td>4.0 µL</td>
<td>3.5 µC</td>
<td>0.93 ppm</td>
</tr>
<tr>
<td>10 µm Array</td>
<td>2.4 µL</td>
<td>2.2 µC</td>
<td>1.0 ppm</td>
</tr>
</tbody>
</table>
Some factors, however, remain unknown and may cause these calculations to differ from the experimental results. These include positioning (i.e. packing geometry) of other ions in solution, such as chloride, that may alter the packing efficiency of Pb(0), and the possibility that islands of Pb(0) may form before uniform monolayer coverage occurs. In addition, the bonding and packing types may vary from our assumptions and could change the calculated Pb(II) concentration for saturation of the electrode surface.

4.3.3. **DPS-ASC of Pb(II) Standards Using a Gold Macroelectrode**

A gold macroelectrode was utilized for the analysis of Pb(II) using DPS-ASC conducted in the coulometry device. This study was performed in duplicate using the same electrode on two different days between which the coulometry device was newly assembled. The data, as presented in crescents, shows that the charge (i.e., crescent size) increased with increasing Pb(II) concentration, agreeing with Faraday’s second law of electrolysis (Figure 38).
Figure 38. DPS-ASC crescents from analysis of 0 ppm to 5 ppm Pb(II) using a gold macroelectrode.

The graph of Pb(II) concentration versus charge is shown in Figure 39. Figure 39 A shows the curve from 1 ppm to 5 ppm Pb(II) with a correlation coefficient ($R^2$) of 0.9899 which indicates linearity throughout this range. As seen in Figure 39 B, the 10 ppm Pb(II) charge did not fit the linear trend, indicating that this concentration was out of the linear range. The calculated monolayer coverage value was 8.0 ppm (Appendix IV), which suggests that the signal is possibly leveling off at 10 ppm due to electrode surface coverage by Pb(0).
Figure 39. Pb(II) concentration versus intercept-corrected charge (IcC) for a gold macroelectrode. A) Pb(II) linear up to 5 ppm, B) Pb(II) loss of linearity above 5 ppm.

Table 11 compares the charge values from Pb(II) analysis by DPS-ASC using the gold macroelectrode. The charges from pulse 2 and pulse 4 were recorded experimentally. The background-corrected charge (Bgc) results from subtraction of pulse 2 charge from pulse 4 charge. The results were highly reproducible with RSD values less than or equal to 1.5%. The intercept-corrected charge (IcC) was determined by
subtracting the 0 ppb Pb(II) BgC (i.e., 3.78 µC) from each BgC value. These values were then compared to the calculated charge values per Faraday’s law. The 1 ppm Pb(II) standard had a high relative error at 43% which results from this concentration being most near the detection limit, calculated using \(3\sigma_{b}\) (Appendix IV) to be 750 ppb. Additionally, the relative error was high at -32% for the 10 ppm Pb(II) standard which results from this concentration being outside of the linear range. Nevertheless, the remaining relative errors were less than 7.5%.

Table 11. DPS-ASC numerical results from analysis of Pb(II) standards from 0 ppm to 10 ppm using a gold macroelectrode in a device of volume 5.5 µL (# trials = 3). Pulse 2 charge obtained after deposition at -400 mV for 0.1 sec. Pulse 4 charge obtained after deposition at -400 mV for 130 sec. Background-corrected (BgC) charge is pulse 4 minus pulse 2 charge. Intercept-corrected (IcC) charge is the BgC charge minus the intercept charge (i.e. the BgC charge for 0 ppm Pb(II)). Calculated (calc) charge is based on Faraday’s law where \(n\) is 2 for Pb(II)/Pb(0), \(F\) is the constant 96,485 C/mol, \(C\) is Pb(II) concentration (mol/L), and \(V\) is 5.5 \(\times\) 10^{-6} L.

<table>
<thead>
<tr>
<th>Pb(II) ppm</th>
<th>Pulse 2 Charge µC</th>
<th>Pulse 4 Charge µC</th>
<th>BgC Charge µC (RSD)</th>
<th>IcC Charge µC</th>
<th>Calc Charge µC</th>
<th>% Error</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>16.10</td>
<td>19.88</td>
<td>3.78 (4.7%)</td>
<td>0.00</td>
<td>0.00</td>
<td>–</td>
</tr>
<tr>
<td>1</td>
<td>16.43</td>
<td>27.54</td>
<td>11.12 (1.4%)</td>
<td>7.34</td>
<td>5.14</td>
<td>43%</td>
</tr>
<tr>
<td>2</td>
<td>16.56</td>
<td>31.34</td>
<td>14.78 (0.4%)</td>
<td>11.00</td>
<td>10.28</td>
<td>7.1%</td>
</tr>
<tr>
<td>3</td>
<td>16.95</td>
<td>36.46</td>
<td>19.51 (1.4%)</td>
<td>15.73</td>
<td>15.42</td>
<td>2.1%</td>
</tr>
<tr>
<td>4</td>
<td>16.92</td>
<td>40.44</td>
<td>23.52 (1.5%)</td>
<td>19.74</td>
<td>20.56</td>
<td>-3.9%</td>
</tr>
<tr>
<td>5</td>
<td>16.96</td>
<td>45.20</td>
<td>28.24 (1.5%)</td>
<td>24.46</td>
<td>25.70</td>
<td>-4.8%</td>
</tr>
<tr>
<td>10</td>
<td>16.62</td>
<td>55.26</td>
<td>38.64 (4.2%)</td>
<td>34.86</td>
<td>51.41</td>
<td>-32%</td>
</tr>
</tbody>
</table>

4.3.4. DPS-ASC of Pb(II) Standards Using Gold Microelectrode Arrays

The gold macroelectrode could only reach a detection limit of 750 ppb, whereas the EPA action level is 15 ppb. Therefore, microelectrode arrays were explored in order to reach lower detection limits. As described in Chapter III, microelectrodes are
advantageous in reducing background charging current, and this should result in less noise and, thus, an improved signal-to-noise ratio. Three different array sizes were considered (50 µm, 20 µm, and 10 µm) which were explored previously in Chapter III. These sizes represent the individual diameter of one microelectrode. The features of each array type were listed in Table 5. Experiments using the 50 µm array were repeated twice using the same 50 µm microelectrode on two different days. Work was repeated four times using the same 20 µm microelectrode on four different days. Analysis of Pb(II) standards using a 10 µm array was repeated twice using the same electrode on two different days.

Pb(II) standards were evaluated by DPS-ASC using a 50 µm microelectrode array. The resulting crescents from the DPS-ASC analysis of Pb(II) using the 50 µm microelectrode array are shown in Figure 40 and demonstrate how the charge increased with concentration. Furthermore, the individual amperograms were found to meet at very fast times (less than 10 msec) for microelectrode arrays compared to the macroelectrode due to the faster charging time associated with the decreased electrode area (i.e., double layer capacitance).
The crescents from the DPS-ASC analysis using the 20 µm microelectrode array are shown in Figure 41. It was speculated that the shape of the crescent was different, as indicated by the uneven curve in the crescents for concentrations above 30 ppb Pb(II), due to coverage of the electrode surface with Pb(0). The gold microelectrodes are more susceptible to coverage with Pb(0) because the electrode area is smaller using the arrays, and it is suggested that the stripping process entails a stripping of Pb(0) from the Pb(0) layer followed by stripping of Pb(0) from the gold electrode, thus giving rise to an uneven curve in the crescents as Pb(II) concentrations increases. The resulting crescents from the DPS-ASC analysis using the 10 µm microelectrode array are shown in Figure 42. Akin to the 20 µm microelectrode array crescents, these crescents also display alteration in curve shape above 20 ppb Pb(II).
Figure 41. DPS-ASC amperograms overlaid to create crescents, representative of the Faradaic charge. Shown here are crescents from analysis of 0 ppb to 90 ppb Pb(II) using a 20 µm gold microelectrode array.

Figure 42. DPS-ASC amperograms overlaid to create crescents, representative of the Faradaic charge. Shown here are crescents from analysis of 0 ppb to 60 ppb Pb(II) using a 10 µm gold microelectrode array.
The 50 µm array electrode was found to have a linear range from 400 ppb to 700 ppb Pb(II) with a $R^2$ value of 0.9701, as seen in Figure 43 A. Due to the smaller electrode area compared to the macroelectrode, it was expected that the microelectrode array surface would become saturated at lower concentrations of Pb(II). The calculated coverage was estimated at 2.2 ppm, but linearity was lost experimentally above 700 ppb (Figure 43 B), indicating a discrepancy in the assumptions and calculation of monolayer coverage (Appendix IV). Nonetheless, lower concentrations near 400 ppb could be detected due to the decrease in charging current.

Figure 44 A demonstrates that the 20 µm microelectrode array was linear from 15 ppb to 90 ppb Pb(II) with a $R^2$ value of 0.9765. The charge began to plateau above 100 ppb Pb(II), as seen in Figure 44 B. This behavior suggests that monolayer coverage of Pb(0) occurred and subsequently decreased further Pb(II) deposition. However, this does not agree with the calculated value for monolayer coverage of 930 ppb.

Figure 45 A shows that the 10 µm microelectrode array was linear from 10 ppb to 60 ppb Pb(II) with a 0.9967 $R^2$ value. Although monolayer coverage was predicted at 1.0 ppm (Appendix IV), the experimental results indicate non-linear behavior beyond 60 ppb (Figure 45 B). In fact, the charge decreased above 60 ppb. One hypothesis for this was that the electrode became fouled after exposure to 60 ppb Pb(II) (i.e. a high concentration for an electrode of small area) and therefore was not performing as expected in ideal conditions.
Figure 43. Pb(II) concentration versus intercept-corrected charge (IcC) for a gold 50 µm array. A) Pb(II) linear up to 700 ppb, B) Pb(II) loss of linearity above 700 ppb.
Figure 44. Pb(II) concentration versus intercept-corrected charge (IcC) for a gold 20 µm array. A) Pb(II) linear up to 90 ppb, B) Pb(II) loss of linearity above 90 ppb.
Figure 45. Pb(II) concentration versus intercept-corrected charge (IcC) for a gold 10 µm array. A) Pb(II) linear up to 60 ppb, B) Pb(II) loss of linearity above 60 ppb.
The large difference between calculated monolayer coverage and experimental results occurred for all three array sizes, possibly indicating there is a difference in monolayer coverage from radial diffusion at the microelectrodes compared to planar diffusion at the macroelectrode. It is proposed that radial diffusion to the microelectrode array sites results in a different packing of Pb(0) atoms other than the hexagonal closed packing geometry and/or different forces exist other than Van der Waals interactions (which were assumed in the monolayer coverage calculation). This, in turn, would alter the concentration value at which monolayer coverage would be expected.

The DPS-ASC charge values from each microelectrode were summarized. In each case, the charges from pulse 2 and pulse 4 were recorded experimentally. The background-corrected charge (BgC) results from subtraction of pulse 2 charge from pulse 4 charge. The intercept-corrected charge (IcC) was determined by subtracting the 0 ppb Pb(II) BgC from each BgC value. And then the IcC values were compared to the calculated charge values per Faraday’s law.

Table 12 compares the charge values from using the 50 µm array electrode for DPS-ASC analysis of concentrations of Pb(II) within the linear range (Figure 43 A), whereas Table 13 lists charge values for Pb(II) standards both inside and outside of the linear range (Figure 43 B). These sets of data were collected using the same electrode on two different days. The BgC values of 0.67 µC and 0.76 µC for a 0 ppb Pb(II) solution were lower than the 0 ppb Pb(II) charge for a macroelectrode because the electrode area was smaller. For Pb(II) samples in the linear range (Table 12), the relative error was highest at -16% for 700 ppb Pb(II), indicating that this concentration is nearing the upper
limit of the linear range. RSD values were less than or equal to 1.5% except for the 0 ppb Pb(II) charge with a 6.6% RSD. The detection limit was 320 ppb Pb(II).

Table 12. DPS-ASC numerical results from analysis of Pb(II) standards from 0 ppb to 700 ppb using a 50 µm array electrode in a device of volume 3.7 µL (# trials = 3). Pulse 2 charge obtained after deposition at -400 mV for 0.1 sec. Pulse 4 charge obtained after deposition at -400 mV for 130 sec. Background-corrected (BgC) charge is pulse 4 minus pulse 2 charge. Intercept-corrected (IcC) charge is the BgC charge minus the intercept charge (i.e. the BgC charge for 0 ppb Pb(II)). Calculated (calc) charge based on Faraday’s law where n is 2 for Pb(II)/Pb(0), F is the constant 96,485 C/mol, C is Pb(II) concentration (mol/L), and V is 3.7 x 10⁻⁶ L.

<table>
<thead>
<tr>
<th>Pb(II) ppb</th>
<th>Pulse 2 Charge µC</th>
<th>Pulse 4 Charge µC</th>
<th>BgC Charge µC (RSD)</th>
<th>IcC Charge µC</th>
<th>Calc Charge µC</th>
<th>% Error</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>6.81</td>
<td>7.48</td>
<td>0.67 (6.6%)</td>
<td>0.00</td>
<td>0.00</td>
<td>–</td>
</tr>
<tr>
<td>400</td>
<td>6.56</td>
<td>8.69</td>
<td>2.14 (1.5%)</td>
<td>1.47</td>
<td>1.38</td>
<td>6.2%</td>
</tr>
<tr>
<td>500</td>
<td>6.57</td>
<td>8.96</td>
<td>2.39 (0.2%)</td>
<td>1.72</td>
<td>1.72</td>
<td>0.0%</td>
</tr>
<tr>
<td>600</td>
<td>6.30</td>
<td>8.84</td>
<td>2.54 (1.3%)</td>
<td>1.87</td>
<td>2.06</td>
<td>-9.3%</td>
</tr>
<tr>
<td>700</td>
<td>6.11</td>
<td>8.81</td>
<td>2.71 (1.2%)</td>
<td>2.04</td>
<td>2.41</td>
<td>-16%</td>
</tr>
</tbody>
</table>

Table 13. DPS-ASC numerical results from analysis of Pb(II) standards from 0 ppm to 5 ppm using a 50 µm array electrode in a device of volume 3.7 µL (# trials = 3). Pulse 2 charge obtained after deposition at -400 mV for 0.1 sec. Pulse 4 charge obtained after deposition at -400 mV for 130 sec. Background-corrected (BgC) charge is pulse 4 minus pulse 2 charge. Intercept-corrected (IcC) charge is the BgC charge minus the intercept charge (i.e. the BgC charge for 0 ppm Pb(II)). Calculated (calc) charge based on Faraday’s law where n is 2 for Pb(II)/Pb(0), F is the constant 96,485 C/mol, C is Pb(II) concentration (mol/L), and V is 3.7 x 10⁻⁶ L.

<table>
<thead>
<tr>
<th>Pb(II) ppm</th>
<th>Pulse 2 Charge µC</th>
<th>Pulse 4 Charge µC</th>
<th>BgC Charge µC (RSD)</th>
<th>IcC Charge µC</th>
<th>Calc Charge µC</th>
<th>% Error</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>6.81</td>
<td>7.57</td>
<td>0.76 (1.9%)</td>
<td>0.00</td>
<td>0.00</td>
<td>–</td>
</tr>
<tr>
<td>250</td>
<td>6.37</td>
<td>8.35</td>
<td>1.98 (3.2%)</td>
<td>1.22</td>
<td>0.86</td>
<td>42%</td>
</tr>
<tr>
<td>500</td>
<td>6.42</td>
<td>8.99</td>
<td>2.56 (1.7%)</td>
<td>1.80</td>
<td>1.72</td>
<td>4.7%</td>
</tr>
<tr>
<td>750</td>
<td>6.59</td>
<td>9.72</td>
<td>3.13 (1.0%)</td>
<td>2.37</td>
<td>2.58</td>
<td>-8.1%</td>
</tr>
<tr>
<td>1,000</td>
<td>5.48</td>
<td>8.30</td>
<td>2.82 (0.5%)</td>
<td>2.06</td>
<td>3.45</td>
<td>-40%</td>
</tr>
<tr>
<td>2,000</td>
<td>6.57</td>
<td>11.42</td>
<td>4.86 (1.9%)</td>
<td>4.10</td>
<td>6.89</td>
<td>-41%</td>
</tr>
<tr>
<td>3,000</td>
<td>6.63</td>
<td>12.49</td>
<td>5.86 (1.1%)</td>
<td>5.10</td>
<td>10.34</td>
<td>-51%</td>
</tr>
<tr>
<td>4,000</td>
<td>6.72</td>
<td>13.81</td>
<td>7.09 (1.8%)</td>
<td>6.33</td>
<td>13.78</td>
<td>-54%</td>
</tr>
<tr>
<td>5,000</td>
<td>6.80</td>
<td>14.94</td>
<td>8.14 (4.6%)</td>
<td>7.38</td>
<td>17.23</td>
<td>-57%</td>
</tr>
</tbody>
</table>
Table 14 compares the charge values from using the 20 µm array electrode within the linear range, whereas Table 15 lists charge values for Pb(II) concentrations outside of the linear range. These sets of data were collected using the same electrode on two different days. The charge was 0.14-0.26 µC for a 0 ppb Pb(II) sample which varied based on the day that the electrode was used. This charge continues to follow the trend that the background charge for a 0 ppb Pb(II) sample is lower with smaller electrode area.

The relative error values were high for this electrode within the linear range (Table 14). However, the error values were consistently high (-29% to 17% relative error) for three different experiments using this electrode across three different days. Due to the reproducibility of this error, it was predicted that there was a difference in the actual device volume for this electrode compared to the volume used in the calculation of charge (i.e. the actual volume within the device is 3.5 µL instead of the calculated 4.0 µL, which would result in lower calculated charge values and thus different relative errors).

Nevertheless, RSD values continue to show decent reproducibility for Pb(II) concentrations in the linear range (Table 14) with values less than 7.5%. The detection limit was determined using 3σ\text{bl} (Appendix IV) as 10 ppb for this 20 µm microelectrode array.
Table 14. DPS-ASC numerical results from analysis of Pb(II) standards from 0 ppb to 90 ppb using a 20 µm array electrode in a device of volume 4.0 µL (# trials = 3). Pulse 2 charge obtained after deposition at -400 mV for 0.1 sec. Pulse 4 charge obtained after deposition at -400 mV for 130 sec. Background-corrected (BgC) charge is pulse 4 minus pulse 2 charge. Intercept-corrected (IcC) charge is the BgC charge minus the intercept charge (i.e. the BgC charge for 0 ppb Pb(II)). Calculated (calc) charge based on Faraday’s law where n is 2 for Pb(II)/Pb(0), F is the constant 96,485 C/mol, C is Pb(II) concentration (mol/L), and V is 4.0 x 10⁻⁶ L.

<table>
<thead>
<tr>
<th>Pb(II) ppb</th>
<th>Pulse 2 Charge µC</th>
<th>Pulse 4 Charge µC</th>
<th>BgC Charge µC (RSD)</th>
<th>IcC Charge µC</th>
<th>Calc Charge µC</th>
<th>% Error</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>2.11</td>
<td>2.24</td>
<td>0.14 (6.7%)</td>
<td>0.00</td>
<td>0.00</td>
<td>–</td>
</tr>
<tr>
<td>15</td>
<td>1.86</td>
<td>2.07</td>
<td>0.21 (7.1%)</td>
<td>0.07</td>
<td>0.06</td>
<td>17%</td>
</tr>
<tr>
<td>30</td>
<td>1.92</td>
<td>2.16</td>
<td>0.24 (1.7%)</td>
<td>0.10</td>
<td>0.11</td>
<td>-9.1%</td>
</tr>
<tr>
<td>45</td>
<td>2.20</td>
<td>2.48</td>
<td>0.28 (1.5%)</td>
<td>0.14</td>
<td>0.17</td>
<td>-18%</td>
</tr>
<tr>
<td>60</td>
<td>2.28</td>
<td>2.60</td>
<td>0.32 (5.3%)</td>
<td>0.18</td>
<td>0.22</td>
<td>-18%</td>
</tr>
<tr>
<td>75</td>
<td>2.43</td>
<td>2.78</td>
<td>0.35 (5.0%)</td>
<td>0.21</td>
<td>0.28</td>
<td>-25%</td>
</tr>
<tr>
<td>90</td>
<td>2.63</td>
<td>3.01</td>
<td>0.38 (1.2%)</td>
<td>0.24</td>
<td>0.34</td>
<td>-29%</td>
</tr>
</tbody>
</table>

Table 15. DPS-ASC numerical results from analysis of Pb(II) standards from 0 ppb to 500 ppb using a 20 µm array electrode in a device of volume 4.0 µL (# trials = 3). Pulse 2 charge obtained after deposition at -400 mV for 0.1 sec. Pulse 4 charge obtained after deposition at -400 mV for 130 sec. Background-corrected (BgC) charge is pulse 4 minus pulse 2 charge. Intercept-corrected (IcC) charge is the BgC charge minus the intercept charge (i.e. the BgC charge for 0 ppb Pb(II)). Calculated (calc) charge based on Faraday’s law where n is 2 for Pb(II)/Pb(0), F is the constant 96,485 C/mol, C is Pb(II) concentration (mol/L), and V is 4.0 x 10⁻⁶ L.

<table>
<thead>
<tr>
<th>Pb(II) ppb</th>
<th>Pulse 2 Charge µC</th>
<th>Pulse 4 Charge µC</th>
<th>BgC Charge µC (RSD)</th>
<th>IcC Charge µC</th>
<th>Calc Charge µC</th>
<th>% Error</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>1.97</td>
<td>2.23</td>
<td>0.26 (1.4%)</td>
<td>0.00</td>
<td>0.00</td>
<td>–</td>
</tr>
<tr>
<td>50</td>
<td>1.90</td>
<td>2.31</td>
<td>0.41 (0.93%)</td>
<td>0.15</td>
<td>0.19</td>
<td>-21%</td>
</tr>
<tr>
<td>100</td>
<td>1.87</td>
<td>2.39</td>
<td>0.53 (2.6%)</td>
<td>0.27</td>
<td>0.37</td>
<td>-27%</td>
</tr>
<tr>
<td>250</td>
<td>1.86</td>
<td>2.59</td>
<td>0.63 (11%)</td>
<td>0.37</td>
<td>0.93</td>
<td>-60%</td>
</tr>
<tr>
<td>500</td>
<td>2.08</td>
<td>2.75</td>
<td>0.67 (3.7%)</td>
<td>0.41</td>
<td>1.86</td>
<td>-78%</td>
</tr>
</tbody>
</table>
Table 16 lists the charge values from using the 10 µm array electrode for DPS-ASC analysis of Pb(II) standards within the linear range (0-60 ppb) and outside of the linear range (75-90 ppb). The charge for 0 ppb Pb(II) was 0.12 µC and this value was subtracted from all BgC values to obtain IcC values. The relative error ranged from 0.0-25% for Pb(II) standards within the linear range and RSD values were less than 8.5%.

The detection limit, calculated using $3\sigma_b I$ (Appendix IV), was 6 ppb Pb(II).

Table 16. DPS-ASC numerical results from analysis of Pb(II) standards from 0 ppb to 90 ppb using a 10 µm array electrode in a device of volume 2.4 µL (# trials = 3). Pulse 2 charge obtained after deposition at -400 mV for 0.1 sec. Pulse 4 charge obtained after deposition at -400 mV for 130 sec. Background-corrected (BgC) charge is pulse 4 minus pulse 2 charge. Intercept-corrected (IcC) charge is the BgC charge minus the intercept charge (i.e. the BgC charge for 0 ppb Pb(II)). Calculated (calc) charge based on Faraday’s law where $n$ is 2 for Pb(II)/Pb(0), $F$ is the constant 96,485 C/mol, $C$ is Pb(II) concentration (mol/L), and $V$ is $2.4 \times 10^{-6}$ L.

<table>
<thead>
<tr>
<th>Pb(II) ppb</th>
<th>Pulse 2 Charge µC</th>
<th>Pulse 4 Charge µC</th>
<th>BgC Charge µC (RSD)</th>
<th>IcC Charge µC</th>
<th>Calc Charge µC</th>
<th>% Error</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>1.12</td>
<td>1.24</td>
<td>0.12 (8.4%)</td>
<td>0.00</td>
<td>0.00</td>
<td>–</td>
</tr>
<tr>
<td>10</td>
<td>1.11</td>
<td>1.25</td>
<td>0.14 (5.6%)</td>
<td>0.02</td>
<td>0.02</td>
<td>0.0%</td>
</tr>
<tr>
<td>20</td>
<td>1.12</td>
<td>1.28</td>
<td>0.17 (4.1%)</td>
<td>0.05</td>
<td>0.04</td>
<td>25%</td>
</tr>
<tr>
<td>30</td>
<td>1.20</td>
<td>1.39</td>
<td>0.19 (5.4%)</td>
<td>0.07</td>
<td>0.07</td>
<td>0.0%</td>
</tr>
<tr>
<td>40</td>
<td>1.25</td>
<td>1.46</td>
<td>0.21 (4.1%)</td>
<td>0.09</td>
<td>0.09</td>
<td>0.0%</td>
</tr>
<tr>
<td>50</td>
<td>1.29</td>
<td>1.53</td>
<td>0.24 (4.0%)</td>
<td>0.12</td>
<td>0.11</td>
<td>9.1%</td>
</tr>
<tr>
<td>60</td>
<td>1.39</td>
<td>1.65</td>
<td>0.26 (2.0%)</td>
<td>0.14</td>
<td>0.13</td>
<td>7.7%</td>
</tr>
<tr>
<td>75</td>
<td>1.48</td>
<td>1.73</td>
<td>0.25 (2.0%)</td>
<td>0.13</td>
<td>0.17</td>
<td>-24%</td>
</tr>
<tr>
<td>90</td>
<td>1.51</td>
<td>1.76</td>
<td>0.25 (3.8%)</td>
<td>0.13</td>
<td>0.20</td>
<td>-35%</td>
</tr>
</tbody>
</table>
4.3.5. Interferents

The deposition of Pb(II) onto a gold electrode will occur simultaneously with other metal ions in solution, such as Ag(I), Cu(II), and Hg(II), that also deposit at -400 mV [60,92,94,95]. Although there are a few metals that could interfere with Pb(II) detection, the focus here is on Cu(II) because Cu(II) is often present in samples also containing Pb(II). This is because both metals are present in the pipes, fittings, and solder used commonly in water lines to transport drinking water. Additionally, the EPA maximum contaminant level for Cu(II) is 1.3 ppm which is much higher than the Pb(II) limit of 15 ppb. Because the levels of Cu(II) can be much higher than other interferents (i.e., Ag(I) and Hg(II)), it is most likely to cause issues and is therefore investigated here.

Two methods were explored in the analysis of Pb(II) in the presence of Cu(II). First, in situ subtraction of Cu(II) was studied. The charge from a DPS-ASC sequence with a deposition potential at -100 mV (Cu(II) only) was subtracted from the charge from a DPS-ASC sequence with a deposition potential of -400 mV (Cu(II) and Pb(II)) to obtain the charge for only Pb(II). The goal of the second method was to remove Cu(II) from the solution before reaching the coulometry device by using an in-line pre-electrolysis device that “trapped” the Cu(0) without affecting the Pb(II).

4.3.5.1. Subtraction of DPS-ASC Charge of Metal Interferents

Solutions of Cu(II) and Pb(II) were evaluated in the coulometry device using a 20 μm gold microelectrode array. These experiments were each performed in duplicate using two different 20 μm gold microelectrodes on different days. The DPS-ASC sequence was first performed with a deposition potential at -100 mV followed by DPS-
ASC with a deposition potential of -400 mV. The charge from DPS-ASC at -100 mV was subtracted from the charge at -400 mV to give the charge due to Pb(II) only.

The impact of 10 ppb Cu(II) on the DPS-ASC response for Pb(II) at 15 ppb, 30 ppb, 45 ppb, and 60 ppb was evaluated, as seen in Figure 46. The charge values for Pb(II) only (after subtraction of -100 mV charge from -400 mV charge) differed slightly from the calculated values per Faraday’s law, with relative errors of -4.5%, -11%, -12%, and -15% for the 15 ppb, 30 ppb, 45 ppb, and 60 ppb Pb(II) samples, respectively, in the presence of 10 ppb Cu(II). The experimental values of Pb(II) charge increased linearly with concentration (Figure 47), resulting in a correlation coefficient ($R^2$) value of 0.9985.

![Figure 46](image.png)

Figure 46. DPS-ASC intercept-corrected charge ($I_{cC}$) values from solutions containing 10 ppb Cu(II) and either 15, 30, 45, or 60 ppb Pb(II). $I_{cC}$ values after deposition at -100 mV for Cu(II) only (blue), deposition at -400 mV for Cu(II) + Pb(II) (yellow), subtracted -100 mV $I_{cC}$ from -400 mV $I_{cC}$ for Pb(II) only (green), and calculated charge based on Faraday’s law for Pb(II) only (black).
Figure 47. Pb(II) concentration in the presence of 10 ppb Cu(II) versus intercept-corrected charge (IcC) as determined by subtraction of -100 mV IcC from -400 mV IcC. These values correspond to the data represented by the green bars in Figure 46.

The addition of Cu(II) at higher concentrations impacted the detection of 15 ppb, 30 ppb, 45 ppb, 60 ppb, and 75 ppb Pb(II). In the presence of 50 ppb Cu(II), the Pb(II) response (after subtraction of -100 mV charge from -400 mV charge) remained static around 0.2 µC indicating that higher levels of Cu(II) influenced analysis of Pb(II) because the charge did not increase as expected per Faraday’s law (Figure 48).
Figure 48. DPS-ASC intercept-corrected charge ($I_cC$) values from solutions containing 50 ppb Cu(II) and either 15, 30, 45, or 60 ppb Pb(II). $I_cC$ values after deposition at -100 mV for Cu(II) only (blue), deposition at -400 mV for Cu(II) + Pb(II) (yellow), subtracted -100 mV $I_cC$ from -400 mV $I_cC$ for Pb(II) only (green), and calculated charge based on Faraday’s law for Pb(II) only (black).

However, it was later found experimentally that the deposition of Pb(II) onto a Cu(0) electrode does not occur until application of -600 mV. The determination of the correct reduction potential for Pb(II) on Cu(0) was performed by linear sweep stripping voltammetry using a Cu(0) electrode (Figure 49). The potential was held at -500 mV, -600 mV, or -700 mV for 20 seconds in a solution of 100 ppm Pb(II). The peak at -425 mV, not observed until a deposition potential of -600 mV was applied, was indicative of oxidation of Pb(0) to Pb(II), which confirmed that deposition of Pb(II) does not occur until -600 mV on a Cu(0) electrode. Kang et al. also found that more negative potentials (i.e. -800 mV) were required for complete deposition of Pb(II) onto a Cu(0) electrode [119]. Thus, it was determined that the Pb(II) charge remained constant in the presence of 50 ppb Cu(II) (Figure 48) because Pb(II) deposition would not occur at -400 mV if there was significant Cu(0) metal on the electrode surface (i.e., the electrode behaved as a
Cu(0) electrode instead of a gold electrode). Executing the DPS-ASC experiment at -600 mV for the deposition of Pb(II) and Cu(II) (instead of -400 mV), then subtracting away the Cu(II)-only charge at -100 mV to obtain the Pb(II)-only charge is expected to overcome the previous issues. However, it is postulated that the charging current would increase with the larger potential step between -600 mV and 500 mV compared to the step from -400 mV to 500 mV per the linear relationship between charging current and potential (Equation 9), and this increase is unfavorable. The increased charging current would worsen the detection limit for Pb(II) because the signal of the blank would be larger due to increased charging current. Additionally, based on the literature, a step to a more negative potential is expected to permit hydrogen evolution at the working electrode which would interfere with electrochemical deposition of Pb(0). Therefore, another method for analysis of Pb(II) in the presence of large amounts of Cu(II) was explored.

Figure 49. Linear sweep stripping voltammetry of 100 ppm Pb(II) on a Cu(0) electrode. Potential held for 20 seconds at -0.5V (solid line), -0.6 V (dashed line), or -0.7 V (dotted line) and then scanned at 0.1 V sec\(^{-1}\) to -0.3 V.
4.3.5.2. Pre-Electrolysis

Another possible method for the selective detection of Pb(II) in the presence of Cu(II) relies on the removal of Cu(II) from the solution via electrolysis. Accordingly, a device was constructed and positioned in-line before the entrance to the coulometry device to “trap” the Cu(II) as Cu(0). The device featured a RVC-carbon cloth electrode with a large surface area. A potential of -500 mV was applied so that Cu(II) would be reduced to Cu(0) on the RVC-carbon cloth. The Cu(0) was retained as solid metal on the electrode surface as the rest of the solution was pumped into the coulometry device. At that point, the sample might be free of Cu(II) without diminishing the Pb(II) which does not deposit at -500 mV on RVC-carbon cloth or Cu(0).

The coulometry device was equipped with a gold macroelectrode, and the samples were pumped through the pre-electrolysis device and into the coulometry device via a syringe pump. Samples containing only 1 ppm Cu(II), only 1 ppm Pb(II), and a mix of 1 ppm Cu(II) and 1 ppm Pb(II) were evaluated in the coulometry device using DPS-ASC with a deposition potential of -400 mV on gold before and after pre-electrolysis (Figure 50), which was performed at -500 mV on the RVC-carbon cloth in the pre-electrolysis device. In addition, samples of 2 ppm Cu(II), a mix of 2 ppm Cu(II) and 1 ppm Pb(II), 10 ppm Cu(II), and a mix of 10 ppm Cu(II) and 1 ppm Pb(II) were analyzed in the coulometry device via DPS-ASC with a deposition potential of -400 mV on gold after pre-electrolysis was performed at -500 mV in the pre-electrolysis device on the RVC-carbon cloth to retain Cu(II) as Cu(0). These analyses were all performed using the gold macroelectrode because these high concentrations of Cu(II) were not in the linear range of the microelectrode arrays.
Figure 50. DPS-ASC intercept-corrected charge (IcC) values after deposition at -400 mV in the coulometry device without pre-electrolysis (solid black) and with pre-electrolysis (striped) for samples of 1 ppm Cu(II), 1 ppm Pb(II), and a mix of 1 ppm Cu(II) with 1 ppm Pb(II). All compared to the calculated charge (dotted) for 1 ppm Pb(II) via Faraday’s law.

The various charge values from the analysis of Cu(II) and Pb(II) solutions with and without pre-electrolysis are listed in Table 17. The IcC for a solution containing only 1 ppm Cu(II) was 14.94 µC without electrolysis and 0.01 µC with electrolysis, indicating near 100% removal of 1 ppm Cu(II) via the RVC-carbon cloth unit. These results were confirmed by performing this experiment in duplicate using the same gold macroelectrode on two different days. Greater than 98% removal efficiency was also confirmed for Cu(II) samples of 2 ppm and 10 ppm, both of which were also analyzed in duplicate. The 2 ppm and 10 ppm Cu(II) solutions were only evaluated after pre-electrolysis was performed because the BgC without pre-electrolysis for these concentrations would exceed 29 µC which is not within the linear range for Cu(II) using a macroelectrode.
Table 17. Numerical results of DPS-ASC charge from experiments without and with pre-electrolysis to remove Cu(II) for targeted Pb(II) detection using a gold electrode in a device of volume 4.9 µL (# trials = 3). Background-corrected (BgC) charge is pulse 4 minus pulse 2 charge. Intercept-corrected (IcC) charge is the BgC charge minus the BgC charge for 0 ppm Pb(II). Calculated (calc) charge is based on Faraday’s law where n is 2 for Cu(II)/Cu(0) or Pb(II)/Pb(0), F is the constant 96,485 C/mol, C is concentration (mol/L) for Cu(II) or Pb(II), and V is 4.9 x 10⁻⁶ L.

<table>
<thead>
<tr>
<th>Sample Description</th>
<th>Without Pre-Electrolysis</th>
<th>With Pre-Electrolysis</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>BgC Charge µC (RSD)</td>
<td>IcC Charge µC</td>
</tr>
<tr>
<td>0 ppm Pb(II)</td>
<td>3.78 (4.1%)</td>
<td>0.00</td>
</tr>
<tr>
<td>1 ppm Cu(II)</td>
<td>18.72 (3.3%)</td>
<td>14.94</td>
</tr>
<tr>
<td>1 ppm Cu(II) + 1 ppm Pb(II)</td>
<td>23.30 (4.0%)</td>
<td>19.52</td>
</tr>
<tr>
<td>1 ppm Pb(II)</td>
<td>8.40 (3.9%)</td>
<td>4.62</td>
</tr>
<tr>
<td>0 ppm Pb(II)</td>
<td>3.78 (4.1%)</td>
<td>0.00</td>
</tr>
<tr>
<td>1 ppm Cu(II)</td>
<td>3.79 (3.1%)</td>
<td>0.01</td>
</tr>
<tr>
<td>1 ppm Cu(II) + 1 ppm Pb(II)</td>
<td>8.32 (4.3%)</td>
<td>4.54</td>
</tr>
<tr>
<td>1 ppm Pb(II)</td>
<td>8.28 (2.9%)</td>
<td>4.50</td>
</tr>
</tbody>
</table>

Solutions of only 1 ppm Pb(II) resulted in charges of 4.62 µC and 4.50 µC, without and with electrolysis, respectively, confirming that the pre-electrolysis device does not capture Pb(II) under these conditions. Then, when mixtures of 1 ppm Cu(II) and 1 ppm Pb(II) were analyzed, the charge without electrolysis was 19.52 µC, which was representative of Cu(II) and Pb(II), whereas the charge after electrolysis was 4.54 µC, which matches well to the experimental charges from only Pb(II) and the calculated
charge of 4.56 µC from Faraday’s law. Furthermore, mixtures of 2 ppm Cu(II) with 1 ppm Pb(II) and 10 ppm Cu(II) with 1 ppm Pb(II) resulted in charges of 5.09 µC and 6.52 µC, respectively, after electrolysis. Although higher than the 4.56 µC expected for Pb(II) per Faraday’s law, the difference is likely due to the < 2% of Cu(II) that was not captured in the pre-electrolysis device for these samples of higher Cu(II) concentration, as seen in the charge of 0.56 µC for 2 ppm Cu(II) and 2.39 µC for 10 ppm Cu(II) after pre-electrolysis.

Overall, this indicates that the pre-electrolysis device was successful in retaining high levels of Cu(II) to permit targeted analysis of Pb(II). Although not explored in this work, it is noted that the pre-electrolysis device could also “trap” other metals that deposit more positively, like Ag(I) and Hg(II).

4.3.6. Analysis of Ohio River Water

To examine the practicality of using DPS-ASC in the detection of Pb(II) in real samples, Ohio River water was sampled and subsequently analyzed using the 20 µm gold microelectrode array due to its combination of linear range and limit of detection. This analysis was executed three times using the same 20 µm array on three different days. DPS-ASC was performed on the Ohio River water after pretreatment (acidification and addition of NaCl), and the results indicated that Pb(II) was not within a reliable detectable window as the charge for the Ohio River water was 0.15 ± 0.00 µC which was not different from the charge of 0.13 ± 0.03 µC for a 0 ppb Pb(II) sample. Therefore, standard addition was utilized to spike 15 ppb, 30 ppb, or 60 ppb Pb(II) into the river water. The samples were subsequently analyzed by DPS-ASC and the results were listed in Table 18. The experimental charges for Pb(II) additions in river water were within -
18% to 17% compared to the calculated charges from Faraday’s law. These findings show that a spike in the Pb(II) levels in river water could be detected, as indicated by an increase in charge with increasing concentration (Figure 51). The standard addition plot in Figure 52 indicated that the pre-treated Ohio river water contained ~10.5 ppb Pb(II), as calculated from the absolute value of the x-intercept of the linear trendline. The charge-concentration response was linear in the standard addition plot with a 0.9904 correlation coefficient, as shown in Figure 52. Further validation of these results could be confirmed by other analytical techniques, such as inductively coupled plasma-atomic emission spectroscopy (ICP-AES) or atomic emission spectroscopy (AAS), but these were not pursued in this work.

Table 18. DPS-ASC numerical results from analysis of Pb(II) in Ohio River water (ORW) using a 20 µm array electrode in a device of volume 4.0 µL (# trials = 3). Pulse 2 charge obtained after deposition at -400 mV for 0.1 sec. Pulse 4 charge obtained after deposition at -400 mV for 130 sec. Background-corrected (BgC) charge is pulse 4 minus pulse 2 charge. Intercept-corrected (IcC) charge is the BgC charge minus the BgC charge for ORW. Calculated (calc) charge is based on Faraday’s law where n is 2 for Pb(II)/Pb(0), F is the constant 96,485 C/mol, C is Pb(II) concentration (mol/L), and V is 4.0 x 10^-6 L.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Pulse 2 Charge µC</th>
<th>Pulse 4 Charge µC</th>
<th>BgC Charge µC (RSD)</th>
<th>IcC Charge µC</th>
<th>Calc Charge µC</th>
<th>% Error</th>
</tr>
</thead>
<tbody>
<tr>
<td>ORW</td>
<td>2.08</td>
<td>2.23</td>
<td>0.15 (3.1%)</td>
<td>0.00</td>
<td>0.00</td>
<td>–</td>
</tr>
<tr>
<td>ORW + 15 ppb Pb(II)</td>
<td>2.07</td>
<td>2.29</td>
<td>0.22 (9.1%)</td>
<td>0.07</td>
<td>0.06</td>
<td>17%</td>
</tr>
<tr>
<td>ORW + 30 ppb Pb(II)</td>
<td>2.25</td>
<td>2.52</td>
<td>0.27 (3.1%)</td>
<td>0.12</td>
<td>0.11</td>
<td>9.1%</td>
</tr>
<tr>
<td>ORW + 60 ppb Pb(II)</td>
<td>2.28</td>
<td>2.61</td>
<td>0.33 (3.6%)</td>
<td>0.18</td>
<td>0.22</td>
<td>-18%</td>
</tr>
</tbody>
</table>
Figure 51. DPS-ASC background-corrected charge (BgC) from analysis of pretreated Ohio River water, a blank solution of 0 ppb Pb(II), and pretreated Ohio River water spiked with 15 ppb, 30 ppb, or 60 ppb Pb(II).

Figure 52. Standard addition of Pb(II) into pretreated Ohio River water as a function of DPS-ASC intercept-corrected charge (IcC).
4.3.7. Longevity Study Using a Gold Macroelectrode

A study of the reliability and consistency of charge measured by DPS-ASC over the course of two weeks was conducted. Unlike the work mentioned previously, these measurements were made using a 3D-printed coulometry device because it facilitated the use of a larger Ag/AgCl reference electrode that was placed into the device using Teflon tape in lieu of silicone grease. It was found that the use of silicone grease in the original coulometry device began to interfere with the DPS-ASC performance after 4-5 days because the grease traveled down into the counter/reference electrode fluidic chamber via gravity, and the silicone grease clogged the membrane. However, this problem was solved by using Teflon tape instead of grease in the 3D-printed device.

Each set of experiments during the longevity study began with cleaning the working electrode with 50 mM H₂SO₄. The working electrode chamber was filled with 50 mM H₂SO₄ and then 50 mM H₂SO₄ was slowly flushed through the chamber as the potential was cycled from 0 mV to 1,400 mV at 100 mV sec⁻¹ for 4-6 scans until cyclic voltammetry traces were reproducible. This was found necessary to return the electrode to optimal operating conditions. Failure to apply this cleaning procedure resulted in lower reproducibility and increased noise.

The stability of the electrochemical sensor was examined over two weeks by analysis of 2 ppm Pb(II) via DPS-ASC. The only exceptions being that data was not collected on the tenth day and only an evening measurement was recorded on the eleventh day. Numerical results from the morning, afternoon, and evening measurements were listed in Table 19 along with the average daily charge and percent error. Figure 53 A shows the experimental average daily charge for 2 ppm Pb(II) as a function of time. The
calculated charge per Faraday’s law for 2 ppm Pb(II) is displayed as the dotted line in Figure 53A for comparison. Figure 53B shows the daily charge response as a function of Pb(II) concentration, wherein the solid line indicates experimental results and the dotted line depicts the calculated concentration that is expected according to Faraday’s law. These results indicate that the device components are reliable for this duration of time with an overall RSD of 7.7% and relative error of -18% to 4.7%.

Table 19. DPS-ASC numerical results of intercept-corrected charge (IcC) from analysis of 2 ppm Pb(II) using a gold macroelectrode in the 3D-printed coulometry device over the course of two weeks. Percent error calculated using the average daily IcC (# trials = 3) and calculated charge of 9.16 µC based on Faraday’s law where n is 2 for Pb(II)/Pb(0), F is the constant 96,485 C/mol, C is 9.65 x 10^-6 mol/L Pb(II), and V is 4.9 x 10^-6 L.

<table>
<thead>
<tr>
<th>Day</th>
<th>Morning IcC µC (RSD)</th>
<th>Afternoon IcC µC (RSD)</th>
<th>Evening IcC µC (RSD)</th>
<th>Average Daily IcC µC</th>
<th>% Error</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>7.28 (1.0%)</td>
<td>7.93 (3.3%)</td>
<td>8.07 (2.3%)</td>
<td>7.76</td>
<td>-15%</td>
</tr>
<tr>
<td>2</td>
<td>10.28 (3.3%)</td>
<td>9.29 (3.3%)</td>
<td>9.21 (1.1%)</td>
<td>9.59</td>
<td>4.7%</td>
</tr>
<tr>
<td>3</td>
<td>8.90 (2.8%)</td>
<td>7.99 (1.6%)</td>
<td>8.03 (1.4%)</td>
<td>8.31</td>
<td>-9.3%</td>
</tr>
<tr>
<td>4</td>
<td>9.45 (2.6%)</td>
<td>9.63 (2.9%)</td>
<td>9.27 (1.9%)</td>
<td>9.45</td>
<td>3.1%</td>
</tr>
<tr>
<td>5</td>
<td>8.78 (0.2%)</td>
<td>9.13 (4.4%)</td>
<td>8.66 (1.2%)</td>
<td>8.86</td>
<td>-3.3%</td>
</tr>
<tr>
<td>6</td>
<td>8.44 (1.7%)</td>
<td>9.71 (8.3%)</td>
<td>8.23 (2.3%)</td>
<td>8.79</td>
<td>-4.1%</td>
</tr>
<tr>
<td>7</td>
<td>8.81 (2.8%)</td>
<td>8.71 (2.1%)</td>
<td>8.45 (2.8%)</td>
<td>8.66</td>
<td>-5.5%</td>
</tr>
<tr>
<td>8</td>
<td>8.39 (2.1%)</td>
<td>8.64 (1.7%)</td>
<td>9.27 (2.8%)</td>
<td>8.77</td>
<td>-4.3%</td>
</tr>
<tr>
<td>9</td>
<td>9.45 (4.1%)</td>
<td>9.55 (6.0%)</td>
<td>9.21 (3.2%)</td>
<td>9.40</td>
<td>2.6%</td>
</tr>
<tr>
<td>11</td>
<td>–</td>
<td>–</td>
<td>8.84 (2.1%)</td>
<td>8.84</td>
<td>-3.5%</td>
</tr>
<tr>
<td>12</td>
<td>9.96 (2.4%)</td>
<td>7.86 (7.0%)</td>
<td>7.91 (6.2%)</td>
<td>8.58</td>
<td>-6.4%</td>
</tr>
<tr>
<td>13</td>
<td>7.84 (2.4%)</td>
<td>6.89 (2.6%)</td>
<td>7.86 (4.5%)</td>
<td>7.53</td>
<td>-18%</td>
</tr>
<tr>
<td>14</td>
<td>7.88 (1.9%)</td>
<td>7.60 (3.1%)</td>
<td>7.50 (6.5%)</td>
<td>7.66</td>
<td>-16%</td>
</tr>
</tbody>
</table>
Figure 53. DPS-ASC results obtained for a 2 ppm Pb(II) solution using a gold macroelectrode (solid line) compared to the calculated results per Faraday’s law (dashed line) over the period of two weeks. Each daily value results from averaging nine measurements (three measurements at three different times each day). Overall RSD 7.7%. Results as A) a function of intercept-corrected charge (IcC) and B) a function of Pb(II) concentration.
Between measurements, pumping valves were left closed and filled with stagnant fluid – the counter/reference electrode compartment was filled with 3 M NaCl, whereas the working electrode compartment was filled with 10 mM HNO$_3$/10 mM NaCl. It is predicted that the working electrode compartment could actually be stored in the analyte solution due to the cleaning step with H$_2$SO$_4$ before each experiment, but it is necessary to fill the counter/reference electrode compartment with 3 M NaCl as that solution is required for proper storage of the Ag/AgCl reference electrode. Because the coulometry device is envisioned for field applications, these are important considerations as multiple solutions (i.e., H$_2$SO$_4$ for cleaning, 3 M NaCl for reference electrode storage, and HNO$_3$ with NaCl for sample pretreatment) will be required on-site. The replenishment of these solutions may be a limiting factor in the length of time the device can operate without human interaction.

4.4. Conclusion

This chapter focused on the detection of Pb(II) considering recent exposures in drinking water in Flint, Michigan, among others. The working electrode explored was gold in both a macroelectrode and microelectrode array configuration. DPS-ASC was optimized as a novel, (re)calibration-free detection method for Pb(II) in water samples.

Pb(II) standards were evaluated to determine optimal solution conditions and deposition/stripping potentials. The supporting electrolyte of 10 mM HNO$_3$/10 mM NaCl contributed to the highest signal for Pb(II), which was supported by the work of other groups that determined chloride is beneficial in the analysis of Pb(II) [60,92]. Deoxygenation of samples was not required. The deposition potential of -400 mV and
stripping potential of 500 mV were determined by linear sweep stripping voltammetry and further utilized for DPS-ASC analysis of Pb(II).

The gold macroelectrode had a linear range of 1 ppm to 5 ppm Pb(II) and calculated detection limit of 750 ppb. Because this limit of detection was much higher than the EPA maximum contaminant level of 15 ppb, microelectrode arrays were explored as they offer lower noise due to smaller charging currents.

The 50 µm array electrode displayed linearity from 400 ppb to 700 ppb with a detection limit of 320 ppb. The 20 µm array electrode and 10 µm array electrode had calculated limits of detection at 10 ppb and 6 ppb, respectively, which are both under the 15 ppb EPA limit. As the electrode area was decreased, the charging current was smaller which resulted in lower noise and thus improved detection limits.

The linear ranges were small, from 15 ppb to 90 ppb for the 20 µm array and 10 ppb to 60 ppb for the 10 µm array. The small linear ranges were attributed to Pb(0) monolayer coverage issues when analyzing large concentrations at these smaller electrode areas. Despite the loss of linearity at high concentrations, these electrode sensors are useful in detecting the presence of high levels of Pb(II) as a screening tool (although non-quantitatively), which could prompt further on-site analysis by a field technician.

The effect of Cu(II) on Pb(II) detection was evaluated. Subtractive DPS-ASC was performed to target the analysis of only Pb(II). This was completed by subtracting the charge at -100 mV (indicative of Cu(II) only) from the charge at -400 mV (Cu(II) and Pb(II) both) to obtain the charge for Pb(II) only. It was determined that 10 ppb Cu(II)
does not interfere with the analysis of low Pb(II) levels and could be subtracted out using this method.

To address samples containing large amounts of Cu(II), an in-line pre-electrolysis device was created that featured a RVC-carbon cloth electrode to trap Cu(II) as Cu(0) by electrochemical deposition and allowed for a simpler, targeted detection of Pb(II). Solutions containing both Cu(II) and Pb(II) were passed through the pre-electrolysis device and near 100% of Cu(II) was removed using that device without affecting the Pb(II) response as measured downstream in the coulometry device.

The practicality of using DPS-ASC for analysis of Pb(II) in real samples was evaluated by examining Ohio River water. Ohio River water was acidified to pH 2 with nitric acid and chloride was added, as these conditions are required for optimal Pb(II) detection. Pb(II) was not at a detectable limit in the river water. Therefore, the samples were spiked with 15 ppb, 30 ppb, or 60 ppb Pb(II). The Pb(II) additions were detected using DPS-ASC, which indicates that this method could be reliable in the field for monitoring environmental spills and spikes of metal contaminants.

Furthermore, the stability of the electrochemical sensor was tested by daily analysis of 2 ppm Pb(II) by DPS-ASC. The new 3D-printed coulometry device coupled with a gold macroelectrode was utilized. Results indicated that this device was reliable for monitoring 2 ppm Pb(II) over the course of two weeks with an overall RSD of 7.7%. It was also determined that the gold electrode must be cleaned each day before use by flushing H₂SO₄ through the device to ensure optimal electrode working conditions.

Herein, a novel electrochemical method, DPS-ASC, has been explored and developed for the (re)calibration-free detection of Pb(II) in water with detection limits.
below the EPA action level. In DPS-ASC, the sample is used as both the blank and the analyte, thus permitting an in situ correction of background signal. The background signal, due to charging current, was decreased with the use of small microelectrode arrays, making them favorable for detection of low levels of analyte. The use of coulometry thus allows for this calibration-free method which could be further combined with engineering components, such as a pumping and data processing system, for use as a remote sensor in which the analytical detector is installed on-site and functions autonomously.
CHAPTER V

BORON DOPED DIAMOND ELECTRODES FOR THE DETECTION OF LEAD BY DPS-ASC

5.1. Introduction

The use of a gold electrode was studied extensively for DPS-ASC analysis of As(III) and Pb(II). However, there are many other electrode materials that ought to be considered, such as platinum, carbon, boron-doped diamond, and modified electrodes. These offer their own advantages, from wider potential windows to increased selectivity toward certain analytes. There are other electrodes, such as mercury, that offer their own unique benefits, but also have key disadvantages to consider (i.e. toxicity and environmental impact). Thus, the decision to explore new electrode materials must be based on both applicability for the analyte of interest and compatibility with the end application for the electrode.

Of the other electrode materials that could be explored beyond gold, boron-doped diamond (BDD) electrodes are considered because BDD is a novel electrode material that offers a wide potential range, low background and capacitive currents, reduced fouling, and mechanical robustness [120–125]. Furthermore, BDD electrodes have several of the characteristics of a mercury electrode without the toxicity, making it a more environmentally friendly alternative.
BDD electrodes have been utilized for the electrochemical determination of a variety of trace metals in water, including As(III)/As(V) [126], Hg(II) [127,128], Pb(II) [102–104], Sb(III) [129], Cu(II) and Pb(II) [111], Cd(II) and Pb(II) [110,130], Cd(II), Cu(II), Pb(II), and Zn(II) [109], and Ag(I), Cd(II), Cu(II), Pb(II), and Zn(II) [131] with sub ppb detection limits. Additionally, BDD electrodes have been used for the detection of trace metals in river sediment [132], contaminated water and soil [133], honey [134], and human blood [135].

As mentioned in Chapter IV, there is a need for remote monitoring of Pb(II) as several instances of Pb(II) contamination of drinking water have occurred throughout the last decade. Progress was made by using a gold electrode in the detection of Pb(II) by DPS-ASC for remote sensing applications (Chapter IV), but there was concern of co-depositing metal interferents, namely Cd(II), that could not be removed by using the pre-electrolysis device. However, oxidation of Cd(0) to Cd(II) and Pb(0) to Pb(II) have been shown to occur at different potentials on BDD electrodes, making targeted detection of Pb(II) feasible [109,110,130,131,133,136]. For this reason, BDD electrodes were evaluated herein for the targeted detection of Pb(II) using DPS-ASC in the coulometry device for remote sensing applications.

5.2. Materials and Methods

5.2.1. Chemicals, Reagents, and Solution Preparation

All reagents were purchased at the highest purity and used without further purification. Nitric acid and AAS 1,000 ppm single element standards were purchased from Sigma Aldrich (St. Louis, MO) for Cd(II) (from Cd(NO$_3$)$_2$) and Pb(II) (from
Pb(NO₃)₂). Sodium chloride, sodium acetate, acetic acid, and hexaamineruthenium(III) chloride were purchased from VWR (Radnor, PA).

All solutions were prepared fresh each day using deionized water. Acetate buffer was made using calculated amounts of sodium acetate and acetic acid, and pH was confirmed using a Thermo pH meter. Nitric acid solutions were made by pipetting the needed volumes of concentrated HNO₃ into volumetric flasks filled halfway with deionized water and subsequently diluting to the mark with deionized water. Cd(II) and Pb(II) standards were prepared by pipetting the appropriate volume of 1,000 ppm AAS into a volumetric flask and diluting to volume with diluent.

Skin contact with Cd(II) and Pb(II) was avoided by wearing protective safety goggles, long-sleeve shirts, long pants, closed-toe shoes, and nitrile examination gloves from VWR (Radnor, PA) during solution preparations. Spills of heavy metal solutions were cleaned with paper towels that were disposed of in appropriate solid waste containers. All solution waste and solid waste was collected and then properly disposed of by the Department of Environmental Health and Safety (DEHS) at the University of Louisville.

5.2.2. Boron Doped Diamond Working Electrodes

BDD electrodes were purchased from Fraunhofer USA Center for Coatings and Diamond Technologies (East Lansing, Michigan) and Sp3 Diamond Technologies (Santa Clara, California). BDD films were grown by each company using microwave plasma-assisted chemical vapor deposition (MPCVD) on 0.5 mm thick silicon substrates.
Electrodes were laser cut into rectangular 30 mm by 13 mm pieces prior to arrival at the University of Louisville.

The working electrode compartment was built directly on top of the 30 mm by 13 mm BDD electrode chips via stamp-and-stick fabrication in the MNCTC at the University of Louisville (Louisville, KY). NOA 68 was spun via Headway Spinners to a 10-20 nm thickness on a bare silicon wafer. An AB-M Inc Aligner was used to transfer a thin layer of NOA 68 onto an 80 µm thick piece of borosilicate glass (30 mm long by 10 mm wide) that had previously been laser cut to pattern the 8 mm by 4 mm ellipse with 5 mm by 1 mm channels into the glass. Then, the NOA 68 coated borosilicate glass was aligned and put into contact with the 30 mm by 13 mm BDD electrode. Finally, the borosilicate glass/electrode combination was exposed to UV light for 300 sec to cure and create electrode sensor chips with permanent, defined volumes.

5.2.3. Coulometry Device

The coulometry device was described previously. It features a working electrode compartment and a counter/reference electrode compartment that are separated by a membrane and have independent sample inlets and outlets with external fluidic flow shut-off valves. A polycarbonate base was recessed to hold the 30 mm by 13 mm working electrode. A 200 MWCO membrane was affixed on top of the working electrode and was replaced after 10-15 days of use. Three laser-cut silicone gaskets, the Panasonic pyrolytic graphite sheet counter, thin laser-cut silicone gasket, and polycarbonate top were added to define the counter/reference electrode compartment. A custom Ag/AgCl reference electrode was inserted through an access hole in the polycarbonate top and sealed in place.
using silicone grease. The polycarbonate pieces were screwed together using four screws, and the torque on each screw was set to a value of 0.51 kg-cm using a torque screwdriver. The device was assembled at the beginning of each day and disassembled at the end of each day.

5.2.4. Electrochemical Measurements

The three-electrode system encompassed a BDD electrode for the working electrode, a custom miniature Ag/AgCl reference electrode, and a pyrolytic graphite sheet as the counter electrode. Reference electrodes were replaced every 5-10 days to ensure proper reference conditions (i.e., no potential drift) and the pyrolytic graphite sheet was replaced after one month of use. A BASi Epsilon potentiostat (West Lafayette, IN) was used for all electrochemical measurements.

The following procedure was utilized for experiments using the coulometry device. First, the counter/reference chamber was filled with diluent (either acetate buffer or HNO₃ solutions) and the fluid valves were closed. This solution was not replaced throughout the experiment. Then, the working electrode chamber was filled with blank or analyte sample, and fluid valves were closed before applying the specified potential(s). Each experiment was repeated at least three times for each sample. Between each experiment, the fluid valves were opened, the working electrode chamber was filled with fresh sample, and then the fluid valves were closed.

Current-time curves (amperograms) were recorded and integrated to obtain charge. All integrations and data processing were done by extracting text files from the BASi Epsilon software and using Microsoft Excel to perform all calculations.
5.3. Results and Discussion

5.3.1. Evaluating Boron Doped Diamond Electrode Characteristics

Electrodes from Fraunhofer and Sp3 were evaluated upon receipt to determine working potential window, electrode kinetics, and monolayer coverage. First, each BDD electrode was placed into a beaker containing 20 mL of 100 mM acetate buffer (pH 5.2), 10 mM HNO₃ (pH 2.0), or 100 mM HNO₃ (pH 1.0). Both acetate buffer and nitric acid solutions were evaluated due to popular use in the literature. A Ag/AgCl reference electrode and platinum wire counter electrode were also positioned in the beaker with the BDD electrode. To determine potential window, an initial potential of 1.0 V vs. Ag/AgCl was applied, and then the potential was scanned at 0.1 V sec⁻¹ in the negative direction. Gradual changes in the negative potential setting were made during cyclic voltammetry to determine the maximum potential window in each solution (i.e., a step to -0.5 V was tested and if no hydrogen evolution was seen then a step to -0.7 V was tested, and so on until hydrogen evolution was reached). As seen in Figure 54, the potential window was largest in the 100 mM acetate buffer (pH 5.2) and smallest in the 100 mM HNO₃ (pH 1.0) for both BDD electrodes. When comparing each BDD electrode in the same analyte, the potential window for the Fraunhofer BDD was nearly 500 mV wider compared to the Sp3 BDD due to the hydrogen termination and surface properties of each electrode.
Cyclic voltammetry of 1 mM Ru(NH$_3$)$_6^{2+/3+}$, a common analyte for testing electrochemical reversibility and electrode kinetics, was then evaluated using each BDD electrode (Figure 55). The potential was cycled from 100 mV to -400 mV to 100 mV vs. Ag/AgCl. The observed voltammograms were similar for both Sp3 BDD and Fraunhofer BDD electrodes with a peak-to-peak separation (ΔE$_p$) of 85 mV, indicating near reversible electrode behavior.
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5.3.2. Electrochemical Detection of Pb(II) Using Boron Doped Diamond Electrodes

The appropriate parameters, such as solution conditions and Pb(II) deposition potential, were investigated for standard Pb(II) solutions using each BDD electrode in a beaker. These conditions were then confirmed through analysis in the coulometry device.

First, Pb(II) was evaluated by linear sweep stripping voltammetry in a solution of 100 mM acetate buffer (pH 5.2) because this solution resulted in the largest potential window (i.e. less concern for hydrogen evolution that may interfere with Pb(II) reduction), and acetate buffer had been reported by others for the successful detection of Pb(II).
determination of Pb(II) in the presence of Ag(I), Cd(II), Cu(II), and Zn(II) using BDD electrodes [104,109,131,133]. The analytes evaluated were 500 ppb Pb(II) in 100 mM acetate buffer (pH 5.2) for the Sp3 BDD electrode and 10 ppm Pb(II) in 100 mM acetate buffer (pH 5.2) for the Fraunhofer BDD electrode. Different deposition potentials were held for 60 sec, and then the potential was scanned at 0.1 V sec$^{-1}$ to -0.3 V to observe stripping of Pb(0), as shown in Figure 56. A deposition potential of -1.3 V vs. Ag/AgCl was found necessary for Pb(II) in 100 mM acetate buffer (pH 5.2) for both the Sp3 BDD electrode and Fraunhofer BDD electrode. Stripping peak current did not increase with application of potentials more negative than -1.3 V vs. Ag/AgCl.
Figure 56. Linear sweep stripping voltammetry of A) 500 ppb Pb(II) in 100 mM acetate buffer (pH 5.2) using the Sp3 BDD electrode and B) 10 ppm Pb(II) in 100 mM acetate buffer (pH 5.2) using the Fraunhofer BDD electrode. Potential was held for 60 sec at -0.9 V (blue), -1.0 V (yellow), -1.1 V (green), -1.2 V (black), or -1.3 V (red) and then scanned at 0.1 V sec\(^{-1}\) to -0.3 V.

As seen in Figure 56 A, a small stripping peak was observed at -0.40 V for the Sp3 BDD electrode in addition to the stripping peak at -0.52 V. Resulting from a change in the deposition morphology of Pb(II), both stripping peaks were attributed to Pb(0) stripping, with the peak at -0.52 V due to stripping of Pb(0) nanoparticles and the peak at
-0.40 V from stripping of Pb(0) thin-film-like structures. This was based on work by Hutton et al. in the examination of BDD electrode surfaces by field emission scanning electron microscopy [137]. This observation was also noticed on the Fraunhofer BDD electrode with a stripping peak at -0.50 V attributed to Pb(0) nanoparticles and a stripping peak at -0.43 V attributed to Pb(0) thin-film-like structures. It was noted that lower concentrations of Pb(II) do not cause this phenomenon [137]. Regardless, experimental findings of a Pb(0) stripping peak near -0.50 V vs. Ag/AgCl in acetate buffer on BDD were consistent with literature reports of Pb(0) stripping peaks at -0.55 V [104], -0.50 V [109], and -0.42 V [133].

Linear sweep stripping voltammetry was then investigated for Pb(II) standards in 10 mM HNO₃. As before, different deposition potentials were applied for 60 sec and then the potential was scanned positively at 0.1 V sec⁻¹ to -0.25 V to observe stripping of Pb(0). A solution of 1 ppm Pb(II) in 10 mM HNO₃ was analyzed using the Fraunhofer BDD in the coulometry device. As seen in Figure 57, the required deposition potential for complete Pb(II) reduction was -0.75 V vs. Ag/AgCl. This agrees closely with literature reporting of -0.66 V vs. Ag/AgCl for Pb(II) deposition in 100 mM HNO₃ [111] (note: literature reports of the deposition potential in 10 mM HNO₃ were not available). Stripping peak current did not increase with application of potentials beyond -0.75 V vs. Ag/AgCl.
Linear sweep stripping voltammetry was performed to evaluate the deposition of Cd(II) and Pb(II) on BDD electrodes, as these two metals were previously inseparable on a gold electrode. The Fraunhofer BDD was placed into a beaker containing 20 mL of 20 ppm Cd(II), 20 ppm Pb(II), or a mixture of both 20 ppm Cd(II) and 20 ppm Pb(II), along with a Ag/AgCl reference electrode and platinum wire counter electrode. The analyte solution media was 10 mM HNO₃/10 mM NaCl. A deposition potential of -1.0 V or -1.2 V vs. Ag/AgCl was applied and held for 60 sec. Then, the potential was scanned at 0.1 V sec⁻¹ toward 0.0 V. As seen in Figure 58 A, deposition at -1.0 V resulted in a Pb(0) stripping peak at -0.41 V vs. Ag/AgCl in both the 20 ppm Pb(II) sample and mixture of Pb(II) and Cd(II). There was not a stripping peak observed for Cd(0), indicating that Cd(II) does not deposit at -1.0 V vs. Ag/AgCl under these conditions. However, deposition at -1.2 V vs. Ag/AgCl did result in a stripping peak for Cd(0) at -0.61 V vs.
Ag/AgCl in the 20 ppm Cd(II) sample. The Cd(0) stripping peak shifted to -0.59 V vs. Ag/AgCl in the mixture of both Pb(II) and Cd(II), as shown in Figure 58 B. The Pb(0) stripping peak remained unchanged at -0.41 V vs. Ag/AgCl in both the 20 ppm Pb(II) solution and mixture of Pb(II) and Cd(II) after deposition at -1.2 V vs. Ag/AgCl.

Figure 58. Linear sweep stripping voltammetry of 20 ppm Pb(II) (dotted), 20 ppm Cd(II) (solid), and a mixture of 20 ppm Pb(II) and 20 ppm Cd(II) (dashed) after deposition at A) -1.0 V vs. Ag/AgCl and B) -1.2 V vs. Ag/AgCl. Deposition potential held for 60 sec, then scanned at 0.1 V sec\(^{-1}\) to 0.0 V. Pb(0) stripping peak at -0.41 V and Cd(0) stripping peak at -0.59 to -0.61 V.
The separation of Pb(II) and Cd(II) was not previously feasible using a gold electrode because Pb(II) and Cd(II) co-deposited. However, it was determined that selective detection of Pb(II) in the presence of Cd(II) would be feasible using BDD electrodes due to the difference in deposition potentials (i.e. Pb(II) was reduced to Pb(0) at -1.0 V, whereas Cd(II) was not reduced at this potential). This finding was not unlike that of Sonthalia et al. who reported on the simultaneous detection of Pb(II) and Cd(II) in 100 mM acetate buffer (pH 4.5) with stripping peak potentials of -0.51 V and -0.75 V vs. Ag/AgCl, respectively (note: individual deposition potentials for Pb(II) and Cd(II) were not reported) [133].

Chronoamperometry was performed using standard Pb(II) solutions to determine if DPS-ASC would be feasible for the detection of Pb(II) using BDD electrodes. The Sp3 BDD electrode was installed in the coulometry device with a Ag/AgCl reference electrode and graphite sheet counter electrode. Solutions of Pb(II) in 10 mM acetate buffer (pH 5.2) were evaluated by applying -1.2 V for 60 sec to reduce Pb(II) to Pb(0) followed by -0.2 V for 10 sec to oxidize Pb(0) to Pb(II). Current versus time was recorded, and the resulting amperograms were integrated to determine charge. The charge increased linearly with a correlation coefficient of 0.9882, as shown in Figure 59. In addition, the RSD values were less than or equal to 2.0%, indicating reproducibility. However, the experimental charge was never more than 75% of the calculated charge per Faraday’s law. This resulted in % error values greater than 25%, as seen in Table 20. These findings were reproducible using the Sp3 BDD electrode (n=8) under these conditions and as acetate buffer concentration, deposition potential, and deposition time were increased.
Figure 59. Pb(II) concentration versus intercept-corrected charge (IcC) from chronoamperometry using the Sp3 BDD electrode.

Table 20. Numerical results from chronoamperometry of Pb(II) in 10 mM acetate buffer (pH 5.2) using the Sp3 BDD electrode in a device of volume 5.9 µL (# trials = 3). Intercept-corrected (IcC) charge is the experimental (Exp) charge minus the Exp charge for 0 ppb Pb(II). Calculated (calc) charge is based on Faraday’s law where n is 2 for Pb(II)/Pb(0), F is the constant 96,485 C/mol, C is Pb(II) concentration (mol/L), and V is 5.9 x 10^{-6} L.

<table>
<thead>
<tr>
<th>Pb(II) ppb</th>
<th>Exp Charge µC (RSD)</th>
<th>IcC Charge µC</th>
<th>Calc Charge µC</th>
<th>% Error</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>1.05 (1.1%)</td>
<td>0.00</td>
<td>0.00</td>
<td>–</td>
</tr>
<tr>
<td>100</td>
<td>1.14 (0.9%)</td>
<td>0.09</td>
<td>0.55</td>
<td>85%</td>
</tr>
<tr>
<td>250</td>
<td>1.58 (2.0%)</td>
<td>0.53</td>
<td>1.37</td>
<td>62%</td>
</tr>
<tr>
<td>500</td>
<td>2.73 (1.6%)</td>
<td>1.68</td>
<td>2.75</td>
<td>39%</td>
</tr>
<tr>
<td>750</td>
<td>3.91 (1.6%)</td>
<td>2.86</td>
<td>4.12</td>
<td>31%</td>
</tr>
<tr>
<td>1,000</td>
<td>4.99 (0.3%)</td>
<td>3.94</td>
<td>5.50</td>
<td>28%</td>
</tr>
</tbody>
</table>

Chronoamperometry using the Fraunhofer BDD electrode was explored using Pb(II) standards in the coulometry device to determine if the same lower-than-expected-charge phenomenon occurs like with the Sp3 BDD electrode. Solutions of Pb(II) in 10 mM HNO₃ were evaluated by applying -1.4 V for 240 sec to reduce Pb(II) to Pb(0)
followed by 0.4 V for 6 sec to oxidize Pb(0) to Pb(II). The charge increased with less linearly than before, with a correlation coefficient of 0.9292 (Figure 60). Yet, the RSD values of less than or equal to 3.6% were still indicative of reproducibility. As before, the experimental charge was less than the calculated charge. The experimental charge was not more than 60% of the calculated charge per Faraday’s law and this resulted in % error values greater than 40%, as seen in Table 21. These findings were reproducible using the Fraunhofer BDD electrode (n=5).

Figure 60. Pb(II) concentration versus intercept-corrected charge (IcC) from chronoamperometry using the Fraunhofer BDD electrode.
Table 21. Numerical results from chronoamperometry of Pb(II) in 10 mM HNO₃ using the Fraunhofer BDD electrode in a device of volume 5.3 µL (# trials = 3). Intercept-corrected (IcC) charge is the experimental (Exp) charge minus the Exp charge for 0 ppm Pb(II). Calculated (calc) charge is based on Faraday’s law where n is 2 for Pb(II)/Pb(0), F is the constant 96,485 C/mol, C is Pb(II) concentration (mol/L), and V is 5.3 x 10⁻⁶ L.

<table>
<thead>
<tr>
<th>Pb(II) ppm</th>
<th>Exp Charge µC (RSD)</th>
<th>IcC Charge µC</th>
<th>Calc Charge µC</th>
<th>% Error</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>3.80 (2.3%)</td>
<td>0.00</td>
<td>0.00</td>
<td>–</td>
</tr>
<tr>
<td>1</td>
<td>4.17 (1.8%)</td>
<td>0.37</td>
<td>4.97</td>
<td>-93%</td>
</tr>
<tr>
<td>2</td>
<td>5.47 (2.4%)</td>
<td>1.67</td>
<td>9.95</td>
<td>-83%</td>
</tr>
<tr>
<td>3</td>
<td>10.20 (3.0%)</td>
<td>6.40</td>
<td>14.92</td>
<td>-57%</td>
</tr>
<tr>
<td>4</td>
<td>14.58 (1.5%)</td>
<td>10.78</td>
<td>19.89</td>
<td>-46%</td>
</tr>
<tr>
<td>5</td>
<td>18.31 (3.6%)</td>
<td>14.51</td>
<td>24.87</td>
<td>-42%</td>
</tr>
</tbody>
</table>

Chronoamperometry experiments were conducted in the coulometry device using the Fraunhofer BDD electrode with a solution of 5 ppm Pb(II) in 10 mM HNO₃, and the charge (from integration of amperograms) was recorded and then plotted as a function of deposition potential applied (Figure 61). The Pb(II) charge increased as up to -1.3 V vs. Ag/AgCl was applied, but then the charge leveled off beyond application of -1.4 V vs. Ag/AgCl, and the charge did not reach the calculated value of 25 µC per Faraday’s law. This indicated that the lower-than-expected charges seen thus far were not due to application of the incorrect deposition potential.
Figure 61. Relationship of applied deposition potential and intercept-corrected charge (IcC) from chronoamperometry of 5 ppm Pb(II) in 10 mM HNO₃ using the Fraunhofer BDD electrode (solid) compared to the calculated charge per Faraday’s law (dashed).

The same trend of lower-than-expected-charge for Pb(II) was observed during the DPS-ASC experiments using the Fraunhofer BDD electrode in the coulometry device. Pb(II) standards in 10 mM acetate buffer (pH 5.2) were evaluated by the DPS-ASC potential sequence listed below. The resulting crescents from overlaying pulses 2 and 4 were integrated, and the Faradaic charge versus Pb(II) concentration was plotted in Figure 62. The charge was linear from 0 to 500 ppb Pb(II) with a correlation coefficient of 0.9805 and RSD values were less than 7.5%. However, when comparing the experimental charges and calculated charges, the % error was greater than -55% (Table 22). As before, the experimental charge was vastly less than the calculated charge.

Pulse 1: -1.5 V for 1 sec
Pulse 2: -0.1 V for 5 sec
Pulse 3: -1.5 V for 120 sec
Pulse 4: -0.1 V for 5 sec
Figure 62. Pb(II) concentration versus intercept-corrected charge (IcC) from DPS-ASC analysis using the Fraunhofer BDD electrode.

Table 22. DPS-ASC numerical results from analysis of Pb(II) standards from 0 ppb to 500 ppb using the Fraunhofer BDD electrode in a device of volume 5.3 µL (# trials = 3). Pulse 2 charge obtained after deposition at -1.5 V for 1 sec. Pulse 4 charge obtained after deposition at -1.5 V for 120 sec. Background-corrected (BgC) charge is pulse 4 minus pulse 2 charge. Intercept-corrected (IcC) charge is the BgC charge minus the intercept charge (i.e. the BgC charge for 0 ppb Pb(II)). Calculated (calc) charge is based on Faraday’s law where \( n = 2 \) for Pb(II)/Pb(0), \( F \) is the constant 96,485 C/mol, \( C \) is Pb(II) concentration (mol/L), and \( V = 5.3 \times 10^{-6} \text{ L} \).

<table>
<thead>
<tr>
<th>Pb(II) ppb</th>
<th>Pulse 2 Charge ( \mu C )</th>
<th>Pulse 4 Charge ( \mu C )</th>
<th>BgC Charge ( \mu C ) (RSD)</th>
<th>IcC Charge ( \mu C )</th>
<th>Calc Charge ( \mu C )</th>
<th>% Error</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>2.63</td>
<td>4.02</td>
<td>1.39 (4.6%)</td>
<td>0.00</td>
<td>0.00</td>
<td>–</td>
</tr>
<tr>
<td>50</td>
<td>2.75</td>
<td>4.08</td>
<td>1.33 (7.2%)</td>
<td>-0.06</td>
<td>0.25</td>
<td>-124%</td>
</tr>
<tr>
<td>250</td>
<td>2.84</td>
<td>4.68</td>
<td>1.84 (2.0%)</td>
<td>0.45</td>
<td>1.25</td>
<td>-64%</td>
</tr>
<tr>
<td>500</td>
<td>2.86</td>
<td>5.27</td>
<td>2.41 (0.8%)</td>
<td>1.02</td>
<td>2.50</td>
<td>-59%</td>
</tr>
</tbody>
</table>
There have been reports in the literature that the Pb(0) stripping charge was less than the Pb(II) deposition charge when using BDD electrodes for anodic stripping experiments [102,137–140]. This has been attributed to poor adhesion of the Pb(0) layer on BDD, incomplete stripping of Pb(0), and hydrogen evolution during deposition. Most recently, Hutton et al. performed atomic force microscopy analysis of BDD electrode surfaces before and after stripping, and they found that a substantial amount of Pb(0) remained on the BDD surface, especially for Pb(II) samples at higher concentrations [137]. This indicates that the stripping charge was less than expected due to incomplete stripping of Pb(0) from the BDD electrode. Hutton et al. proposed an in situ cleaning procedure in which a potential of 1.24 V vs. Ag/AgCl was applied for 10 min to rid of remaining Pb(0) deposits [137]. However, while successful, this procedure is not feasible in the coulometry device as bubbles of oxygen are formed at this potential and these bubbles could get lodged in the working electrode chamber of the coulometry device. These bubbles could cause an increased resistance between the working electrode and reference electrode as less solution contact is maintained between electrodes. In addition, the bubbles are likely to inhibit the accurate measurement of Pb(0) stripping signal.

Therefore, while BDD electrodes have been shown practical for the targeted detection of Pb(II) in the presence of Cd(II), the inadequate stripping of Pb(0) at reasonable potentials (which contributes to the lower-than-expected charges) does not make BDD electrodes feasible for Pb(II) analysis by DPS-ASC.
5.4. Conclusion

BDD electrodes were evaluated for the analysis of Pb(II) because BDD electrodes offer wide potential windows, low background and capacitive currents, reduced fouling, and mechanical robustness. Although DPS-ASC analysis of Pb(II) was feasible using a gold electrode (Chapter IV), BDD electrodes were investigated because Cd(II) and Pb(II) (which co-deposit on gold) were expected to be resolved from one another on BDD.

Both acetate buffer and HNO₃ were evaluated and found suitable as media for the chronoamperometric detection of Pb(II). Conditions such as deposition time and deposition/stripping potentials were then optimized using BDD electrodes from Sp3 and Fraunhofer. Then, linear sweep stripping voltammetry of a mixture of Pb(II) and Cd(II) was performed, and it was determined that the deposition potentials of Pb(II) and Cd(II) differed enough such that selective detection of Pb(II) could be completed in the presence of Cd(II) using BDD electrodes, which was not feasible on gold electrodes.

However, despite several months of changing deposition potentials, times, and supporting electrolytes, the Pb(II) charge was always lower than the calculated charge per Faraday’s law. The experimental charge for Pb(II) never exceeded 75% of the calculated charge. It was determined that this phenomenon occurred because the Pb(0) was not fully being stripped from the BDD electrode surface. However, the required potential to fully strip Pb(0) from the BDD surface is not suitable for the coulometry device because oxygen bubbles form at this potential and could become lodged in the working electrode chamber and interfere with the stripping analysis of the Pb(0). Due to low Pb(II) charge and the inability to resolve this issue using the coulometry device, BDD electrodes were not considered further for DPS-ASC detection of Pb(II).
CHAPTER VI

CONCLUSION

6.1. Summary of Research

The overall focus of this dissertation is in utilizing double potential step-anodic stripping coulometry (DPS-ASC) for the electrochemical detection of heavy metals in water. Various improvements herein have tackled some of the challenges in getting our electrochemical sensor ready for field use and are briefly summarized below.

The focus of Chapter II was to explore methods for the creation of electrochemical sensors with fixed and controllable volumes. Controlling the volume is critical as it plays a role in the experimental determination of concentration via Faraday’s law. Stamp-and-stick fabrication was found most successful in creating devices of varied thicknesses and, thus, volumes. As the volume was increased, the detection limit for As(III) improved to 20 ppb. However, this was at the cost of increasing the experiment time as the diffusion distance was lengthened.

The emphasis on As(III) detection continued in Chapter III as the use of microelectrode arrays was explored because a decrease in electrode area was expected to reduce noise due to charging current. Gold microelectrode arrays were fabricated, and the limit of detection for As(III) was 4 ppb when using the 10 µm diameter array, which meets the WHO safety standard guideline. The limit of detection for As(III) was 8 ppb in
the presence of 5 ppb Cd(II), 2 ppb Hg(II), and 15 ppb Pb(II) low-level interferents, which still meets the WHO safety standard guideline.

Chapter IV turned the attention away from As(III) and instead focused on Pb(II). A gold macroelectrode was useful for detection of high Pb(II) levels (greater than 1 ppm), whereas the gold 10 µm array electrode had a detection limit of 6 ppb that met the 15 ppb EPA limit. A device was then developed to capture metal interferents by electrolysis and near 100% of Cu(II) was trapped within the device so that the detection of Pb(II) downstream in the coulometry device was largely unaffected. Pb(II) was then detected in Ohio River water after 15 ppb, 30 ppb, or 60 ppb Pb(II) was introduced. Last, the stability of the electrochemical sensor was evaluated over the course of two weeks by daily analysis of Pb(II) charge with a 7.7% RSD.

Then, boron-doped diamond (BDD) electrodes were explored for the detection of Pb(II) in Chapter V. BDD electrodes are advantageous in that the deposition and stripping of Cd(II) and Pb(II) is resolved. However, the removal of Pb(0) from the BDD surface was incomplete, and the procedure for complete stripping of Pb(0) was not feasible in the coulometry device.

Overall, a novel method referred to as DPS-ASC was critically evaluated in the selective detection of As(III) and Pb(II) for applications in remote sensing. A summary of the key findings is listed in Table 23, which includes the linear range and detection limit for the gold electrodes mentioned in this dissertation for both As(III) and Pb(II). As noted in the table, some linear ranges may exceed those values listed because analyte levels outside of those ranges were not examined.
Table 23. Summary of results from DPS-ASC analysis of As(III) and Pb(II) using gold electrodes in the coulometry device.

<table>
<thead>
<tr>
<th>Electrode Type</th>
<th>Volume $\mu$L</th>
<th>Analyte</th>
<th>Linear Range $ppb$</th>
<th>Detection Limit $ppb$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Macroelectrode</td>
<td>4.3</td>
<td>As(III)</td>
<td>50-1,000*</td>
<td>55</td>
</tr>
<tr>
<td>Macroelectrode</td>
<td>5.6</td>
<td>As(III)</td>
<td>50-1,000*</td>
<td>32</td>
</tr>
<tr>
<td>Macroelectrode</td>
<td>6.8</td>
<td>As(III)</td>
<td>50-1,000*</td>
<td>20</td>
</tr>
<tr>
<td>50 µm Array</td>
<td>2.5</td>
<td>As(III)</td>
<td>50-1,000*</td>
<td>25</td>
</tr>
<tr>
<td>20 µm Array</td>
<td>2.3</td>
<td>As(III)</td>
<td>5-100</td>
<td>6</td>
</tr>
<tr>
<td>10 µm Array</td>
<td>2.2</td>
<td>As(III)</td>
<td>5-100</td>
<td>4</td>
</tr>
<tr>
<td>Macroelectrode</td>
<td>5.5</td>
<td>Pb(II)</td>
<td>1,000-5,000</td>
<td>750</td>
</tr>
<tr>
<td>50 µm Array</td>
<td>3.7</td>
<td>Pb(II)</td>
<td>400-700</td>
<td>320</td>
</tr>
<tr>
<td>20 µm Array</td>
<td>4.0</td>
<td>Pb(II)</td>
<td>15-90</td>
<td>10</td>
</tr>
<tr>
<td>10 µm Array</td>
<td>2.4</td>
<td>Pb(II)</td>
<td>10-60</td>
<td>6</td>
</tr>
</tbody>
</table>

*Indicates that the linear range was not tested beyond the values listed and thus may actually exceed the values listed herein.

From these results, it was concluded that the best electrode for As(III) determination by DPS-ASC was the 10 µm array gold electrode due to its 4 ppb detection limit which surpassed the 10 ppb WHO safety standard. Although this electrode does not perform linearly above 100 ppb As(III), this electrode would function properly within a warning device to notify personnel if concentrations exceed the 10 ppb WHO safety standard or not.

Furthermore, the optimal electrode for Pb(II) detection by DPS-ASC was also concluded to be the 10 µm array gold electrode. The 6 ppb limit of detection for Pb(II) remained under the criteria of 15 ppb set forth by the EPA. As before, this electrode is sensitive to small concentration changes but does not produce linear results above 60 ppb. However, with a target application of remote sensing of drinking water and a maximum contamination level of 15 ppb, any concentrations above that value require action and
remediation. Ultimately, for our application it is more important to have sensitivity at lower concentrations (as with the 10 µm array) than to have a linear range at concentrations that surpass the regulated limits.

6.2. Concluding Discussions and Considerations

The goal of DPS-ASC is to perform in situ background correction that voids the requirement for (re)calibration (i.e. instrument calibration is not required after the initial electrode calibration is complete to determine device volume) which is otherwise not feasible using traditional instrumentation. While it is true that the potential step sequence corrects for most of the charging current in situ, the correction is not 100% as indicated by the non-zero charge for a 0 ppb solution. It was determined that the 0 ppb charge was minimal (as low as 0.05 µC) when using electrodes of a smaller area, such as the microelectrode arrays. Reduction of the 0 ppb charge is further expected from creation and use of electrodes of even smaller area. Thus, while DPS-ASC is a novel advancement toward (re)calibration-free detection, it is not yet ready for autonomous use as the in situ correction is not 100%. However, practical use of the DPS-ASC method as-is could continue in the field by sequential analysis of a 0 ppb solution (stored on-site) followed by analyte solution. The resulting 0 ppb charge could be subtracted from the analyte charge to give a more accurate representation of metal analyte charge, as performed in this work.

Another consideration of the DPS-ASC method is sensitivity and selectivity. The sensitivity (discussed here as detection limit based on 3σbl) of DPS-ASC for both As(III) and Pb(II) was herein advanced by using a larger sample volume and/or smaller electrode
area to increase signal or decrease noise, respectively, to meet the WHO and EPA requirements. Sensitivity was therefore determined acceptable for single element solutions of As(III) and Pb(II). However, sensitivity becomes more challenging in multi-elemental solutions due to intermetallic interactions and co-deposition/stripping. This gives way to selectivity, which is a common challenge in the field of electrochemistry. Selectivity is difficult as the application of one deposition potential may simultaneously deposit many different metals. In addition to other metals in solution, selectivity also factors in other variables such as the solution conditions (i.e., pH) and presence of organics, for example. Selectivity was addressed in Chapter IV by the fabrication of an in-line electrolysis device to capture interferents that deposit more positively than the target analyte. However, there is the concern that as interferent metals (i.e., Hg(II)) are “captured” in the electrolysis device, that a new metal (i.e., Hg(0)) electrode forms in the electrolysis device. If the target analyte (which does not normally deposit in the electrolysis device) does deposit on the new metal electrode, then the target analyte will also be “captured” in the electrolysis device and will not be detected in the coulometry device. Similarly, there is still progress needed for analytes that co-deposit, such as Cd(II) and Pb(II), which cannot be resolved using the electrolysis device. However, the result from DPS-ASC analysis of a solution of metals that cannot be separated using the electrolysis device is currently just a larger charge due to the stripping of several metals. Although not specific, this larger charge is useful as a warning system that can trigger further on-site analytical testing, indicating that current “failures” of the electrolysis device are not detrimental.
As DPS-ASC is envisioned for remote sensing applications, one must consider the reliability of the electrodes to ensure that daily measurements are consistent for extended periods of time. From findings in Chapter IV, it was determined that one electrode was consistent over the course of two weeks with a 7.7% RSD when the coulometry device was kept assembled. Furthermore, when an electrode was newly assembled each day into the coulometry device, one electrode was utilized for up to 26 non-consecutive days (Appendix III) with only using the general H₂SO₄ cleaning procedure before signs of fouling were discovered. Both findings indicate strong reliability of a single gold electrode. Stamp-and stick-fabrication largely attributed to the enhancement of reliability by improving the volume reproducibility between assemblies of the coulometry device. In addition, stamp-and stick made it possible to improve the variability between different electrodes such that electrode-to-electrode reproducibility was increased.

Another consideration of electrode reliability focuses on reliable electrode behavior at high concentrations of metals. As discussed throughout this work, metal coverage of the electrode surface was a contributing factor to the linear range of each electrode. It was found that the charge was non-linear above certain concentrations, indicating that electrode reliability was reliant on analyte concentration. To ensure reliability, proper electrode maintenance (i.e., cleaning) must be performed, and the electrode should only be used for samples in which the expected concentrations fall within the linear range.

For the coulometry device to move outside of the laboratory and into the field, there are critical considerations and components needed for remote sensing. The necessary functions for sensor feasibility need to facilitate sample pretreatment, waste
collection, fluid handling, electrochemical measurements via a custom potentiostat, on-line data processing, power supply, and GPS tracking.

Sample pretreatment steps are critical to ensure accurate analysis. First, particulates and debris should be filtered out of the sample to avoid clogging the instrument tubing. UV light may be required for sanitation and removal of bacteria or algae. The sample is typically acidified for metal analyses to avoid metal complexes characteristically found at neutral pH levels, and the addition of chloride has been found necessary for As(III) and Pb(II) analyses. Sample acidification and addition of salt require that concentrated acids and salt solutions are stored in the field. Appropriate volumes of these solutions would need to be added to aliquots of sample and mixed thoroughly to create the ideal conditions for DPS-ASC. Minimal additions of sample pretreatment solutions are preferred as the analyte of interest will become more dilute with extensive sample prep. Note that acidification and addition of sodium chloride is required for DPS-ASC of As(III) and Pb(II), however other analytes of interest may require different solution conditions.

If the sample is heavily pretreated (i.e., pH significantly lowered), suitable sample posttreatment (i.e., addition of base) must occur before releasing the sample back into the water source. In lieu of an appropriate posttreatment process, waste could be collected and stored on-site. However, this means that a technician must return to the field periodically to collect this waste, unless large containers are stored on-site.

The fluidic pumping system needs to incorporate small, low pressure, in-line pumps that can be controlled remotely for fluid intake and outtake. In addition, there may be the requirement for the fluidic flow to be stopped, so in-line fluidic shut-off valves
may be needed. The pumping system and valves must be able to communicate with the instrument and function accordingly to draw in sample before each analysis and flush out the sample after the experiment. Once the fluid is in the coulometry device, there must be a reliable instrument to perform DPS-ASC and analyze the data. Therefore, a miniaturized potentiostat is required that can meet the needs of DPS-ASC in measuring fast and instantaneous currents. Additionally, a system must be in place that can wirelessly transmit the collected data to a central laboratory for further analysis.

Furthermore, the remote sensor must contain its own power supply as it cannot rely on traditional wall plugs and power outlets (i.e., infrastructure main power sources) used by instrumentation in a central laboratory. Energy must be supplied by batteries or harvested using solar or other techniques. Although the energy usage is minimal, it is critical to have a method in place to facilitate the operation of the remote sensor from fluidic pumps to data acquisition software.

The last consideration is that of a GPS tracking system for the device. Not only can the location information be connected to the data acquired for easy analysis, but GPS would also be helpful in the unlikely event of the movement of the remote sensor due to strong weather events or unexpected relocation by an animal or human.

In summary, there are both advantages and disadvantages to consider for this novel electrochemical method, and this project offers the potential for improvement and many new experiments beyond those mentioned here. Ultimately, this technology holds great promise for the field of remote sensing and advancement of electrochemical analyses.
REFERENCES


[89] P. Delahay, M. Pourbaix, P. Van Rysselberghe, Potential-pH diagram of lead and its applications to the study of lead corrosion and to the lead storage battery, J. Electrochem. Soc. 98 (1951) 57–64.


[113] V. Vicente, S. Bruckenstein, Rotating ring-disk electrode study of the adsorption of lead on gold in 0.05 M potassium chloride, Anal. Chem. 45 (1973) 2036–2043. doi:10.1021/ac60334a008.


APPENDIX I

REFERENCE TABLES

1.1. Standard Reduction Potentials for As(III) in Aqueous Solutions [64]

<table>
<thead>
<tr>
<th>Reaction</th>
<th>Potential vs. Ag/AgCl, V</th>
</tr>
</thead>
<tbody>
<tr>
<td>$\text{AsO}_2^- + 2\text{H}_2\text{O} + 3e^- \to \text{As} + 4\text{OH}^-$</td>
<td>-0.885</td>
</tr>
<tr>
<td>$\text{As}_2\text{O}_3 + 6\text{H}^+ + 6e^- \to 2\text{As} + 3\text{H}_2\text{O}$</td>
<td>0.025</td>
</tr>
<tr>
<td>$\text{H}_3\text{AsO}_3 + 3\text{H}^+ + 3e^- \to \text{As} + 3\text{H}_2\text{O}$</td>
<td>0.035</td>
</tr>
<tr>
<td>$\text{HAsO}_2 + 3\text{H}^+ + 3e^- \to \text{As} + 2\text{H}_2\text{O}$</td>
<td>0.045</td>
</tr>
<tr>
<td>$\text{AsO}^+ + 2\text{H}^+ + 3e^- \to \text{As} + \text{H}_2\text{O}$</td>
<td>0.045</td>
</tr>
<tr>
<td>$\text{AsO}_2^- + 4\text{H}^+ + 3e^- \to \text{As} + 2\text{H}_2\text{O}$</td>
<td>0.225</td>
</tr>
</tbody>
</table>

1.2. Standard Reduction Potentials for Pb(II) in Aqueous Solutions [72]

<table>
<thead>
<tr>
<th>Reaction</th>
<th>Potential vs. Ag/AgCl, V</th>
</tr>
</thead>
<tbody>
<tr>
<td>$\text{Pb}^{2+} + 2e^- \to \text{Pb}$</td>
<td>-0.330</td>
</tr>
<tr>
<td>$\text{PbO}_2 + 4\text{H}^+ + 2e^- \to \text{Pb}^{2+} + 2\text{H}_2\text{O}$</td>
<td>1.265</td>
</tr>
<tr>
<td>$\text{PbO}_2 + \text{SO}_4^{2-} + 4\text{H}^+ + 2e^- \to \text{PbSO}_4 + 2\text{H}_2\text{O}$</td>
<td>1.495</td>
</tr>
<tr>
<td>$\text{PbSO}_4 + 2e^- \to \text{Pb} + \text{SO}_4^{2-}$</td>
<td>-0.556</td>
</tr>
</tbody>
</table>
APPENDIX II

MICROFABRICATION TECHNIQUES

2.1. Fabrication of Gold Macroelectrodes

A 4-inch oxidized silicon wafer was placed into a Lesker PVD-75 thin film deposition system to deposit 20 nm of titanium followed by 120 nm of gold onto the wafer to create a thin-film gold macroelectrode. The following procedure was used in operation of the Lesker PVD-75.

Lesker PVD-75 Operation:
1. Turn on the Lesker PVD-75 and press “start PC vent”
2. Load the metal targets and substrate into the system:
   a. Source 1: 3 inch gold target
   b. Source 3: 4 inch titanium target
   c. Substrate: oxidized silicon wafer
3. Check the conductivity between targets and dark space shield
4. Close the door and press “start PC pump”
5. Wait at least 45 minutes, until pressure is at or below $1.8 \times 10^{-6}$ torr
6. On the vacuum page, turn on “PC high vac throttle”
7. On the platen motion page, select “FWD”
8. On the gas page, complete the following tasks:
   a. Turn on source 3 gas
   b. Set “capman pressure SP” to 5 mtorr
   c. Set “MFC1 mode” to 4
9. On the deposition page, perform the following functions:
   a. Set “power supply 4” at 300 Watts
   b. Turn on “source SW8 (source 3)”
   c. Turn on “power supply 4”
   d. Open source 3 shutter
   e. Perform 1 minute sputter to remove oxide on titanium target
   f. Open the substrate shutter
   g. Perform 2 minute sputter to deposit ~ 20 nm of titanium
   h. Close substrate shutter
   i. Close source 3 shutter
   j. Turn off “power supply 4”
   k. Turn off “source SW8 (source 3)”

10. On the gas page, turn off source 3 gas and turn on source 1 gas

11. On the deposition page, perform the following functions:
   a. Turn on “source SW6 (source 1)”
   b. Turn on “power supply 4”
   c. Open source 1 shutter
   d. Open the substrate shutter
   e. Perform 2.5 minute sputter to deposit ~ 120 nm of gold
   f. Close substrate shutter
   g. Close source 1 shutter
   h. Turn off “power supply 4”
   i. Turn off “source SW6 (source 1)”

12. Press “start PC vent” and unload the metal targets and substrate

13. Press “start PC pump” and shut down the Lesker PVD-75
## 2.2. Processing of SU-8

<table>
<thead>
<tr>
<th>Process</th>
<th>Equipment</th>
<th>Parameters</th>
<th>Goal</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rinsing</td>
<td>Fume Hood</td>
<td>Acetone for 1 min Water for 1 min</td>
<td>Clean wafer</td>
</tr>
<tr>
<td>Drying</td>
<td>Fume Hood</td>
<td>Nitrogen gas</td>
<td>Dries wafer</td>
</tr>
<tr>
<td>Dehydration Bake</td>
<td>Hot Plate</td>
<td>30 minutes at 115°C Then cool to room temp on counter</td>
<td>Rid of water on wafer</td>
</tr>
<tr>
<td>Spinning SU-8</td>
<td>Headway Spinners</td>
<td>1. Apply 4 mL of SU-8 2. 500 rpm at 100 rpm/sec for 10 sec 3. 3,000 rpm at 300 rpm/sec for 30 sec</td>
<td>Spin layer of 100 um SU-8</td>
</tr>
<tr>
<td>Soft Bake</td>
<td>Polymid Oven</td>
<td>Start 50°C Ramp for 30 min up to 115°C Hold for 30 min at 115°C Ramp for 45 min down to 50°C</td>
<td>Reduce solvent content to promote adhesion</td>
</tr>
<tr>
<td>Expose</td>
<td>Suss Mask Aligner</td>
<td>Top-side alignment (TSA) + soft contact H-line filter 405 nm 12.1 mV/cm² for 65 seconds</td>
<td>Imprint ellipse, channels, and contact pad</td>
</tr>
<tr>
<td>Post-Exposure Bake</td>
<td>Polymid Oven</td>
<td>Start 50°C Ramp for 30 min up to 115°C Hold for 30 min at 115°C Ramp for 45 min down to 50°C</td>
<td>Ensure exposure</td>
</tr>
<tr>
<td>Development</td>
<td>Fume Hood</td>
<td>SU-8 Developer Time: 10-15 minutes</td>
<td>Remove unexposed SU-8</td>
</tr>
<tr>
<td>Rinsing</td>
<td>Fume Hood</td>
<td>Isopropanol for 1 min Water for 1 min</td>
<td>Rid of SU-8 developer and cleans wafer</td>
</tr>
<tr>
<td>Drying</td>
<td>Fume Hood</td>
<td>Nitrogen gas</td>
<td>Dries wafer</td>
</tr>
<tr>
<td>Hard Bake</td>
<td>Polymid Oven</td>
<td>Ramp to 200°C Hold at 200°C for 10 min Ramp down to 95°C</td>
<td>Strengthens resist</td>
</tr>
<tr>
<td>Inspection</td>
<td>Optical Microscope</td>
<td>Visually inspect for errors in pattern</td>
<td>Check etching</td>
</tr>
<tr>
<td>Dektak</td>
<td></td>
<td>Measure film thickness</td>
<td>Determine SU-8 height</td>
</tr>
<tr>
<td>Dicing</td>
<td>Dicing Saw</td>
<td>Submit for 30mm x 13mm dicing</td>
<td>Creates individual chips</td>
</tr>
</tbody>
</table>
# 2.3. Processing of SU-8 using OmniCoat

<table>
<thead>
<tr>
<th>Process</th>
<th>Equipment</th>
<th>Parameters</th>
<th>Goal</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rinsing</td>
<td>Fume Hood</td>
<td>Acetone for 1 min, Water for 1 min</td>
<td>Clean wafer</td>
</tr>
<tr>
<td>Drying</td>
<td>Fume Hood</td>
<td>Nitrogen gas</td>
<td>Dries wafer</td>
</tr>
<tr>
<td>Dehydration Bake</td>
<td>Hot Plate</td>
<td>30 minutes at 115°C, Then cool to room temp. on counter</td>
<td>Rid of water on wafer</td>
</tr>
</tbody>
</table>
| Spinning OmniCoat        | Headway Spinners | 1. Apply 4 mL of OmniCoat  
2. 500 rpm at 100 rpm/sec for 5 sec  
3. 3,000 rpm at 300 rpm/sec for 30 sec  
4. Remove wafer from spinners | Spin layer of 13 nm OmniCoat          |
|                          | Hot Plate     | 1 minute at 200°C, Then cool to room temp. on counter                     | Bakes on 1st layer                      |
|                          | Hot Plate     |                                                                             | ***Repeat spin + heating two more times*** |
| Spinning SU-8            | Headway Spinners | 1. Apply 4 mL of SU-8  
2. 500 rpm at 100 rpm/sec for 10 sec  
3. 3,000 rpm at 300 rpm/sec for 30 sec | Spin layer of 100 um SU-8             |
| Soft Bake                | Polymid Oven  | Start 50°C, Ramp for 30 min up to 115°C, Hold for 30 min at 115°C, Ramp for 45 min down to 50°C | Reduce solvent content to promote adhesion |
| Expose                   | Suss Mask Aligner | Top-side alignment (TSA) + soft contact  
H-line filter 405 nm  
12.1 mV/cm² for 65 seconds | Imprint ellipse, channels, and contact pad |
| Post-Exposure Bake       | Polymid Oven  | Start 50°C, Ramp for 30 min up to 115°C, Hold for 30 min at 115°C, Ramp for 45 min down to 50°C | Ensure exposure                         |
| Development              | Fume Hood     | SU-8 Developer, Time: 10-15 minutes                                         | Remove unexposed SU-8                   |
| Rinsing                  | Fume Hood     | Isopropanol for 1 min, Water for 1 min                                     | Rid of SU-8 developer and cleans wafer  |
| Drying                   | Fume Hood     | Nitrogen gas                                                               | Dries wafer                             |
| OmniCoat Development     | Option 1: Fume Hood | Agitate in MCC 101 Developer for 1 min, Then DI water for 2 min           | Removes OmniCoat                        |
|                          | Option 2: Fume Hood | Agitate in Microposit MF 319 for 30 sec, Then DI water for 2 min          |                                         |
| Hard Bake                | Polymid Oven  | Ramp to 200°C, Hold at 200°C for 10 min, Ramp down to 95°C                | Strengthens resist                      |
| Inspection               | Optical Microscope | Visually inspect for errors in pattern                                     | Check etching                           |
|                          | Dektak        | Measure film thickness                                                     | Determine SU-8 height                   |
| Dicing                   | Dicing Saw    | Submit for 30mm x 13mm dicing                                              | Creates individual chips                |
## 2.4. Fabrication of Gold Microelectrode Arrays

<table>
<thead>
<tr>
<th>Process</th>
<th>Equipment</th>
<th>Parameters</th>
<th>Goal</th>
</tr>
</thead>
<tbody>
<tr>
<td>Clean Wafer</td>
<td>QDR/Spin Dry</td>
<td>Water</td>
<td>Clean and dry wafer</td>
</tr>
<tr>
<td>Dehydration Bake</td>
<td>Hot Plate</td>
<td>3 minutes at 115°C Then cool to room temp</td>
<td>Rid of water on wafer</td>
</tr>
<tr>
<td>Spinning 1827</td>
<td>Headway Spinners</td>
<td>Program 1</td>
<td>Spin layer of 1827 photoresist</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1. 500 rpm at 500 rpm/sec for 1 sec</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>2. 4,000 rpm at 500 rpm/sec for 10 sec</td>
<td></td>
</tr>
<tr>
<td>Soft Bake</td>
<td>Hot Plate</td>
<td>1.5 minute at 115°C Then cool to room temp</td>
<td>Reduce solvent content to promote adhesion</td>
</tr>
<tr>
<td>Mask Alignment</td>
<td>Suss Mask Aligner MA 6</td>
<td>Top-side alignment (TSA) Hard contact 12.1 mV/cm² for 20 seconds</td>
<td>Microarray pattern</td>
</tr>
<tr>
<td>Development</td>
<td>Hood</td>
<td>MF-319 Time: 60 seconds</td>
<td>Removes exposed 1827</td>
</tr>
<tr>
<td>Clean Wafer</td>
<td>QDR/Spin Dry</td>
<td>Water</td>
<td>Rid of MF-319</td>
</tr>
<tr>
<td>Inspection</td>
<td>Optical Microscope</td>
<td>Visually inspect for errors in pattern</td>
<td>Check development</td>
</tr>
<tr>
<td>Hard Bake</td>
<td>Hot Plate</td>
<td>5 minutes at 115°C Then cool to room temp</td>
<td>Ensure development</td>
</tr>
<tr>
<td>Dicing</td>
<td>Dicing Saw</td>
<td>Have Curt McKenna dice wafer</td>
<td>Cut 13 x 30 mm chips</td>
</tr>
<tr>
<td>Nitride Etch</td>
<td>Hood</td>
<td>Adhere chip to a weigh boat Invert over liquid buffered oxide etch Let etch occur over 15 hours</td>
<td>Etches silicon nitride to expose gold arrays</td>
</tr>
<tr>
<td>Inspection</td>
<td>Multimeter</td>
<td>Check for conductivity of gold</td>
<td>Check etching</td>
</tr>
<tr>
<td>Inspection</td>
<td>Optical Microscope</td>
<td>Visually inspect for errors in pattern</td>
<td>Check etching</td>
</tr>
<tr>
<td>Strip 1827</td>
<td>Hood</td>
<td>Acetone</td>
<td>Rid of 1827 photoresist</td>
</tr>
<tr>
<td>Clean Wafer</td>
<td>QDR/Spin Dry</td>
<td>Water</td>
<td>Clean and dry wafer</td>
</tr>
</tbody>
</table>

Begin with silicon wafer with native oxide, 20 nm titanium, 200 nm gold, 300 nm silicon nitride ordered from the University of Michigan.
APPENDIX III

OVERVIEW OF WORKING ELECTRODES

Below is a listing of the various gold macroelectrodes and gold microelectrode arrays that were fabricated in-house and used throughout this dissertation. The electrodes were assigned names for ease of use. The number of days each electrode was used in the coulometry device along with the volume of the electrode chip is listed. The results presented in this dissertation were determined by using the electrodes in the tables that are highlighted in yellow.

3.1. Gold Macroelectrodes

<table>
<thead>
<tr>
<th>Name of Electrode</th>
<th>Days Used</th>
<th>Volume</th>
</tr>
</thead>
<tbody>
<tr>
<td>001</td>
<td>1</td>
<td>6.2 µL</td>
</tr>
<tr>
<td>003</td>
<td>1</td>
<td>4.5 µL</td>
</tr>
<tr>
<td>005</td>
<td>2</td>
<td>4.5 µL</td>
</tr>
<tr>
<td>007</td>
<td>7</td>
<td>4.3 µL</td>
</tr>
<tr>
<td>008</td>
<td>7</td>
<td>4.8 µL</td>
</tr>
<tr>
<td>010</td>
<td>6</td>
<td>5.3 µL</td>
</tr>
<tr>
<td>012</td>
<td>6</td>
<td>5.5 µL</td>
</tr>
<tr>
<td>013</td>
<td>16</td>
<td>4.9 µL</td>
</tr>
<tr>
<td>101</td>
<td>3</td>
<td>6.9 µL</td>
</tr>
<tr>
<td>102</td>
<td>4</td>
<td>6.3 µL</td>
</tr>
<tr>
<td>103</td>
<td>5</td>
<td>4.3 µL</td>
</tr>
<tr>
<td>105</td>
<td>6</td>
<td>6.8 µL</td>
</tr>
<tr>
<td>106</td>
<td>7</td>
<td>5.6 µL</td>
</tr>
</tbody>
</table>
### 3.2. Gold Microelectrode Arrays

<table>
<thead>
<tr>
<th>Name of Electrode</th>
<th>Days Used</th>
<th>Volume</th>
</tr>
</thead>
<tbody>
<tr>
<td>10 µm A</td>
<td>1</td>
<td>3.2 µL</td>
</tr>
<tr>
<td>10 µm C</td>
<td>3</td>
<td>2.4 µL</td>
</tr>
<tr>
<td>10 µm E</td>
<td>9</td>
<td>2.2 µL</td>
</tr>
<tr>
<td>10 µm F</td>
<td>3</td>
<td>2.4 µL</td>
</tr>
<tr>
<td>10 µm H</td>
<td>2</td>
<td>2.9 µL</td>
</tr>
<tr>
<td>20 µm C</td>
<td>3</td>
<td>2.2 µL</td>
</tr>
<tr>
<td>20 µm E</td>
<td>26</td>
<td>2.3 µL</td>
</tr>
<tr>
<td>20 µm H</td>
<td>6</td>
<td>4.0 µL</td>
</tr>
<tr>
<td>50 µm A</td>
<td>2</td>
<td>2.1 µL</td>
</tr>
<tr>
<td>50 µm D</td>
<td>5</td>
<td>2.5 µL</td>
</tr>
<tr>
<td>50 µm H</td>
<td>8</td>
<td>3.7 µL</td>
</tr>
</tbody>
</table>
APPENDIX IV
EXAMPLE CALCULATIONS

4.1. Arsenic Monolayer Coverage on a Gold Electrode

The concentration at which As(0) saturates a gold electrode surface (macroelectrode and microelectrode arrays) via monolayer coverage was calculated to better understand impacts on the linear range of detection. This is important as the deposition of As(III) onto electrodeposited As(0) reaches a limiting thickness due to the increased resistance of the As(0) layer and further prohibits detection of remaining As(III) [66]. Van der Waals interactions (As radius 185 pm), hexagonal closed packing (74% packing efficiency), and monolayer coverage were assumed in these calculations.

For a gold macroelectrode of 35.1 mm² electrode area and 6.8 μL volume:

1) Area of one As atom = \( \pi (185 \text{ pm})^2 = 107,521 \text{ pm}^2 = 1.08 \times 10^{-13} \text{ mm}^2 \)

2) Number of As atoms = \( \frac{35.1 \text{ mm}^2}{1.08 \times 10^{-13} \text{ mm}^2} \times 74\% = 2.41 \times 10^{14} \text{ atoms} \)

3) Moles of As = \( 2.41 \times 10^{14} \text{ atoms} \times \frac{1 \text{ mol}}{6.022 \times 10^{23} \text{ atoms}} = 4.00 \times 10^{-10} \text{ mol} \)

4) Mass of As = \( 4.00 \times 10^{-10} \text{ mol} \times \frac{74.9216 \text{ g}}{1 \text{ mol}} = 2.99 \times 10^{-8} \text{ g} \)

5) Concentration of As = \( \frac{2.99 \times 10^{-8} \text{ g}}{6.8 \mu \text{L}} \times \frac{1,000 \text{ mg}}{1 \text{ g}} \times \frac{1 \times 10^6 \mu \text{L}}{1 \text{ L}} = 4.4 \text{ ppm} \)
For a 50 µm gold microelectrode of 6.6 mm² electrode area and 2.5 µL volume:

1) Area of one As atom = \(\pi (185 \text{ pm})^2 = 107,521 \text{ pm}^2 = 1.08 \times 10^{-13} \text{ mm}^2\)

2) Number of As atoms = \(\frac{6.6 \text{ mm}^2}{1.08 \times 10^{-13} \text{ mm}^2} \times 74\% = 4.5 \times 10^{13}\) atoms

3) Moles of As = \(4.5 \times 10^{13} \text{ atoms} \times \frac{1 \text{ mol}}{6.022 \times 10^{23} \text{ atoms}} = 7.5 \times 10^{-11} \text{ mol}\)

4) Mass of As = \(7.5 \times 10^{-11} \text{ mol} \times \frac{74.9216 \text{ g}}{1 \text{ mol}} = 5.6 \times 10^{-9} \text{ g}\)

5) Concentration of As = \(\frac{5.6 \times 10^{-9} \text{ g}}{2.5 \mu \text{L}} \times \frac{1,000 \text{ mg}}{1 \text{ g}} \times \frac{1 \times 10^6 \mu \text{L}}{1 \text{ L}} = 2.3 \text{ ppm}\)

For a 20 µm gold microelectrode of 3.1 mm² electrode area and 2.3 µL volume:

1) Area of one As atom = \(\pi (185 \text{ pm})^2 = 107,521 \text{ pm}^2 = 1.08 \times 10^{-13} \text{ mm}^2\)

2) Number of As atoms = \(\frac{3.1 \text{ mm}^2}{1.08 \times 10^{-13} \text{ mm}^2} \times 74\% = 2.1 \times 10^{13}\) atoms

3) Moles of As = \(2.1 \times 10^{13} \text{ atoms} \times \frac{1 \text{ mol}}{6.022 \times 10^{23} \text{ atoms}} = 3.5 \times 10^{-11} \text{ mol}\)

4) Mass of As = \(3.5 \times 10^{-11} \text{ mol} \times \frac{74.9216 \text{ g}}{1 \text{ mol}} = 2.6 \times 10^{-9} \text{ g}\)

5) Concentration of As = \(\frac{2.6 \times 10^{-9} \text{ g}}{2.3 \mu \text{L}} \times \frac{1,000 \text{ mg}}{1 \text{ g}} \times \frac{1 \times 10^6 \mu \text{L}}{1 \text{ L}} = 1.1 \text{ ppm}\)
For a 10 µm gold microelectrode of 2.0 mm² electrode area and 2.2 µL volume:

1) Area of one As atom = \( \pi (185 \text{ pm})^2 = 107,521 \text{ pm}^2 = 1.08 \times 10^{-13} \text{ mm}^2 \)

2) Number of As atoms = \( \frac{2.0 \text{ mm}^2}{1.08 \times 10^{-13} \text{ mm}^2} \times 74\% = 1.4 \times 10^{13} \text{ atoms} \)

3) Moles of As = \( 1.4 \times 10^{13} \text{ atoms} \times \frac{1 \text{ mol}}{6.022 \times 10^{23} \text{ atoms}} = 2.3 \times 10^{-11} \text{ mol} \)

4) Mass of As = \( 2.3 \times 10^{-11} \text{ mol} \times \frac{74.9216 \text{ g}}{1 \text{ mol}} = 1.7 \times 10^{-9} \text{ g} \)

5) Concentration of As = \( \frac{1.7 \times 10^{-9} \text{ g}}{2.2 \mu \text{L}} \times \frac{1,000 \text{ mg}}{1 \text{ g}} \times \frac{1 \times 10^6 \mu \text{L}}{1 \text{ L}} = 0.77 \text{ ppm} \)

### 4.2. Limit of Detection

The IUPAC standards were followed in determining detection limits. The calculation, seen below, of the detection limit signal \( (S_{dl}) \) involves the signal of the blank \( (S_{bl}) \) and the standard deviation of the blank \( (\sigma_{bl}) \). The linear regression equation from the calibration curves were then used to relate \( S_{dl} \) to the limit of detection (LOD) using the slope \( (m) \) and y-intercept \( (b) \). This conversion was necessary as the \( S_{dl} \) had units of charge \( (\mu \text{C}) \) whereas the LOD needed to be reported as a concentration (ppb or ppm).

1) \( S_{dl} = S_{bl} + 3\sigma_{bl} \)

2) \( \text{LOD} = \frac{S_{dl} - b}{m} \)
4.3. Lead Monolayer Coverage on a Gold Electrode

The concentration at which Pb(0) saturates a gold electrode surface (macroelectrode and microelectrode arrays) via monolayer coverage was calculated to better understand impacts on the linear range of detection. Van der Waals interactions (Pb radius 254 pm), hexagonal closed packing (74% packing efficiency), and monolayer coverage were assumed in these calculations. Covalent bonding was considered but resulted in tighter packing that does not agree with experimental results of electrode saturation.

For a gold macroelectrode of 35.1 mm² electrode area and 5.5 µL volume:

1) Area of one Pb atom = \( \pi (254 \text{ pm})^2 = 202,683 \text{ pm}^2 = 2.03 \times 10^{-13} \text{ mm}^2 \)

2) Number of Pb atoms = \( \frac{35.1 \text{ mm}^2}{2.03 \times 10^{-13} \text{ mm}^2} \times 74\% = 1.28 \times 10^{14} \text{ atoms} \)

3) Moles of Pb = \( 1.28 \times 10^{14} \text{ atoms} \times \frac{1 \text{ mol}}{6.022 \times 10^{23} \text{ atoms}} = 2.13 \times 10^{-10} \text{ mol} \)

4) Mass of Pb = \( 2.13 \times 10^{-10} \text{ mol} \times \frac{207.2 \text{ g}}{1 \text{ mol}} = 4.40 \times 10^{-8} \text{ g} \)

5) Concentration of Pb = \( \frac{4.40 \times 10^{-8} \text{ g}}{5.5 \mu L} \times \frac{1,000 \text{ mg}}{1 \text{ g}} \times \frac{1 \times 10^6 \mu L}{1 \text{ L}} = 8.0 \text{ ppm} \)
For a 50 µm gold microelectrode of 6.6 mm² electrode area and 3.7 µL volume:

1) Area of one Pb atom = \( \pi(254 \text{ pm})^2 = 202,683 \text{ pm}^2 = 2.03 \times 10^{-13} \text{ mm}^2 \)

2) Number of Pb atoms = \( \frac{6.6 \text{ mm}^2}{2.03 \times 10^{-13} \text{ mm}^2} \times 74\% = 2.4 \times 10^{13} \text{ atoms} \)

3) Moles of Pb = \( 2.4 \times 10^{13} \text{ atoms} \times \frac{1 \text{ mol}}{6.022 \times 10^{23} \text{ atoms}} = 4.0 \times 10^{-11} \text{ mol} \)

4) Mass of Pb = \( 4.0 \times 10^{-11} \text{ mol} \times \frac{207.2 \text{ g}}{1 \text{ mol}} = 8.3 \times 10^{-9} \text{ g} \)

5) Concentration of Pb = \( \frac{8.3 \times 10^{-9} \text{ g}}{3.7 \mu \text{L}} \times \frac{1,000 \text{ mg}}{1 \text{ g}} \times \frac{1 \times 10^6 \mu \text{L}}{1 \text{ L}} = 2.2 \text{ ppm} \)

For a 20 µm gold microelectrode of 3.1 mm² electrode area and 4.0 µL volume:

1) Area of one Pb atom = \( \pi(254 \text{ pm})^2 = 202,683 \text{ pm}^2 = 2.03 \times 10^{-13} \text{ mm}^2 \)

2) Number of Pb atoms = \( \frac{3.1 \text{ mm}^2}{2.03 \times 10^{-13} \text{ mm}^2} \times 74\% = 1.1 \times 10^{13} \text{ atoms} \)

3) Moles of Pb = \( 1.1 \times 10^{13} \text{ atoms} \times \frac{1 \text{ mol}}{6.022 \times 10^{23} \text{ atoms}} = 1.8 \times 10^{-11} \text{ mol} \)

4) Mass of Pb = \( 1.8 \times 10^{-11} \text{ mol} \times \frac{207.2 \text{ g}}{1 \text{ mol}} = 3.7 \times 10^{-9} \text{ g} \)

5) Concentration of Pb = \( \frac{3.7 \times 10^{-9} \text{ g}}{4.0 \mu \text{L}} \times \frac{1,000 \text{ mg}}{1 \text{ g}} \times \frac{1 \times 10^6 \mu \text{L}}{1 \text{ L}} = 0.93 \text{ ppm} \)
For a 10 \( \mu \)m gold microelectrode of 2.0 mm\(^2\) electrode area and 2.4 \( \mu \)L volume:

1) Area of one Pb atom = \( \pi (254 \text{ pm})^2 = 202,683 \text{ pm}^2 = 2.03 \times 10^{-13} \text{ mm}^2 \)

2) Number of Pb atoms = \( \frac{2.0 \text{ mm}^2}{2.03 \times 10^{-13} \text{ mm}^2} \times 74\% \times 74\% = 7.3 \times 10^{12} \text{ atoms} \)

3) Moles of Pb = \( 7.3 \times 10^{12} \text{ atoms} \times \frac{1 \text{ mol}}{6.022 \times 10^{23} \text{ atoms}} = 1.2 \times 10^{-11} \text{ mol} \)

4) Mass of Pb = \( 1.2 \times 10^{-11} \text{ mol} \times \frac{207.2 \text{ g}}{1 \text{ mol}} = 2.5 \times 10^{-9} \text{ g} \)

5) Concentration of Pb = \( \frac{2.5 \times 10^{-9} \text{ g}}{2.4 \text{ } \mu \text{L}} \times \frac{1,000 \text{ mg}}{1 \text{ g}} \times \frac{1 \times 10^6 \text{ } \mu \text{L}}{1 \text{ L}} = 1.0 \text{ ppm} \)
4.4. Lead Monolayer Coverage on a Boron-Doped Diamond Electrode

The concentration at which Pb(0) saturates a boron-doped diamond macroelectrode via monolayer coverage was calculated to understand impacts on the linear range of detection. Van der Waals interactions (Pb radius 254 pm) and hexagonal closed packing (74% packing efficiency) were assumed in these calculations.

For the Fraunhofer BDD macroelectrode of 35.1 mm$^2$ electrode area and 5.3 µL volume:

1) Area of one Pb atom = \( \pi (254 \text{ pm})^2 = 202,683 \text{ pm}^2 = 2.03 \times 10^{-13} \text{ mm}^2 \)

2) Number of Pb atoms = \( \frac{35.1 \text{ mm}^2}{2.03 \times 10^{-13} \text{ mm}^2} \times 74\% = 1.28 \times 10^{14} \) atoms

3) Moles of Pb = \( 1.28 \times 10^{14} \) atoms \( \times \frac{1 \text{ mol}}{6.022 \times 10^{23} \text{ atoms}} = 2.13 \times 10^{-10} \) mol

4) Mass of Pb = \( 2.13 \times 10^{-10} \) mol \( \times \frac{207.2 \text{ g}}{1 \text{ mol}} = 4.40 \times 10^{-8} \) g

5) Concentration of Pb = \( \frac{4.40 \times 10^{-8} \text{ g}}{5.3 \text{ µL}} \times \frac{1,000 \text{ mg}}{1 \text{ g}} \times \frac{1 \times 10^6 \text{ µL}}{1 \text{ L}} = 8.3 \text{ ppm} \)

For the Sp3 BDD macroelectrode of 35.1 mm$^2$ electrode area and 5.9 µL volume:

1) Area of one Pb atom = \( \pi (254 \text{ pm})^2 = 202,683 \text{ pm}^2 = 2.03 \times 10^{-13} \text{ mm}^2 \)

2) Number of Pb atoms = \( \frac{35.1 \text{ mm}^2}{2.03 \times 10^{-13} \text{ mm}^2} \times 74\% = 1.28 \times 10^{14} \) atoms

3) Moles of Pb = \( 1.28 \times 10^{14} \) atoms \( \times \frac{1 \text{ mol}}{6.022 \times 10^{23} \text{ atoms}} = 2.13 \times 10^{-10} \) mol

4) Mass of Pb = \( 2.13 \times 10^{-10} \) mol \( \times \frac{207.2 \text{ g}}{1 \text{ mol}} = 4.40 \times 10^{-8} \) g

5) Concentration of Pb = \( \frac{4.40 \times 10^{-8} \text{ g}}{5.9 \text{ µL}} \times \frac{1,000 \text{ mg}}{1 \text{ g}} \times \frac{1 \times 10^6 \text{ µL}}{1 \text{ L}} = 7.5 \text{ ppm} \)
CURRICULUM VITAE

Kelsey Lynn Kaht
www.linkedin.com/in/kelseykaht/

HIGHLIGHTS

Detail-oriented scientist utilizing leadership and planning skills to move projects from idea generation to implementation and analysis, while rigorously considering evidence and data.

Well-educated in biology and chemistry with expert training in analytical chemistry and emphasis in environmental analyses, trace metal detection, data analytics, and analytical instrumentation (e.g. GC-MS, HPLC, ICP-AES, IR).

EDUCATION

PhD Chemistry, University of Louisville, Louisville, KY
Sep 2017 – present

MS Chemistry, University of Louisville, Louisville, KY
Aug 2014 – Sep 2017

BA Biology, BA Chemistry, Thomas More College, Crestview Hills, KY
Aug 2010 – May 2014

DISSERTATION

Title: “An Electrochemical Instrument for the Analysis of Heavy Metals in Water via Anodic Stripping Coulometry for Applications in Remote Sensing”

Committee: Richard Baldwin, Craig Grapperhaus, Robert Keynton, John Price, Thomas Roussel, Frank Zamborini
RESEARCH EXPERIENCE

Analytical Chemistry Graduate Research, University of Louisville
Aug 2014 – May 2019
• Developed an exhaustive coulometry method for in situ blank correction which permits operator-free analyses of trace metals, such as arsenic and lead, in water at sub ppm levels.
• Collaborated with a team of engineers to create technology for the remote deployment of our electrochemical instrument out into the field.

Analytical Chemistry Undergraduate Research, Thomas More College
Jun 2012 – Jun 2014
• Utilized inductively coupled plasma-atomic emission spectroscopy (ICP-AES) to distinguish between samples of human cremated remains and cement powder by trace metal analysis.
• Performed extensive statistical tests to determine the trace metal “fingerprint” of human cremated remains compared to cement powder.
• This project was inspired by the Tri-State Crematorium Incident of 2002 in which families were unjustly given cement powder in place of human cremated remains of their loved ones.

PUBLICATIONS

Kaht, K.; Marei, M.; Roussel, T.; Keynton, R.; Baldwin, R. Detection of As(III) by in situ, background corrected, double potential step-anodic stripping coulometry: investigating the use of a larger sample volume or smaller electrode area to increase sensitivity. In preparation for submission to Sens Actuators B Chem.


CONFERENCE PRESENTATIONS


**Kaht, K.** (Apr 2018). How much arsenic is in that water? Developing an electrochemical sensor for continuous, on-site analysis of arsenic. Seminar at GRADtalks Series, Louisville, KY.


**Kaht, K.; Jeffries, M.; Roussel, T.; Keynton, R.; Baldwin, R.** (Mar 2017). Advances in electrochemical instrumentation for the continuous, on-site monitoring of trace metals in water. Poster at the Graduate Student Regional Research Conference, Louisville, KY.


Farwick, C.; **Sparks, K.**; Wetzel, W.; Currie, C. (May 2016). Mathematical strategies for the analysis of human cremated remains. Poster at the 47th American Chemical Society Central Regional Meeting, Covington, KY.
Sparks, K.; Wetzel, W.; Currie, C. (Apr 2014). Trace metal analysis of bone ash, Portland cement, and human cremated remains by ICP-AES. Poster at the Butler University Undergraduate Research Conference, Indianapolis, IN.


WORK EXPERIENCE

Chemist III, Oakwood Labs, Cleveland, OH  
Jul 2019 – present  
- Analytical scientist in the research and development of pharmaceutical products encapsulated in microspheres for extended release applications.

Graduate Student Researcher, University of Louisville, Louisville, KY  
Aug 2014 – May 2019  
- Developing an innovative electrochemical instrument for operator-free, in situ detection of trace metals in water, such as arsenic and lead.  
- Collaborating with a team of engineers to manufacture components for the remote deployment of our system out into the field.

Graduate Teaching Assistant, University of Louisville, Louisville, KY  
Aug 2014 – Dec 2018  
- Selected as Lead TA in Fall 2018 and tasked with redesigning and improving the laboratory manual used by over 800 students annually.  
- Instructed and supervised up to 5 analytical chemistry laboratory classes weekly, each comprised of up to 20 undergraduate students.

Pharmacy Technician III, St. Elizabeth Hospital, Edgewood, KY  
Jun 2012 – Jun 2014  
- Operated effectively in a fast-paced team environment to deliver medications and prepare IVs to meet patient needs.
Undergraduate Student Researcher, Thomas More College, Crestview Hills, KY
Jun 2012 – Jun 2014
- Analytical chemistry research utilizing inductively coupled plasma-atomic emission spectroscopy (ICP-AES) to distinguish between human cremated remains and cement powder by trace metal analysis, inspired by the 2002 Tri-State Crematorium Incident.

Chemistry Tutor, Thomas More College, Crestview Hills, KY
Aug 2012 – May 2014
- Mentored and advised freshmen and sophomore college students in general and organic chemistry on a weekly basis.

Pharmacy Technician, Remke-Biggs Market, Florence, KY
Jul 2010 – Jun 2012
- Answered questions from customers and prepared prescription orders.

LEADERSHIP AND SERVICE

Student Government Association, University of Louisville, Louisville, KY
Aug 2017 – May 2019
- One of five Student Senators who represent the entire graduate student population at the University of Louisville to Student Government.

Graduate Student Ambassador Program, University of Louisville, Louisville, KY
Jun 2015 – May 2019
- Selected as one of few students to serve as an Ambassador for the Graduate School.

Graduate Student Council, University of Louisville, Louisville, KY
Aug 2014 – May 2019
- Elected President, May 2018 – May 2019
  o Graduate student body president and the sole graduate student spokesperson to higher administration.
- Elected Vice President of External Affairs, May 2017 – May 2018
  o Chair of event programming for over 5,000 graduate students.
- Elected Senator-Elect, May 2015 – May 2017
  o Supporting role in all event planning and meetings, assisting the Vice President of External Affairs.

Student Activities Board, University of Louisville, Louisville, KY
May 2017 – May 2018
- Selected as the Graduate Student Representative who managed and oversaw the Student Activities Board programming meetings.
Graduate Network in Arts and Sciences, University of Louisville, Louisville, KY
Aug 2014 – May 2018
  • Elected President, May 2016 – May 2018
    o Organized and ran monthly meetings of graduate students from the 20 departments within the College of Arts and Sciences.
  • Elected Vice President, May 2015 – May 2016
    o Worked alongside the President to assist with planning monthly meetings and event programming.

Chemistry Graduate Student Association, University of Louisville, Louisville, KY
Aug 2014 – May 2017
  • Elected President, May 2016 – May 2017
    o Directed the 2017 Derby Lecture Series in which Dr. Peter Agre, 2003 Nobel Laureate in Chemistry, was the invited guest. This entailed planning Dr. Agre’s trip from travel reservations and auditorium bookings for his seminars, to leading the awards banquet. The event concluded with a day at the Kentucky Derby with Dr. Agre.
  • Elected Co-President, May 2015 – May 2016
    o Worked as a team alongside my co-president to organize the 2016 Derby Lecture Series which featured distinguished chemist, Dr. Joseph Franklin.

Intramural Soccer, University of Louisville, Louisville, KY
Aug 2014 – Jan 2015
  • Team Captain of “The Atoms Family” soccer team composed of chemistry graduate students.

Student Ambassador Program, Thomas More College, Crestview Hills, KY
Sep 2012 – May 2014
  • Chosen to serve as an Ambassador for new STEM majors which entailed meeting with prospective students and answering their questions.

Biology Club, Thomas More College, Crestview Hills, KY
Aug 2010 – May 2014
  • Elected Co-President, Aug 2013 – May 2014
    o Led bimonthly meetings of over 30 undergraduate biology majors and organized events committed to service, like volunteer days at Hosea House and Ronald McDonald House.
  • Elected Secretary, Aug 2012 – May 2013
    o Maintained and recorded all meeting notes and attendance records from bimonthly meetings.
Chemistry Club, Thomas More College, Crestview Hills, KY
Aug 2010 – May 2014
• Elected President, Aug 2012 – May 2014
  o Ran monthly meetings of undergraduate chemistry majors and coordinated student volunteers to serve as judges during science fairs at local elementary schools.

Math and Physics Club, Thomas More College, Crestview Hills, KY
Aug 2010 – May 2014
• Volunteered at astronomy nights to educate the public about space and participated in group events, including a COSI museum trip.

American Cancer Society, Thomas More College, Crestview Hills, KY
Apr 2011 – Apr 2014
• Volunteered on the committee that planned the Relay for Life overnight fundraiser walk on the Thomas More College campus.
• Team captain of “The Science Team” in Apr 2013 and Apr 2014.

Susan G. Komen Foundation, Cincinnati, OH
Sep 2009 – Sep 2013
• Volunteered to setup for the annual Race for the Cure and also participated in 5K walk/run fundraisers in honor of family friends.

STEM Summer Camp, Thomas More College, Crestview Hills, KY
Jul 2012 & Jul 2013
• Mentored high school students as part of a week-long STEM summer camp, which entailed guiding students between daily activities and supervising them overnight.
• Assisted students in the collection of Ohio River water samples and analysis of the water using ICP-AES.

St. Elizabeth Hospital Volunteer Program, Florence, KY and Edgewood, KY
• Volunteered over 200 hours per year between the pharmacy, emergency room, coumadin clinic, and same day surgery unit.
AWARDS AND HONORS

Graduate Dean’s Citation Award  May 2019
John Richard Binford Memorial Award  May 2019
Doctoral Dissertation Completion Award  Jan 2019 – May 2019
Outstanding Contribution in Reviewing  Oct 2016
Graduate Student Council Outstanding Service Award  May 2015 – May 2016
Chemistry Department Outstanding Senior Award  May 2014
Dean’s Honor List  Aug 2013 – May 2014
Outstanding Service as Club President  Aug 2013 – May 2014
Thomas More Leadership Roundtables  Sep 2010 – May 2014
Dean’s Award of Excellence in Science (1st Place)  Mar 2014
Distinguished Leadership and Service Award  Aug 2012 – May 2013
Outstanding Service as Club President  Aug 2012 – May 2013
Dean’s Honor List  Aug 2010 – Dec 2010

GRANTS AND SCHOLARSHIPS

Graduate Teaching Assistantship  Aug 2014 – Dec 2018
Chemistry Departmental Travel Grant  Mar 2018
Graduate Network in Arts and Sciences Travel Grant  Mar 2018
Graduate Student Council Travel Grant  Mar 2018
Arts and Sciences Creative Research Grant  Jun 2016 – Jun 2017
Chemistry Departmental Travel Grant  Mar 2017
Graduate Network in Arts and Sciences Travel Grant  Mar 2017
Graduate Student Council Travel Grant  Mar 2017
Charles B’ Hymer Scholarship  Aug 2013 – May 2014
Chemistry Departmental Scholarship  Aug 2010 – May 2014
Women Leading Kentucky Scholarship  Aug 2012 – May 2013
Zembrodt Chemistry Scholarship  Aug 2012 – May 2013

PROFESSIONAL AFFILIATIONS

American Chemical Society Member  Jan 2018 – present
Golden Key International Honor Society Member  Nov 2016 – present
Kentucky Academy of Science Member  Mar 2015 – present
Electrochemical Society Member  Nov 2014 – present
Tri-Beta Biological Honors Society Member  Apr 2011 – present
National German Honors Society Member  May 2010 – present