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Bridging Inductively Coupled Plasma - Mass Spectrometry (ICP-MS)
from Metalloproteomics to the Undergraduate Curriculum

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Abstract

Inductively Coupled Plasma-Mass Spectrometry (ICP-MS) has been a cornerstone of elemental analysis for the past three decades. The field of metallomics has emerged as the study of metals and their use for growth and survival within biological organisms.\textsuperscript{1-3} More recently, the field of metalloproteomics has harnessed the power of separation techniques coupled to metal detection for the study of metal transport, storage, and use by proteins, and this has expanded protein studies considerably.\textsuperscript{2-3} The process of lysing cells to analyze metalloproteins requires examination of current cell lysis techniques and the development of new methods in order to retain non-covalently bound metals and this remains a major challenge.\textsuperscript{4} In \textbf{Chapter 2}, various cell lysis techniques were investigated in an effort to analyze the metalloproteome of dimorphic fungus \textit{Histoplasma capsulatum}, expanding knowledge of microbial metalloproteomes which are largely uncharacterized.\textsuperscript{5} Specifically, zinc metalloproteins were of greatest interest as zinc plays a critical role in immune response.\textsuperscript{6,7} The work of \textbf{Chapter 2} was conducted with the assistance of three undergraduate researchers, Stephanie Lewis, Taylor Tocash, and Sami Abraham. As part of their undergraduate research experience, they developed materials for sample preparation, instrumental use, and data analysis for High Performance Liquid Chromatography (HPLC) and ICP-MS and they assisted in experimental design. These materials have been used by graduate students and undergraduates in research labs and at the undergraduate teaching lab at the University of Cincinnati. In addition, these materials provided the foundation for the development of the undergraduate laboratory experiment in \textbf{Chapter 3} in which students determined the concentration of arsenic in commercial sinus wash and tap water using two modes of ICP-MS and compared the results to federal regulations. The addition of the ICP-MS experiment to the CHEM 3030L Instrumental Analysis Laboratory course was one component of a larger course redesign which included the revision and addition of laboratory experiments as well as efforts to address course learning outcomes and improve students’ laboratory report writing skills. A Scientific Reasoning Tool (SRT) was developed based on McNeill’s Claim Evidence Reasoning Model as a way to scaffold the
process of organizing information before writing a laboratory report as described in **Chapter 4**. To determine the impact of the SRT on student laboratory reports, the ICP-MS experiment laboratory reports were independently analyzed, student surveys were administered, and instructor interviews were conducted. This work seeks to demonstrate how the use of ICP-MS in research laboratories can be accessible to undergraduate researchers as well as undergraduate students through the design of new experiments. The exposure to modern, complex instrumentation such as ICP-MS is not yet common at colleges and universities and presents an opportunity for integration in the curriculum to prepare students for careers in Science Technology Engineering and Math (STEM) fields.
Acknowledgements and Dedications

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came as a surprise to so many of us and I am so glad I had the opportunity to work with you, even if it was only for a few years.

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…

You are a child of the universe,

no less than the trees and the stars;

you have a right to be here.

And whether or not it is clear to you,

no doubt the universe is unfolding as it should.

Therefore be at peace with God,

whatever you conceive Him to be,

and whatever your labors and aspirations,

in the noisy confusion of life keep peace with your soul.

With all its sham, drudgery, and broken dreams,

it is still a beautiful world.

Be cheerful.

Strive to be happy.

-Max Ehrmann, Desiderata, Copyright 1952
Contents

Abstract.............................................................................................................................................................. ii

Acknowledgements and Dedications .................................................................................................................. v

List of Tables ................................................................................................................................................... xii

List of Figures ................................................................................................................................................ xiii

Chapter 1: Introduction ....................................................................................................................................... 14

  1.1 Introduction to Research ........................................................................................................................ 14

  1.2 Inductively Coupled Plasma – Mass Spectrometry ............................................................................... 14

    1.2.1 Sample Introduction ......................................................................................................................... 15

    1.2.2 Ionization ........................................................................................................................................ 16

    1.2.3 Plasma Interface/Ion Focusing ......................................................................................................... 17

    1.2.4 Interference Removal ....................................................................................................................... 17

    1.2.5 Mass Filtering/Detection ................................................................................................................. 18

    1.2.6 Instrument Capabilities Agilent 7700 and Agilent 8800 ................................................................. 18

  1.3 Research Overview .................................................................................................................................. 20

Chapter 2: Development of Cell Lysis Methods for Metalloproteomics Analysis, a Study of Histoplasma capsulatum .............................................................................................................................................. 22

  2.1 Introduction .............................................................................................................................................. 22

  2.2 Materials and Methods ............................................................................................................................ 26

    2.2.1 Reagents ......................................................................................................................................... 26

    2.2.2 Instrumentation ............................................................................................................................... 27

    2.2.3 Preparation of Histoplasma capsulatum ....................................................................................... 27
Chapter 3: Determination of Arsenic in Sinus Wash and Tap Water by Inductively Coupled Plasma – Mass Spectrometry, An Instrumental Analysis Laboratory Experiment .............................................................. 47

3.1 Introduction ....................................................................................................................................... 47

3.2 Experimental ..................................................................................................................................... 50

3.3 Hazards ............................................................................................................................................. 51

3.4 Discussion ......................................................................................................................................... 51

3.5 Conclusion ........................................................................................................................................ 57

Chapter 4: Impact of a Scientific Reasoning Tool on ICP-MS Laboratory Reports in an Instrumental Analysis Laboratory Course ....................................................................................................................... 59

4.1 Introduction ....................................................................................................................................... 59

4.1.1 CHEM 3030L Instrumental Analysis Course Redesign ............................................................ 60

4.1.2 Development of Scientific Reasoning Tool ............................................................................... 64

4.2 Materials and Methods ...................................................................................................................... 66

4.2.1 Experiment Design ..................................................................................................................... 66

4.2.2 Materials .................................................................................................................................... 68

4.3 Results and Discussion ..................................................................................................................... 79
List of Tables

Table 2.1 Comparison of total protein concentration in cell lysates by various cell lysis methods using Qubit® assay........................................................................................................................................32

Table 2.2 Comparison of zinc in cell lysates by various cell lysis methods..................................................34

Table 3.1 Example of student generated results for the concentration of arsenic in Sinus Wash and Tap Water..................................................................................................................................................56

Table 4.1 Course Learning Outcomes for CHEM 3030L as of Fall 2016......................................................63

Table 4.2 McNeill’s definitions of Claim, Evidence, and Reasoning.................................................................65

Table 4.3 Results from the SRT Impact Rubric..................................................................................................86
List of Figures

Figure 1.1 Schematic of the Agilent 7700.................................................................16

Figure 1.2 Schematic of the Agilent 8800.................................................................19

Figure 2.1 Graphical abstract for the Development of Cell Lysis Methods for Metalloproteomics Analysis, a Study of *Histoplasma capsulatum*.................................................................22

Figure 2.2 Comparison of $^{66}$Zn SEC-HPLC-ICP-MS chromatograms using a) Glass Homogenizer b) Bead Beater c) Sonication Probe.................................................................36

Figure 2.3 Comparison of Mn, Co, Cu, and Zn SEC-HPLC-ICP-MS signal using a Sonication Probe and lysis buffer with 1% Triton x-100.................................................................38

Figure 2.4 Comparison of Zn SEC-HPLC-ICP-MS chromatograms using a) Vortex, and Lysis buffer with 1% Triton x-100 b) Sonication Bath, Vortex with 1% Triton x-100 c) Vortex, and no detergent d) Sonication Bath, Vortex, and no detergent.................................................................39

Figure 2.5 Comparison of distribution of molecular weight (MW) bound zinc among various cell lysis methods.................................................................42

Figure 2.6 Comparison of Fe, Cu, and Zn SEC-HPLC-ICP-MS chromatograms using the Vortex with 1% Triton x-100 method (V, T).................................................................43

Figure 3.1 Graphical abstract for Determination of Arsenic in Sinus Wash and Tap Water by Inductively Coupled Plasma – Mass Spectrometry, An Instrumental Analysis Laboratory Experiment.................47

Figure 3.2 Example of student generated arsenic calibration curve using germanium internal standard for no gas mode and He mode.................................................................53

Figure 3.3 Example of student generated Internal Standard Recovery Plot.................................................................55
Figure 4.1 Graphical abstract for the Impact of a Scientific Reasoning Tool on ICP-MS Laboratory Reports in an Instrumental Analysis Laboratory Course……………………………………………………………………59

Figure 4.2 Scientific Reasoning Tool (SRT)………………………………………………………………69

Figure 4.3 Scientific Reasoning Tool with directions………………………………………………70

Figure 4.4 Scientific Reasoning Tool with example experiment and data…………………………71

Figure 4.5 Laboratory report rubric for instrumental laboratory experiments in CHEM 3030L……73

Figure 4.6 SRT Impact Rubric. Rubric to assess impact of the Scientific Reasoning Tool (SRT) on the ICP-MS laboratory report……………………………………………………………………………………………75

Figure 4.7 Student surveys for sections that did not use the SRT and section that did use the SRT……77

Figure 4.8 CHEM 3030L Faculty Interview Questions……………………………………………………78

Figure 4.9 Comparison of SRT Impact Rubric Criteria Results for reports with the SRT and without the SRT…………………………………………………………………………………………………………………………84

Figure 4.10 Student survey results from January and March 2016 comparing sections that used the SRT and did not use the SRT………………………………………………………………………………………………88
Chapter 1: Introduction

1.1 Introduction to Research

This dissertation seeks to demonstrate how the use of Inductively Coupled Plasma-Mass Spectrometry (ICP-MS) in research laboratories can be accessible to undergraduate students through research as well as coursework via laboratory experiments. Innovations in chemical instrumentation change the nature of scientific research and production of consumer goods. As such, colleges and universities must adapt to prepare students for their careers in an ever-changing world. Increasing student exposure to complex, modern instrumentation through undergraduate research and the undergraduate curriculum are two modes that are well established in academia, but continue to be refined. Such exposure not only impacts future chemists, but also influences future chemical engineers, operations management, and more.

1.2 Inductively Coupled Plasma – Mass Spectrometry

ICP-MS has been the cornerstone of elemental analysis for three decades. High sensitivity, large dynamic range, and low limits of detection have propelled ICP-MS to a place of prominence for trace metal detection.\textsuperscript{4-10} A wide range of metals and non-metals can be selectively detected virtually simultaneously. The flexibility of ICP-MS allows for stand-alone operation for total metal analysis or coupling to a variety of separation techniques, including, but not limited to Capillary Electrophoresis (CE), Gas Chromatography (GC), and Liquid Chromatography (LC). ICP-MS is now an academic, government, and industry workhorse for a wide variety of applications in areas such as biological, environmental, and pharmaceutical analysis. In academia, primarily graduate students and those with advanced degrees operate the ICP-MS likely due to the cost, maintenance, and technical skill needed for troubleshooting. In government and industry,
research and development using ICP-MS is typically conducted by those with advanced degrees, but routine analysis may be conducted by those with high school, associates, and bachelor’s degrees especially as software and hardware become more accessible.

1.2.1 Sample Introduction

Solid, liquid, and gaseous samples can be analyzed by ICP-MS, each requiring a different sample introduction. Liquid sampling is the most common and the work of this dissertation will focus on introduction of liquid samples using an auto-sampler as well as High Performance Liquid Chromatography (HPLC). The main purpose of the sample introduction system is to maintain a robust plasma throughout the analysis by diverting most of the sample to waste and limiting the amount of sample that enters the instrument.

For total metal analysis, the amount of sample that is delivered to the sample introduction system is dependent on the peristaltic pump. Sensitivity and plasma stability are dependent on the sample uptake rate and typical sample uptake rate is 0.4 mL/min as not to overload the plasma. The type of nebulizer used depends on the sample being analyzed, but the most common nebulizer is the concentric nebulizer which consists of a sample capillary sheathed in argon with a flow rate of 1 mL/min. The sample exits the tip of the nebulizer creating an aerosol in the spray chamber. The Scott double pass spray chamber is most common in which smaller droplets (<10 µm) pass through the spray chamber and larger droplets condense in the cooled spray chamber and pass through to the waste as shown in Figure 1.1.
1.2.2 Ionization

Once the small droplets pass through the spray chamber, the aerosol enters the torch where desolvation, vaporization, atomization, and ionization take place. The quartz torch consists of three concentric tubes which carry the aerosol, auxiliary gas, and plasma gas. The end of the torch is located within a cooled copper coil which carries a high electrical current created by a radio frequency (RF) generator at 1500W. The strong magnetic field created causes free electrons and argon atoms to collide which results in argon ions and electrons. Collisions continue until a stable plasma is formed with a temperature up to 10,000 K. The sample atoms are ionized by the plasma by removing an electron. Ionization efficiency is dependent on the first ionization potential of an element as well as the plasma temperature and electron density.

Figure 1.1 Schematic of the Agilent 7700.15
1.2.3 Plasma Interface/Ion Focusing

The sample ions created in the torch are at atmospheric pressure and pass through an interface into the ion focusing chamber. The interface is composed of a large metallic sampler cone which is typically made of copper with a nickel center containing a 1 mm diameter hole. A smaller skimmer cone, typically made of nickel, rests directly behind the sampler cone and has a 0.4 mm diameter hole. The sample and skimmer cones serve to extract the richest part of the ion packet. The ion packet is further focused through the ion lenses which are a set of electrostatic plates that use applied voltages to focus the ion packet into a tight ion beam. In the Agilent 7700 and Agilent 8800 used in this dissertation, the ion lenses are off-axis, and therefore, do not allow photons and neutral species to pass through to the high vacuum region. The plasma interface and ion focusing chamber allow for high ion transmission over a wide mass range to the high vacuum region.

1.2.4 Interference Removal

Removing spectral interferences is essential to the accuracy of the analysis. In the Agilent 7700 and the 8800 mass spectrometers, this takes place in the collision reaction cell (CRC) which consists of an octopole ion guide pressurized with a gas. Typical gasses for the CRC include hydrogen, helium, and oxygen, depending on the application. As a collision cell, helium collides with polyatomic interferences which lose energy with each collision. The polyatomic interferences are larger and collide more often with helium as compared to the analyte, resulting in a greater loss of energy. This difference in kinetic energy allows for kinetic energy discrimination created by the exit lens of the CRC which has a negative potential bias relative to the entrance optics voltage of the quadrupole. Only ions that have sufficient kinetic energy can pass through the barrier. As a reaction cell, hydrogen or oxygen is used to react with analytes causing a mass
shift. The use of the CRC as a collision cell as well as a reaction cell and the difference between the Agilent 7700 and Agilent 8800 will be further discussed.

1.2.5 Mass Filtering/Detection

As only one analyte can be detected at a time by the electron multiplier (EM), mass filtering must take place prior to detection. In the Agilent ICP-MS models used in this dissertation, mass filtering is performed by a quadrupole mass analyzer in the high-pressure chamber; species are separated based on mass-to-charge ratios at approximately \(10^{-6}\) torr. Two pairs of cylindrical rods make up the quadrupole where varying alternating current (AC) and direct current (DC) voltages are applied. Ions in a certain mass range can pass through to the detector while other ions are lost through an unstable trajectory. While only one analyte can be detected at a time by the EM, ions from 2 to 260 amu can be scanned in a very short time resulting in nearly simultaneous detection. The EM provides low background noise, high sensitivity, and a wide linear dynamic range contributing to the power and flexibility of ICP-MS. The analyte signal is directly proportional to the amount of sample introduced, producing a spectrum which can be used for quantitative analysis.

1.2.6 Instrument Capabilities Agilent 7700 and Agilent 8800

The evolution of ICP-MS over the past decade has included instrumentation with single and multiple quadrupole mass analyzers. The introduction of the CRC and multiple quadrupoles in the ICP-MS has expanded the range of analytes and lowered the detection limits in complex matrices. Analysis of sulfur and phosphorus have resulted in novel applications, particularly for biological and pharmaceutical research. This dissertation research focuses on interference removal using helium in the CRC as a collision gas. The
The main difference between the Agilent 7700 and Agilent 8800 is the placement of the CRC and the number of quadrupole mass analyzers. The Agilent 7700 employs a single quadrupole which is placed after the CRC as seen in Figure 1.1. The Agilent 8800 employs two quadrupoles as seen in Figure 1.2, one between the ion lenses and the CRC and the other between the CRC and EM detector. The additional quadrupole allows for selective filtering of a certain mass-to-charge, such as 32 for sulfur detection, in the first quadrupole. Dioxygen (O$_2^+$) at $m/z$ 32 is a major interference for the major isotope of sulfur ($^{32}$S$^+$) and also passes through the first quadrupole. In the CRC, oxygen is introduced as a reaction gas, producing $^{32}$S$^{16}$O$^+$ with a $m/z$ of 48. The second quadrupole can be used to selectively filter $m/z$ 48, thereby reducing interferences significantly. In this dissertation, the CRC was used in collision mode with helium gas in the Agilent 8800 which does not have a dramatic detection difference compared to the Agilent 7700 but demonstrates flexibility of the instrument. For example, detection of $^{66}$Zn in the Agilent 7700 involves sample ions and interferences colliding with helium in the CRC followed by selective filtering of $m/z$ 66 in the quadrupole. Using the Agilent 8800, ions with a $m/z$ of 66 are selectively filtered through the first quadrupole, collide with helium in the CRC followed by selective filtering of $m/z$ 66 in the second quadrupole.

Figure 1.2 Schematic of the Agilent 8800.
1.3 Research Overview

This dissertation demonstrates how the use of ICP-MS can be accessible to undergraduate students through research as well as through laboratory experiments as part of the curriculum. Historically, ICP-MS has been largely absent from the undergraduate experience, likely due to the high cost, maintenance requirements, and complexity of operation. While these factors are true concerns, there are several ways to incorporate ICP-MS into the undergraduate curriculum that address such factors in a way that is flexible and resourceful. Opportunities for undergraduate research using ICP-MS over a semester or a year allows for a small number of undergraduates to become familiar with ICP-MS and engage in novel scientific research. Incorporation of ICP-MS in the undergraduate curriculum allows for a larger number of students to become familiar with ICP-MS, but does not allow for the same amount of depth as undergraduate research. Modern textbooks used for analytical chemistry lecture courses include ICP-MS \(^\text{17-19}\) but there are few resources for incorporation of ICP-MS into laboratory courses. The addition of new laboratory experiments in a course should warrant a close examination of the course design and student performance as it is a natural point in time to make changes to the overall structure of the course. This dissertation describes such a process in the Department of Chemistry at the University of Cincinnati.

This first part of this work involves the development of a suitable cell lysis method for metalloproteomics studies of \textit{Histoplasma capsulatum} as described in Chapter 2. Seven cell lysis methods were compared including chemical and mechanical techniques as well as combinations of techniques. Considerations were made to maximize the preservation of metalloproteins such as the choice of cell lysis buffer components and separation technique. Cell lysis methods were evaluated using a combination of ICP-MS, SEC-HPLC-ICP-MS, and the Qubit® assay. This work was conducted with the assistance of undergraduate researchers who played an active role in the creation of materials used to conduct research as well as experiment design.
using ICP-MS. Currently, this work has been submitted to *Analytical and Bioanalytical Chemistry* and is under review.

The second part of this work involves the development of an undergraduate laboratory experiment for the determination of arsenic in sinus wash and tap water by ICP-MS as described in **Chapter 3**. This experiment used internal standard calibration and demonstrated matrix effects, specifically the high salt matrix of sinus wash. Specific interferences and reduction of such interferences using the CRC in no gas mode and helium mode was a major component of the experiment. Similar to other laboratory experiments, the students were also required to describe the content in the context of instrument applications and the significance of their results. This experiment is the first ICP-MS undergraduate chemistry laboratory experiment ever published. It was published in the *Journal of Chemical Education*.20

The third part of this work, described in **Chapter 4**, involves evaluating the creation, implementation, and impact of the Scientific Reasoning Tool (SRT) on the ICP-MS laboratory reports in an Instrumental Analysis Laboratory course using the experiment described in the second part of this work. The SRT was developed to support students in writing laboratory reports and achieving course learning outcomes, particularly scientific argumentation skills. The creation of the undergraduate ICP-MS laboratory experiment as well as the SRT were part of a larger course redesign of the Instrumental Analysis Laboratory Course, CHEM 3030L, at the University of Cincinnati. This course redesign is ongoing and the results of the SRT study will be published in the next few years.
Chapter 2: Development of Cell Lysis Methods for Metalloproteomics Analysis, a Study of *Histoplasma capsulatum*

**Figure 2.1** Graphical abstract for the Development of Cell Lysis Methods for Metalloproteomics Analysis, a Study of *Histoplasma capsulatum*.

### 2.1 Introduction

The field of metallomics has emerged over the past few decades as the study of metals and their use for growth and survival within biological organisms.\(^1\)-\(^3\) Many times, metals are associated with a given protein and it is estimated that one third of all proteins contain a metal.\(^2\) Within metallomics, the study of metal transport, storage, and use by proteins, known as metalloproteomics, has expanded protein studies considerably.\(^2\)-\(^3\) Investigations range from pure inquiry into the metalloproteome as a whole or targeting a specific metal, as the metal may be used by enzymes as cofactors to catalyze reactions, the metal may be
necessary for protein function, or the metal may play key structural roles within a protein.\textsuperscript{22-25} Other studies probe metal-protein binding as an assessment of environmental exposure\textsuperscript{26-28} or disease pathogenesis.\textsuperscript{29,30} Still others seek to elucidate the mechanism of metallodrugs and their fate within cells.\textsuperscript{31,32}

Most microbial metalloproteomes are, as of yet, largely uncharacterized.\textsuperscript{33} \textit{H. capsulatum}, in particular, is a dimorphic fungus and causes a respiratory infection known as Histoplasmosis which may develop into a progressive infection, especially for immunocompromised individuals.\textsuperscript{34} One of the first lines of defense against \textit{H. capsulatum} is macrophage (MØ), yet \textit{H. capsulatum} has the ability to avoid host immune defenses by replicating within the MØ.\textsuperscript{35} One of the body’s responses to this infection, using the \textit{H. capsulatum} strain G217B, is the simultaneous sequestration of zinc and generation of Reactive Oxygen Species (ROS) through NADPH within MØ.\textsuperscript{35,36} This immune response indicates that the micronutrient zinc is essential to \textit{H. capsulatum}.

In some metalloproteomic studies, the element of interest is bound within the primary structure of the protein, such as selenoproteins in which selenium replaces the sulfur in cysteine amino acids. However, in the vast majority of cases, the metal ion is held within the protein structure by non-covalent forces.\textsuperscript{37} One of the greatest challenges in the experimental analysis of the metalloproteome of a given organism within the latter case is the development of an optimal cell lysis method.\textsuperscript{38} The strength of the fungal cell wall varies between species and poses and added challenge in choosing a cell lysis method as compared to cells with weaker membrane structures such as mammalian cells.\textsuperscript{39} When choosing a method for an organism that contain cell walls, the method must be strong to break the cell wall, allowing the release of the cytosolic proteins. At the same time, it cannot be so harsh that it denatures the protein as this leads to loss of the associated metal.\textsuperscript{37,40} As a result, established physical and chemical cell lysis techniques need to be carefully
evaluated to determine if they can be used in such studies. Klimek-Ochab showed fungal yeast may be better suited for extraction with glass beads, but few fungal species have been studied in depth.

Several cell lysis techniques have been developed over the years to disrupt cells and the majority of them can be classified as chemical or mechanical. Chemical cell lysis techniques often involve detergents, enzymes, and/or salts. Detergents are commonly used for cell lysis techniques to study proteins. Detergents contain hydrophobic tails and hydrophilic heads, allowing for the disruption of the hydrophobic-hydrophilic interface of biological membranes. Non-ionic detergents, such as Triton x-100, tend to be gentler and generally do not disrupt the native structure of proteins. Other non-ionic detergents may be suitable for metalloproteomic studies such as Triton x-114, Brij-35, Brij-58, Tween 20, Tween 80, Octyl glucoside, and Octyl thioglucoside. Salts are used in cell lysis buffers to maintain the pH and osmolarity of the cell lysate. Salts, such ethylenediaminetetraacetic acid (EDTA) disodium salt, are not suitable for metalloproteomic experiments due to metal binding properties. Buffer agents such as Tris(hydroxymethyl)aminomethane hydrochloride (Tris-HCl) and 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES) are also often used in cell lysis solutions. The NP-40 lysis buffer, which can be used with the detergent Triton x-100, is fairly mild and is common for whole cell or soluble cytosolic proteins and was deemed suitable for this study. Several mechanical examples include bead beater, homogenizer, french press, and freeze-thaw. Bead beaters use the mechanical shear of small glass beads while homogenizers use the shear created by a ground glass tight fitting mortar and pestle. French press uses shear and decompression by forcing the sample through a small orifice under high pressure. Freeze-thaw involves rapid cycling of sample temperature to disrupt cells through ice crystal formation. Both the chemical and mechanical cell lysis methods described were originally designed for the study of proteins without consideration of metal-binding and they may not be suitable for metalloproteomics experiments. Further, commercial cell lysis products contain proprietary formulas that may produce denaturing conditions and/or metal contamination.
Therefore, it is essential to assess cell lysis methods and optimize protocols to preserve the metalloproteome.

In addition to preservation of the metalloproteome, the chosen cell lysis technique needs to be compatible with the analytical scheme. As noted by Hagege et. al., losses of metal-protein complexes must be minimized by the separation technique.\textsuperscript{42} Size Exclusion Chromatography (SEC) is routinely used to roughly separate metalloproteins by hydrodynamic radius.\textsuperscript{28,32} While not a particularly powerful separation technique, it can be carried out under non-denaturing conditions, although there may be components in the lysis buffer that interfere with the analysis such as certain detergents that absorb in the ultraviolet region when using UV detection. Further, SEC typically offers complete recovery of all species due to the absence of secondary separation principles such as absorption onto the stationary phase.\textsuperscript{43} A common second dimension of separation is Ion Exchange Chromatography (IEX). Lysis buffer components that are charged, such as the anionic detergent SDS, can interfere with this separation and should therefore be removed from all steps of sample preparation. For both separation procedures, Inductively Coupled Plasma-Mass Spectrometry (ICP-MS) is commonly employed for detection due to its multi-element capability and low detection limits.\textsuperscript{28,32}

In this work, several cell lysis techniques and two lysis buffers were investigated to evaluate the preservation of the zinc metalloproteome of \textit{H. capsulatum} while maintaining compatibility with the analytical techniques employed. Investigation of the \textit{H. capsulatum} zinc metalloproteome will expand knowledge of zinc pathways and the role of zinc in the survival of the fungus. Further characterization and quantification of the zinc metalloproteome could be used to assess changes to the fungus under zinc stress, mimicking the immune response. The cell lysis methods investigated were evaluated based the total protein
concentration, the total concentration of metal, the molecular weight distribution of the metal, and additional factors pertinent to sample preparation.

2.2 Materials and Methods

2.2.1 Reagents

All solutions were prepared using double de-ionized (DDI) water (18 Ω cm-1, produced by the Nanopure treatment system purchased from Sybron Barnstead, Boston, MA, USA). Labware was acid washed with 10% nitric acid (Fisher Scientific, Fair Lawn, New Jersey, USA) to remove metals. Cells were washed with Phosphate-Buffered Saline (PBS) without added calcium and magnesium (Mediatech, Inc. A Corning Subsidiary, Manassas, VA, USA). Lysis buffer components included sodium chloride (Fisher Scientific, Fair Lawn, New Jersey, USA), Triton x-100 (Sigma-Aldrich, St. Louis, MO, USA), tris(hydroxymethyl)aminomethane (Tris) (Acros Organics, Morris Plains, NJ, USA), and Mini EDTA-free Protease Inhibitor Cocktail Tablets (Roche Life Science, Indianapolis, IN, USA). The mobile phase for SEC contained 50 mM ammonium acetate (Fisher Scientific, Fair Lawn, New Jersey, USA) and 0.5 % methanol (Fisher Scientific, Fair Lawn, New Jersey, USA) in DDI water, adjusted to pH 7.4 using ammonium hydroxide (Fisher Scientific, Fair Lawn, New Jersey, USA). The Gel Filtration Standard (Bio-Rad Laboratories, Inc., Hercules, CA, USA) used for SEC was a lyophilized mixture of molecular weight markers at 1.35kDa, 17kDa, 44kDa, 158kDa, and 670kDa which was dissolved in DDI water. Total protein concentration was determined using a Qubit® Protein Assay Kit (Thermo Fisher Scientific, Waltham, MA, USA).
2.2.2 Instrumentation

Trace elemental analysis was conducted using an Agilent 8800 ICP-MS (Agilent Technologies, Santa Clara, CA, USA). The sample introduction system for the ICP-MS included a MicroMist nebulizer (Glass Expansion, Pocasset, MA), a Scott double-channel spray chamber (2 °C), and a shield torch. The ICP-MS was used in collision mode with helium gas at 3.0 mL/min to reduce isobaric interferences. Isotopes monitored included $^{55}$Mn, $^{56}$Fe, $^{59}$Co, $^{63}$Cu, and $^{66}$Zn. For total metal analysis, an Agilent ASX-500 Autosampler was employed and $^{45}$Sc was used as internal standards. For cell lysate separation, an Agilent 1100 HPLC (Agilent Technologies, Santa Clara, CA, USA) with a Zorbax GF-250 column (Agilent Technologies, Santa Clara, CA, USA) was used. The HPLC was equipped with a vacuum membrane degasser system, binary pump, cooled autosampler, a temperature controlled column compartment (held at 25 °C), and a diode array detector where the UV absorbance was recorded at 280nm. The integrated area of the Gel Filtration Standard was used to normalize the ICP-MS signal from day to day. The HPLC was coupled to the ICP-MS allowing for nearly simultaneous UV detection and metal detection. Total protein concentration was determined using a Qubit® 2.0 Fluorometer (Thermo Fisher Scientific, Waltham, MA, USA).

2.2.3 Preparation of *Histoplasma capsulatum*

*H. capsulatum* yeast (strain G217B) were prepared by inoculating 25 ml of Ham’s F-12 medium set at pH 7.5, as previously described, with $3 \times 10^6$ cells/ml from five day old slants. After 30 hours, samples were centrifuged for five minutes at 1500 g, the supernatant was removed, and the cells were rinsed with PBS, centrifuged again, and the supernatant was removed.
2.2.4 Cell Lysis Techniques

For each cell lysis technique, the yeast cell count was used and the ratio of yeast cells to volume of lysis buffer was held constant in order to normalize data and compare chromatographic areas.

Glass Homogenizer:

Glass homogenizers are primarily used for tissue homogenization, but have occasionally been used with yeast cells.45,47 Due to the operator dependence and lack of reproducibility, it was expected that this method would not extract the greatest amount of protein or metal and was investigated as a comparison to other methods better suited for yeast cell lysis.45 A 15 mL glass Glass Homogenizer (H) with tight fitting pestle was used to grind $3 \times 10^8$ yeast cells and 0.5mL of lysis buffer (1% Triton x-100, 50mM Tris, 100mM NaCl, EDTA-free protease inhibitor) with 10 even rotations. The mixture was transferred to a 1.5 mL plastic vial, and chilled on ice for five minutes. The homogenate was centrifuged for five minutes at 12,000 g and the supernatant transferred to an HPLC vial.

Bead Beater:

Bead beaters have been used extensively to disrupt microorganisms using small glass beads with several examples involving yeast.47,50 A BioSpec 3110 BX Mini-BeadBeater (BB) (BioSpec, Bartlesville, OK) was used with 500 µm diameter glass beads and 2 mL screw cap vials with o-ring seals with straight walls and a sharp conical bottom. The vials were filled with 0.5 mL of lysis buffer (1% Triton x-100, 50mM Tris, 100mM NaCl, EDTA-free protease inhibitor), $3 \times 10^8$ yeast cells, and nitric acid-washed 500 µm glass beads to the brim of the vial. The Bead Beater was operated at 4800 rpm for two minutes, the vial was removed and put on ice for two minutes, and the cycle was repeated with two minutes on the BeadBeater,
two minutes on ice, and finally one minute on the BeadBeater and one minute on ice. The homogenate was removed and centrifuged for five minutes at 12,000 g and the supernatant transferred to an HPLC vial.

Sonication Probe:

Cell lysis by sonication probe has been used in yeast using short pulse times to limit heat generation. The Sonication Probe method was based on the work of Neppiras et. al. A Q125 Qsonica Sonicator (Qsonica, Newtown, CT) equipped with a 1/8” diameter probe (SP) was operated at an amplitude of 20% of 65W. A total of 3.0x10^8 yeast cells were combined with 0.5 mL of lysis buffer (1% Triton x-100, 50mM Tris, 100mM NaCl, EDTA-free protease inhibitor) in a 1.5 mL plastic vial that was suspended in an ice bath and subjected to a one second pulse followed by 10 seconds of rest and another one second pulse. The homogenate was centrifuged for five minutes at 12,000 g and the supernatant transferred to an HPLC vial.

Vortex with Glass Beads:

There are several examples of the vortex and glass bead cell lysis technique for protein extraction in yeast. The Vortex with Glass Beads method (V) involved one mL of 500 µm glass beads and 0.5 mL of lysis buffer combined with 3.0x10^8 yeast cells in a 1.5 mL plastic vial which was chilled at 4 ºC for 15 minutes. Two lysis buffers were evaluated: one included detergent (1% Triton x-100, 50mM Tris, 100mM NaCl, EDTA-free protease inhibitor) (T) and the other contained no detergent (50mM Tris, 100mM NaCl, EDTA-free protease inhibitor) (NT). The vials were vortxed for 30 seconds, placed on ice for 30 seconds and the cycle was repeated for a total of 10 minutes. The homogenate was removed and centrifuged for five minutes at 12,000g and the supernatant transferred to an HPLC vial.
Sonication Bath and Vortex with Glass Beads:

Sonication baths have been used in yeast to improve the reproducibility of protein extraction and was combined with the Vortex with Glass Bead method in this study to determine if it had a similar effect. The Sonication Bath and Vortex with Glass Beads method (SB,V) involved one mL of 500 µm glass beads and 0.5 mL of lysis buffer combined with $3.0 \times 10^8$ yeast cells in a 1.5mL vial which was chilled at 4 ºC in a refrigerator for 15 minutes. Again, two lysis buffers were evaluated, one containing detergent (T) and the other with no detergent (NT). A Sonication Bath was chilled with ice while the samples chilled in the refrigerator and the ice was removed prior to sonicating the samples. The vials were placed in the Sonication Bath for 10 minutes and placed on ice for two minutes. The vials were then vortexed for 30 seconds, placed on ice for 30 seconds and the cycle of vortexing and ice was repeated for a total of 10 minutes. The homogenate was removed, centrifuged for five minutes at 12,000 g, and the supernatant transferred to an HPLC vial.

2.3 Results and Discussion

In this study, mechanical and chemical lysis techniques were investigated as well as various combinations of techniques. For the chemical lysis techniques, the NP-40 lysis buffer using the non-ionic detergent Triton x-100 was chosen to limit protein denaturation. In addition, to assess the impact of the detergent, a lysis buffer containing the same ingredients and no detergent was explored. Several mechanical techniques were also employed. Seven lysis methods were considered and these included: Glass Homogenizer (H), Bead Beater (BB), Sonication Probe (SP), Vortex with 1% Triton x-100 (V, T), Vortex with no Triton x-100 (V, NT), Sonication Bath, Vortex, and 1% Triton x-100 (SB, V, T) and Sonication Bath, Vortex, and no Triton x-100 (SB, V, NT).
Total protein concentration provides a limited amount of information to determine the most appropriate cell lysis method because it does not take into account if the proteins contain metals nor if they are denatured. Conventional cell lysis methods employ assays such as Bradford to determine total protein concentration. However, for our work, one of the disadvantages of such an assay is the incompatibility with detergents such as Triton x-100. Other assays are compatible with detergents, but do require significant dilution such as the Qubit® assay which was used in this work. Results for the concentration of protein by Qubit® assay are shown in Table 2.1. The methods that resulted in the greatest extracted protein concentration include Sonication Probe (SP) 215.73 µg/mL, Vortex, Triton x-100 method (V, T) 197.08 µg/mL, and Sonication Bath, Vortex and 1% Triton x-100 (SB, V, T) 165.71 µg/mL. An ANOVA was used to determine difference between groups ($F(2,12)=5.9984, p=0.0156$). A Tukey HSD Post-hoc Test determined significant different between the Sonication Bath, Vortex, and 1% Triton x-100 method (SB, V, T) and the Sonication Probe method (SP) ($p=0.0129$) but there was not a significant difference between the Sonication Probe method (SP) and the Vortex, 1% Triton x-100 method (V, T) ($p=0.4335$) or Sonication Bath, Vortex, 1% Triton x-100 method (SB, V, T) and the Vortex, 1% Triton x-100 method (V, T) ($p=0.1214$). The 280nm signal collected using the UV detector of the HPLC might also be used for total protein comparison, as this wavelength is routinely used for protein concentration measurement, but it also presented a major challenge due to the Triton x-100 lysis buffer. Triton x-100 absorbs in the ultraviolet region of the spectrum leading to enhanced readings at this wavelength. Therefore, the UV signal was used to monitor the retention times of the protein standard mixture, but not used to compare protein concentration of lysate samples.
<table>
<thead>
<tr>
<th>Cell lysis method</th>
<th>Total protein concentration, Qubit® Assay</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Average protein concentration (µg/mL)</td>
</tr>
<tr>
<td>H</td>
<td>116.16</td>
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<tr>
<td>BB</td>
<td>145.71</td>
</tr>
<tr>
<td>SP</td>
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</tr>
<tr>
<td>V, T</td>
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</tr>
<tr>
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<tr>
<td>V, NT</td>
<td>145.31</td>
</tr>
<tr>
<td>SB, V, NT</td>
<td>137.68</td>
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</table>

**Table 2.1** Comparison of total protein concentration in cell lysates by various cell lysis methods using Qubit® assay. All data is normalized to 3.0x10^8 *H. capsulatum* yeast and an HPLC injection volume of 100uL. n=5. Glass Homogenizer (H), Bead Beater (BB), Sonication Probe (SP), Vortex with 1% Triton x-100, (V, T), Vortex with no Triton x-100 (V, NT), Sonication Bath, Vortex, and 1% Triton x-100 (SB, V, T) and Sonication Bath, Vortex, and no Triton x-100 (SB, V, NT).

Total metal analysis of the cell lysates was conducted by ICP-MS and is reported as ng Zn/100uL lysate in **Table 2.2**. The Vortex, Triton x-100 method (V, T) produced 18.51 ng Zn /100uL lysate, the Sonication Bath, Vortex, Triton x-100 method (SB, V, T) produced 13.95 ng Zn/ 100uL lysate, and the Sonication Probe method (SP) produced 10.92 ng Zn/100uL lysate which demonstrate the highest zinc concentrations. An ANOVA was used to determine difference between groups (*F*(2,12)=19.2197, *p*=0.00002). A Tukey
HSD Post-hoc Test determined significant different between the Vortex, and 1% Triton x-100 method (V, T) and the Sonication Probe method (SP) ($p=0.0001$) and the Sonication Bath, Vortex, and 1% Triton x-100 method (SB, V, T) ($p=0.0079$) but there was not a significant difference between the Sonication Probe method (SP) and the Sonication Bath, Vortex, and 1% Triton x-100 method (SB, V, T) ($p=0.0720$). However, this data is limited in value as it does not provide information regarding free and bound metals or molecular weight distribution.
<table>
<thead>
<tr>
<th>Cell lysis method</th>
<th>Total metal analysis, ICP-MS</th>
<th>Zn chromatogram, SEC-HPLC-ICP-MS</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Average ng Zn / 100 µL cell lysate</td>
<td>Standard Deviation</td>
</tr>
<tr>
<td>H</td>
<td>8.50</td>
<td>1.14</td>
</tr>
<tr>
<td>BB</td>
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<td>1.18</td>
</tr>
<tr>
<td>SP</td>
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<tr>
<td>V, T</td>
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<td>SB, V, T</td>
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<tr>
<td>V, NT</td>
<td>7.63</td>
<td>1.39</td>
</tr>
<tr>
<td>SB, V, NT</td>
<td>8.91</td>
<td>1.23</td>
</tr>
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</table>

Table 2.2 Comparison of zinc in cell lysates by various cell lysis methods. All data is normalized to 3.0x10⁸ H. capsulatum yeast and an HPLC injection volume of 100µL. n=5. Data in column 6 is the ratio of ng of Zn in a 100 µL volume was divided by the µg of protein in a 100 µL volume as determined by Qubit®. Glass Homogenizer (H), Bead Beater (BB), Sonication Probe (SP), Vortex with 1% Triton x-100, (V, T), Vortex with no Triton x-100 (V, NT), Sonication Bath, Vortex, and 1% Triton x-100 (SB, V, T), and Sonication Bath, Vortex, and no Triton x-100 (SB, V, NT).

SEC-HPLC-ICP-MS was used for the chromatographic analysis of all lysis samples and the zinc signal was integrated using Origin Pro 9 (Origin Lab Corporation, Northampton, MA, USA) to compare the amount of bound zinc, indicating the strong possibility of zinc metalloproteins. Molecular weight markers from the
protein standard mixture are indicated by arrows. A zinc calibration curve was analyzed by SEC-HPLC-ICP-MS to calculate the concentration of Zn in each zinc chromatogram, corresponding to 100 µL of cell lysate injected, reported as ng Zn/100 µL cell lysate. The average and standard deviation of replicates were calculated for all experiments and results can be found in Table 2.2. These data were then used to calculate the ratio of the zinc concentration by the protein concentration determined by the Qubit® assay. Summarily, the ng of Zn in a 100 µL volume was divided by the µg of protein in a 100 µL volume and these results are shown in Table 2.2, column 6. Based on this combination of data, there may be a greater probability of zinc proteins using the Vortex and Triton x-100 method (V, T) and the Sonication Bath, Vortex, and 1% Triton x-100 method (SB, V, T), but molecular weight distribution was deemed necessary to compare cell lysis methods.

The first cell lysis technique investigated was a Glass Homogenizer (H) as it is common for tissue grinding, and in a way, mimics mortar and pestle action. However, with the small sample size of H. capsulatum yeast cells, the Glass Homogenizer was cumbersome and it was difficult to remove all of the cell lysate after grinding. Figure 2.2 shows the SEC-HPLC-ICP-MS signal intensity for 66Zn likely bound to proteins in the 1.35kD to 670kD molecular weight region extracted using the Glass Homogenizer (H) corresponding to 8.24 ng Zn/100 µL cell lysate as shown in Table 2.2.
Figure 2.2 Comparison of $^{65}$Zn SEC-HPLC-ICP-MS chromatograms using a) Glass Homogenizer (H) b) Bead Beater (BB) c) Sonication Probe (SP). Lysis buffer contained 1% Triton x-100. Each sample contained $3.0 \times 10^8$ *H. capsulatum* yeast. n=5. Arrows indicate molecular weight markers 670 kDa, 158 kDa, 44 kDa, 17 kDa, and 1.35 kDa.

A Bead Beater (BB) was used with the lysis buffer containing 1% Triton x-100 to take advantage of the mechanical shear of small glass beads in conjunction with the lysing power of the detergent. This technique seemed promising as metal contamination could be controlled through acid washing the glass beads and
the samples could be contained within individual vials for the duration of the lysis which limits sample transfer. This technique did result in $^{66}\text{Zn}$ peaks at a range of molecular weights as shown in Figure 2.2 b, but a surprisingly low amount of bound zinc, 7.66 ng Zn was extracted as shown in Table 2.2. While the concentrations of zinc by HPLC-SEC-ICP-MS in the cell lysates for the Glass Homogenizer (H) and the Bead Beater (BB) methods were similar as determined by a one-way ANOVA ($F(1,8)=0.3982, p=0.5456$), the chromatograms provide a comparison of molecular weight distribution. The Glass Homogenizer (H) zinc chromatogram, as shown in Figure 2.2 a has very little high molecular weight bound zinc (8.85 min to 11.13 min) as compared to the Bead Beater (BB) in Figure 2.2 b. This may indicate that the Bead Beater method (BB) preserves high molecular weight zinc proteins to a greater degree than the Glass Homogenizer method (H).

A Sonication Probe (SP) was used in combination with the 1% Triton x-100 lysis buffer. Initially, a longer pulse time was used (10 second pulse, 30 second rest, 10 second pulse) which resulted in a single large peak at 22 minutes, corresponding to approximately 1.35 kDa as shown in Figure 2.3. With a lack of mid and high low molecular weight proteins, it is highly unlikely that only low molecular weight proteins were extracted but rather, the metals dissociated from the proteins in the extraction process. This was supported by examining the metal signal of $^{55}\text{Mn}$, $^{59}\text{Co}$, and $^{63}\text{Cu}$ which have a similar pattern as shown in Figure 2.3. As a result, the pulse time was changed to one second followed by 10 seconds of rest and another one second pulse, which drastically reduced the amount of metalloprotein denaturation as seen in Figure 2.2. Short pulse times limited sample heating and the detergent did not lead to excessive foaming. A limited amount of high molecular weight bound zinc can be observed for the Sonication Probe (SP), which may indicate a certain amount of protein denaturation. The Sonication Probe method (SP) produced an integrated Zn concentration of 12.75 ng Zn/100 µL cell lysate.
Figure 2.3 Comparison of Mn, Co, Cu, and Zn SEC-HPLC-ICP-MS signal using a Sonication Probe (SP) and lysis buffer with 1% Triton x-100. Data was collected using a TOSOH TSKgel G3000SW column (Tosoh Bioscience, King of Prussia, PA, USA) with a flow rate of 0.5mL/min during method development. Samples were subjected to a 10 second pulse, 30 second rest, and a 10 second pulse. Arrows indicate molecular weight markers 670 kDa, 158 kDa, 44 kDa, 17 kDa, and 1.35 kDa.

A hybrid Bead Beater method was developed to maximize simultaneous sample preparation and to take advantage of cell-bead interactions which may increase lysis efficiency of the cell wall in *H. capsulatum*. A vortex was used to mechanically agitate the sample with a lysis buffer containing 1% Triton x-100 (V,T) and glass beads with a 500 µm diameter were compared to 5 mm diameter glass beads (data not shown). A variety of containers were tested including 10 mL glass digestion vials, 15mL plastic conical vials, 14 mL plastic round bottom capped test tubes, and 1.5 mL plastic conical vials (data not shown). The 500 µm diameter glass beads in 1.5mL plastic conical vials, referred to as the Vortex with 1% Triton x-100 method (V,T), produced the highest average integrated zinc concentration of 20.31 ng Zn/100 µL cell lysate and this was significantly higher than the Sonication Probe method (SP) as determined by one-way ANOVA ($F(1,8)=14.7872, p=0.0049$). The efficiency of this combination is likely due to the limited amount of
available volume and small bead size, causing an increase in the collisions of the glass beads with the *H. capsulatum* yeast.

**Figure 2.4** Comparison of Zn SEC-HPLC-ICP-MS chromatograms using a) Vortex, 1% Triton x-100 (V, T) b) Sonication Bath, Vortex with 1% Triton x-100 (SB, V, T) c) Vortex, and no detergent (V, NT) d) Sonication Bath, Vortex, and no detergent (SB, V, NT). Each sample contained $3.0 \times 10^8$ *H. capsulatum* yeast. n=5. Arrows indicate molecular weight markers 670 kDa, 158 kDa, 44 kDa, 17 kDa, and 1.35 kDa.
While the Sonication Probe (SP) technique may have been too harsh to keep the maximum amount of metalloproteins intact, a Sonication Bath is much gentler than a Sonication Probe. In an attempt to further improve the lysis efficiency, a Sonication Bath was employed prior to the vortex to take advantage of multiple mechanical techniques. Heating of the sample remained a concern so ice was added to the water in the Sonication Bath while the samples remained under refrigeration and the ice was removed before the samples were sonicated. The resulting cold water bath provided an environment where heat generated through sonication was absorbed by the water bath, limiting the amount of thermal protein denaturation. Results from the Sonication Bath, Vortex, Triton x-100 method (SB, V, T) are show in Figure 2.4 and Table 2.2 and demonstrate that despite employing a gentler sonication water bath to provide an additional mechanism, sonication by water bath does not provide an added advantage in the lysis of *H. capsulatum*. Although the total amount of Zn extracted determined by ICP-MS using the Vortex, Triton x-100 method (V, T) was significantly higher than the Sonication Bath, Vortex, Triton x-100 method, as determined by one-way ANOVA ($F(1,8)=12.6973$, $p=0.0074$), the results by HPLC-ICP-MS were not statistically different ($F(1,8)=4.9133$, $p=0.0575$).

To ensure that the use of Triton x-100 was more effective than no detergent, the two most promising techniques, Vortex and Sonication Bath then Vortex were employed without the addition of detergent (V, NT and SB, V, NT) and results are shown in Figure 2.4 c,d. Concentrations of Zn by HPLC-SEC-ICP-MS are comparable, as determined by a one-way ANOVA ($F(1,8)=1.8385$, $p=0.2122$), at 7.55 ng Zn/ 100 µL cell lysate and 8.69 ng Zn/ 100 µL cell lysate and, respectively, as shown in Table 2.2. While the zinc traces follow a similar pattern for each technique with and without detergent, using 1% Triton x-100 in the lysis buffer resulted in a significant increase in the amount of zinc extracted as compared to a simple salt buffer with no detergent using both techniques. The concentration of zinc by SEC-HPLC-ICP-MS for the Vortex, 1% Triton x-100 method (V, T) was significantly higher than the Vortex, no Triton method (V, NT) as determined by a one-way ANOVA ($F(1,8)=51.3928$, $p=0.0001$). Similarly, the concentration of zinc by
SEC-HPLC-ICP-MS for the Sonication Bath, Vortex, 1% Triton x-100 method (SB, V, T) was significantly higher than the Sonication Bath, Vortex, no Triton method (SB, V, NT) as determined by a one-way ANOVA ($F(1,8)=33.8942, p=0.0004$).

To further quantitatively probe the differences in molecular weight fractions produced by each of these techniques, specific regions in the zinc Size Exclusion Chromatograms were separately integrated. Three regions were examined and results are reported as a percentage of the total zinc extracted which was set to 100% and graphically presented in Figure 2.5. The Bead Beater method (BB) produced the largest percentage of low molecular weight zinc (15.8 min to 18.8 min) which may indicate slight denaturation of zinc metalloproteins. This may be a result of sample heating when using the Bead Beater (BB) in this application.
Figure 2.5 Comparison of distribution of molecular weight (MW) bound zinc among various cell lysis methods. High MW – 8.85 min to 11.13 min, Mid MW – 11.13 min to 15.8 min, Low MW – 15.8 min to 18.8 min. Glass Homogenizer (H), Bead Beater (BB), Sonication Probe (SP), Vortex with 1% Triton x-100, (V, T), Vortex with no Triton x-100 (V, NT), Sonication Bath, Vortex, and 1% Triton x-100 (SB, V, T), and Sonication Bath, Vortex, and no Triton x-100 (SB, V, NT).

Comparing total protein concentration by Qubit® assay, zinc concentration by total metal analysis using ICP-MS, zinc concentration by SEC-HPLC-ICP-MS, molecular weight distribution, and other contributing factors such as potential contamination sources, vortexing *H. capsulatum* yeast cells with 500 µm glass beads in a 1% Triton x-100 lysis buffer (V, T) delivered the most potential zinc metalloproteins. This method produced the greatest concentration of zinc in the cell lysate by ICP-MS and HPLC-SEC-ICP-MS as well as one of the highest concentrations of protein by Qubit® Assay. Further, the ratio of zinc to total protein was the highest among the seven cell lysis methods studied, suggesting the greatest probability of zinc proteins. The molecular weight distribution of zinc for the Vortex, 1% Triton method (V, T) suggests limited protein denaturation and the sample preparation allows for less sample handling and contamination.
Future studies will involve multi-dimensional separation and identification of metalloproteins in *H. capsulatum* and expansion to iron and copper proteins based on SEC-HPLC-ICP-MS results as shown in Figure 2.6. In addition, quantification of zinc metalloproteins in *H. capsulatum* grown under varying levels of zinc stress will provide insight into the response of the fungus during MO zinc sequestration.

![Figure 2.6](image)

**Figure 2.6** Comparison of Fe, Cu, and Zn SEC-HPLC-ICP-MS chromatograms using the Vortex with 1% Triton x-100 method (V, T). Each sample contained $3.0 \times 10^8$ *H. capsulatum* yeast. n=5. Arrows indicate molecular weight markers 670 kDa, 158 kDa, 44 kDa, 17 kDa, and 1.35 kDa.

### 2.4 Conclusion

In order to address common challenges within metalloproteomics analysis, designing the cell lysis method is an important step that influences the entire analysis. For organisms with additional challenges such as the strong cell walls of fungi, cell lysis methods must be assessed to optimize the amount of metalloprotein extracted. Comparing the total protein concentration by Qubit® protein assay provides a simple comparison
between cell lysis methods, but lacks information about protein-bound metals. Comparing the concentration of metal in cell lysates by total metal analysis using ICP-MS and the metal chromatogram by SEC-HPLC-ICP-MS allows for additional comparison between different cell lysis methods. Closer examination of the SEC metal chromatograms allows for comparison of molecular weight distribution which provides information regarding protein preservation. Additional factors such as sample heating and potential contamination sources also need to be considered when developing cell lysis methods. The combination of these factors can be used to assess cell lysis methods suitable for a particular sample. We found that a cooled non-ionic detergent based lysis buffer containing 1% Triton x-100 combined with 500 µm glass beads in a 1.5 mL conical plastic vial that is vortexed (V, T) provides the greatest amount of potential zinc proteins with a wide distribution of molecular weights in *H. capsulatum*. As the field of metalloproteomics continues to grow and more metalloproteomes are examined, optimizing cell lysis methods for different types of samples is as important as developing separation, identification, and quantification methods. We hope that this work provides one example of the process which can be adapted for future metalloproteomic studies.

2.5 Undergraduate Researchers

2.5.1 Materials Creation

This work was completed with the assistance of undergraduate researchers. As part of their capstone research experience, I wanted to expose them to all aspects of a research laboratory and conducting research. Together, with the undergraduates, we identified several “procedures” we commonly use in the lab including acid washing and drying vials, preparing samples for digestion, hot block digestion, and preparing calibration curves. We created our own set of Standard Operating Procedure (SOPs) with specific references to the laboratory space in which they were working, such as “The nitric acid is located in the blue cabinet.
near the closet”. I also wanted the undergraduate students to operate the HPLC, ICP-MS, analyze the data, and create meaningful tables and figures from the data. I developed a Quick Guide for the ICP-MS which the undergraduates refined based on their experience as novices and we adapted this quick guide for the different models of the Agilent ICP-MS. This document for the Agilent 7700 can be found in Appendix A. This was also used in the CHEM 3030L Instrumental Analysis Laboratory Course for the experiment described in Chapter 3. We also created SOPs for preparing samples for SEC-HPLC, operating the HPLC, analyzing total metal analysis data, HPLC-ICP-MS data, and how to create figures and tables for this data using Origin software. The undergraduates enjoyed taking an active role in all aspects of the research process and also felt more valued as a result of creating the SOPs and seeing them implemented. The undergraduate researchers also felt comfortable enough to make revisions to the SOPs based on their experience such as centrifuging digestion vials for a few minutes after the hot block digestion to make sure sample was not lost through droplets on the caps. As other undergraduates started working in the laboratory, the more experienced undergraduates were able to help them get acquainted and they were able to finish their training and begin their work much faster. By exposing the undergraduate researchers to industry and government standards such as SOPs, they not only gained skills applicable to their careers, but they also gained confidence and independence in their work.

2.5.2 Experiment Design

In addition to involving the undergraduate researchers to SOPs, I wanted to allow them the freedom to design their own experiments. In the development of the cell lysis methods, the undergraduates developed several methods which we tested and refined. One of the undergraduates commented that she had never had the opportunity to just “play” with things in the lab and try things out. Her past experiences with science had involved procedures and materials lists with little room for true inquiry. A few of the undergraduate researchers’ ideas were used for the final comparison of lysis methods for *H. capsulatum* as described in
this chapter such as cooling the sonication bath with ice to reduce protein denaturation. While I designed most of the experiments that the undergraduates carried out, their contributions to this work were invaluable. Working very closely with several undergraduate researchers provided me with a different perspective on conducting research which ultimately influenced the creation of the laboratory experiment described in Chapter 3.
3.1 Introduction

Arsenic is of concern due to its toxic and carcinogenic properties. The toxicity of arsenic is related directly to its binding form, as inorganic forms are more toxic than organic forms. Arsenic exists in nature in a variety of oxidation states: +5 (arsenate), +3 (arsenite), 0 (arsenic), and –3 (arsine). In aqueous environments, which are the focus of this experiment, arsenic is often in the +5 and +3 states as well as arsenous acid (As(III)), arsenic acid (As(V)), and their corresponding salts. Chronic arsenic exposure can lead to poisoning with symptoms including weakness, loss of reflexes, fatigue, gastritis, colitis, anorexia,
Further, there is a well-established link between arsenic and skin, lung, and bladder cancer.\textsuperscript{61} At a molecular level, As(III) can bond to the sulfur atom in biological proteins containing cysteine residues, disrupting tertiary structure and function.\textsuperscript{62} The U.S. Environmental Protection Agency (EPA) has established a maximum contaminant level of 10 part per billion (ppb) in drinking water to protect the public from long term exposure of arsenic.

Illustration of spectroscopic techniques is fundamental to the undergraduate instrumental analysis course. Atomic spectroscopy is used for elemental analysis in a host of industries including food, energy, environmental, agricultural, and pharmaceutical.\textsuperscript{4-10, 63} Several instruments are employed including the Atomic Absorption Spectrometer (AAS) and the Inductively Coupled Plasma-Optical Emission Spectrometer (ICP-OES). Laboratory experiments utilizing each have been published in \textit{J. Chem. Educ.} (AAS\textsuperscript{64-66} ICP-OES\textsuperscript{67-71}) and collectively demonstrate individual strengths and limitations. A third instrument used for elemental analysis, the Inductively Coupled Plasma-Mass Spectrometer (ICP-MS), has become an academic, industry, and government workhorse for the rapid and nearly simultaneous analysis of most elements in the periodic table at part per trillion (ppt) detection limits or lower.\textsuperscript{4-10, 63} The experiment described in this work teaches students several important aspects of actual use of this instrument in the laboratory.

The principles of ICP-MS have been previously reviewed\textsuperscript{70} and the present laboratory experiment emphasizes the fact that ICP-MS is a powerful tool for rapid, sensitive, multi-element analysis over a wide concentration range. In addition, three specific objectives were established for this laboratory experiment. The first objective was to introduce the use of internal standard calibration. Harvey reported on the importance of student understanding of matrix effects and options for calibration methods to improve accuracy of analytical results.\textsuperscript{71} The sinus wash samples show a decreased signal response for the analyte
of interest, arsenic, and associated internal standard, germanium, as compared to tap water. Students were asked to use their data to describe how the matrix might affect the choice of calibration method.

The second objective was to demonstrate the presence of specific interferences in analytical techniques. In the present case, use of an ICP-MS necessitates the consideration of polyatomic ions formed in the plasma. In a high chloride matrix, the ArCl⁺ ion may be produced in the plasma, overlapping the analyte’s only isotope at m/z = 75. Sinus wash has a high saline content and therefore illustrates the inflated high arsenic level that may be inaccurately reported in a high chloride matrix. A collision cell is employed in this experiment to eliminate the interfering ArCl⁺ ion and students are able to view results without the collision cell in no gas mode and with the collision cell using helium. It should be noted that standard addition does not compensate for the error caused by the ArCl⁺ ion, thus connecting the two objectives.

The third objective was to relate the experimental results to EPA regulations for arsenic in drinking water. Arsenic was partially chosen as the analyte of interest due to its relation with human health and the environment. Students were required to research the EPA’s regulation for arsenic in drinking water either on a computer in the lab or outside of class and comment on their results with respect to the regulation. Many students discussed the health effects of arsenic poisoning and the need to correctly analyze the concentration of arsenic to maintain public safety. Connecting the EPA regulation with their experimental results provided students with relevant context, encouraging student engagement within the course.
3.2 Experimental

This experiment was employed in an undergraduate Instrumental Analysis Laboratory class at The University of Cincinnati. The experiment manual can be found in Appendix B and instructor notes can be found in Appendix C. The course is populated largely by senior chemistry and chemical engineering students. Students worked in groups of three in a class sections of approximately 24 students. This experiment was one of eight that each group performed under a weekly rotation schedule. Lab periods were scheduled on two days each week and for a period of three and a half hours each day. Due to the rapid sample analysis of the ICP-MS and the design of the laboratory experiment, students were generally able to complete this laboratory within the first laboratory period.

An Agilent 7700 equipped with an Octopole Reaction System, as the collision cell, was used in this experiment, although it could be adapted to any ICP-MS system with collision cell capability. Helium was used as the collision gas and the 7700 Series MassHunter software was employed for data manipulation. Specific instrument conditions are detailed in the Appendix B and C.

Procedurally, students were asked to prepare an eight-point calibration curve for arsenic including a blank. The range extended from 0 to 25 ppb and the working solution was a 100 ppb arsenic solution previously prepared by the graduate student Teaching Assistants (TAs). Within each calibration point, the students were required to add germanium at a final concentration of 1 ppb and the germanium working solution supplied was at a concentration of 40 ppb and was also previously prepared by the TAs. Finally, students were asked to ensure that their final calibration standards contained nitric acid at a concentration of 20 mL of concentrated (70%) nitric acid per liter. All calculations for standard preparation were required to be complete by the start of the lab.
Two sample types were investigated for the presence of arsenic and these included commercially-purchased sinus wash and local tap water. The students were asked to collect a tap water sample prior to their laboratory period and this exercise sparked their curiosity. Student groups collected water from a variety of sources (home, the lab, the water fountain, and filtered tap water), further connecting the experiment to the greater world. Students were asked to prepare each sample type in quadruplicate and discuss the importance of replicates and outliers in the lab reports.

3.3 Hazards

Caution and care must be exercised when working with nitric acid; it is a strong, corrosive acid and appropriate Personal Protective Equipment (PPE) and safety precautions must be employed. Students were required to wear goggles, gloves, and work in a fume hood. Care was taken when preparing solutions, as arsenic is toxic and carcinogenic. Arsenic and germanium standards were 1000 ppm commercially purchased stock solutions. These stock solutions were diluted by the TAs prior to the actual lab to concentrations of 100 ppb (arsenic) and 40 ppb (germanium) for use by the students. All waste should be disposed of in a proper manner according to institutional waste policy protocol.

3.4 Discussion

This laboratory experiment was performed for the first time during the 2015 spring semester course 3030L, Instrumental Analysis Laboratory, at the University of Cincinnati. A total of 120 chemistry and chemical engineering seniors participated in this course and performed this laboratory experiment working in small groups to promote collaborative learning.
During the laboratory period, the TA discussed hazards and precautions regarding nitric acid, checked the student group’s dilution calculations, and turned on the instrument with the students. After the student group prepared all standards and samples, the TA showed the students how to tune the instrument. Additionally, the TA discussed principles of operation as detailed in the Supporting Info as well as individual instrumental components and functions (peristaltic pump, nebulizer, spray chamber, torch, etc). The students then set up a sequence to analyze their blank, calibration standards and samples in both no gas mode and He mode. Student generated calibration curves are shown in Figure 3.2; R² values were exceptional and generally at 0.9997 or higher, demonstrating that the students accurately prepared their calibration standards. Students were able to view the m/z 75 signal in both collision cell modes, promoting an initial conversation regarding the difference in signal for a given sample.
Figure 3.2 Example of student generated arsenic calibration curve using germanium internal standard for no gas mode and He mode.
The internal standard method offers high accuracy because it alleviates errors introduced by sample introduction, matrix and/or variations in detector response. Germanium was chosen as the internal standard due to its closeness in mass to arsenic on the periodic table as well as absence in the sample matrix. As demonstrated in Figure 3.3, a graph of internal standard recovery, the students’ were able to see the depressed germanium signal in the sinus wash matrix, illustrating the importance of matrix consideration when choosing a calibration method. In the laboratory report, students were asked to comment on the advantages of internal standard calibration. Without using internal standard, the arsenic signal in sinus wash would be depressed and the incorrect concentration would be calculated. When using internal standard, the same amount of internal standard is added to each standard and sample. In this way, the ratio of internal standard to analyte is plotted and can be used to compensate for matrix effects. As shown in the calibration curve graphs in Figure 3.2, students were able to see a plot of the ratio to the concentration generated by the software.
Figure 3.3 Example of student generated Internal Standard Recovery Plot. S1-S4 refer to Sinus Wash replicates. T1-T4 refer to Tap Water replicates.

Depending on the matrix or the specific isotopes, interferences may disrupt the analysis of particular elements and employment of a collision cell is useful for their removal. Arsenic is one such element, as it is monoisotopic (75 amu) and in a high chloride matrix (such as some drinking waters), it is difficult to measure at low levels because of the formation of ArCl⁺ in the plasma, a polyatomic with the same m/z. In this experiment, a collision cell was employed to facilitate the low level detection of this harmful element. Students were able to observe the results generated both with and without the use of the collision cell. The students were also asked to consider the sinus wash vs. the tap water in both modes (no gas mode and He mode) in their laboratory report as part of a hypothesis and in the discussion. Table 3.1 illustrates the results of the quantification of arsenic in both sinus wash and tap water both with and without using the collision
cell, allowing observation of the presence of the ArCl⁺ interferant in the no gas mode analysis of the sinus wash.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Sinus Wash</th>
<th>Tap Water</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No Gas</td>
<td>Helium</td>
</tr>
<tr>
<td>Replicate 1 (ppb As)</td>
<td>6.52</td>
<td>0.26</td>
</tr>
<tr>
<td>Replicate 2 (ppb As)</td>
<td>6.69</td>
<td>0.33</td>
</tr>
<tr>
<td>Replicate 3 (ppb As)</td>
<td>6.75</td>
<td>0.24</td>
</tr>
<tr>
<td>Replicate 4 (ppb As)</td>
<td>6.83</td>
<td>0.39</td>
</tr>
<tr>
<td>Average concentration of As (ppb As)</td>
<td>6.7</td>
<td>0.31</td>
</tr>
<tr>
<td>Standard Deviation (ppb As)</td>
<td>0.13</td>
<td>0.07</td>
</tr>
<tr>
<td>% Relative Standard Deviation (%)</td>
<td>1.95</td>
<td>22.62</td>
</tr>
</tbody>
</table>

**Table 3.1** Example of student generated results for the concentration of arsenic in Sinus Wash and Tap Water.

Students were asked to prepare samples in quadruplicate (See Replicate 1-4 in **Table 3.1**) to allow them to calculate the precision in their results and comment on the sample preparation in the lab report. Overall, students were able to explain the advantage of preparing samples in quadruplicate over triplicate, given enough sample. A few students used the Grubbs test to determine if any of the replicates were outliers. This
activity informed students on how measurement variability can be addressed in the development of an analytical method.

Before students analyzed their samples, many looked up the federal regulation for arsenic in drinking water and formed their hypothesis. Almost all students believed that the arsenic concentration in tap water and sinus wash would fall below the EPA’s 10 ppb arsenic regulation. At first this seemed to be obvious conclusion to students, but further research of arsenic led to concerns over environmental contamination and human health. Students were able to go beyond performing the laboratory experiment and discuss in their lab reports why such an analysis can benefit the wider community by monitoring arsenic and other toxic metals in drinking water and consumer products. This connection between the course and the greater world not only fosters student engagement, but also creates a lasting connection between the classroom experience and industrial applications.

3.5 Conclusion

A laboratory experiment employing ICP-MS with collision cell to determine low part-per-billion levels of arsenic in sinus wash and tap water was successfully developed and implemented in an undergraduate instrumental analysis laboratory course. It is the hope that collectively, with this laboratory experiment and the other seven experimental procedures taught in this course, students will be able to evaluate the limitations and capabilities of different analytical instrumentation as well as fully understand the application of these instruments based on the principles of operation. Specific to this experiment, ICP-MS is a standard tool in industry and it is critical that chemists and chemical engineers have an understanding of the principles of its operation as well as practical hands-on experience. During this experiment, students learned that sample matrix can significantly affect the results of a given analytical method. The use of the
germanium internal standard in the sinus wash samples illustrated the necessity of matrix consideration when choosing the calibration method. Further, specific matrices may mask certain analytes, yielding biased analytical results. These interferences must be approached and considered with the utmost care to avoid the reporting of false positives and the use of a collision cell in ICP-MS is one approach to address such problems. Finally, students were able to relate their results to current EPA regulations and discussed the importance of monitoring toxic metals, further connecting the classroom experience to the greater world.
Chapter 4: Impact of a Scientific Reasoning Tool on ICP-MS Laboratory Reports in an Instrumental Analysis Laboratory Course

Figure 4.1 Graphical abstract for the Impact of a Scientific Reasoning Tool on ICP-MS Laboratory Reports in an Instrumental Analysis Laboratory Course

4.1 Introduction

The ICP-MS laboratory experiment described in Chapter 3 was part of a larger initiative to redesign the CHEM 3030L Instrumental Analysis Course for chemistry and chemical engineering majors at the University of Cincinnati. This course is designed to provide hands-on experience with many of the instruments that are covered in the preceding lecture course. The course redesign process took place during the Fall 2014 semester which included the revision and addition of laboratory experiments and changes to course materials to create a more consistent assessment strategy. The development of the Scientific
Reasoning Tool (SRT) was one of the course materials created to support the development of students’ scientific argumentation skills and an initial study was conducted to determine its impact on student achievement of course learning outcomes in student laboratory reports.

4.1.1 CHEM 3030L Instrumental Analysis Course Redesign

In the 2012-2013 academic year, the Instrumental Analysis course was taught in the fall semester and student lab reports were written individually and graded by Teaching Assistants (TAs). A short list of lab report guidelines (see Appendix D) was provided to students to help them structure the lab reports. In addition, the TAs used the lab report guidelines to grade reports, but they mainly focused on making sure that students included required sections in their lab reports including abstract, introduction, materials and methods, data, discussion, conclusion. Based on interviews, the instructors reported that during this time, a select group of students complained to the instructors about the grades they received and how their lab reports were graded by the TA. As a result, the instructor and TA had to discuss how the report was graded which sometimes led to adjusting grades.

Between 2012 and 2013, the University of Cincinnati transitioned from a quarter system to a semester system and the Instrumental Analysis Laboratory course was changed from the fall to the spring. In addition, students previously took the lecture and associated laboratory course simultaneously. This was changed to the CHEM 3030 lecture course being offered in the fall and the CHEM 3030L laboratory course being offered in the spring in the semester calendar. This resulted in chemistry majors enrolling in the course during the spring of their third year and chemistry engineering majors enrolling in the course during the spring of their final semester before graduation. Based on instructor interviews, part way through the semester in spring 2014, students from different sections complained to the instructors that lab reports were
not graded in the same way in different sections of the course. During the spring 2014, TAs were responsible for grading all of the laboratory reports for the section they were assigned to but did not use rubrics which likely explains these complaints.

To prepare for the spring 2015 semester, the course was redesigned which included the revision and addition of laboratory experiments and changes were made to create a more consistent assessment strategy. During fall 2014, thorough lab report guidelines were created (see Appendix E) to provide a detailed description of how to write a lab report including language and style, structure, and formatting. Policies regarding plagiarism and paraphrasing were also included. These lab report guidelines were adapted from a document created by Ms. Tardalo at North Lenoir High School in La Grange, NC for her AP Biology Course. In addition, a detailed laboratory report rubric was created to assess student lab reports. The lab report criteria were created based on the lab report guidelines, instructor, and TA input.

From 2012-2014, instructors and TAs commented that while some students were able to construct testable hypotheses and write strong lab reports, they felt that many students needed to develop their laboratory writing skills. This difference may partially be due to the variance in experience between chemistry and chemical engineering majors as well as disciplinary writing differences. In addition to creating thorough laboratory report guidelines and a laboratory report rubric, it was suggested that a tool used to scaffold the process of writing a laboratory report may aid students in strengthening argumentation and writing skills. We felt that the tool should address all of the course learning outcomes and support students in achieving these outcomes which should correspond to higher final course grades.

The course learning outcomes for CHEM 3030L are shown in Table 4.1. The aim of this intervention was to determine if use of a laboratory report scaffolding tool would strengthen students’ argumentation skills
through written lab reports. The ICP-MS experiment was chosen for comparison purposes. One of the main reasons why the ICP-MS experiment was chosen was flexibility that the experiment offered. While the experiment was designed to determine arsenic in sinus wash and tap water using two modes of the ICP-MS, a number of hypotheses could be created for the experiment. In addition, the students were not aiming for one “right” answer, unlike traditional “cookbook” style experiments. While the ICP-MS experiment cannot be considered an inquiry-based experiment, elements of such an experiment were incorporated into the design to allow for student interest and exploration. This flexibility allows for a range of hypotheses backed by scientific arguments at various levels. This work received a “Not Human Subjects Determination” from the Institutional Review Board at the University of Cincinnati.
At the conclusion of this course, students will be able to:

1. Competently utilize the chemical literature.

2. Effectively communicate scientific information and results in written format.

3. Understand the principles/applications of and gain experience with the following instruments. (Gas Chromatography, High Pressure Liquid Chromatography, Atomic Adsorption Spectroscopy, Inductively Coupled Plasma Mass Spectrometry, Gas Chromatography Mass Spectrometry, Diode Array Spectrophotometry, Fluorescence spectroscopy)

4. Understand and perform the mathematical calculations used in determining unknown concentrations, including statistical analysis in order to critically evaluate results.

Table 4.1 Course Learning Outcomes for CHEM 3030L as of Fall 2016. The course outcomes are currently being revised to replace understand with action verbs that are measurable and to include additional experiments for Infrared Spectroscopy and Raman Spectroscopy.

The laboratory report scaffolding tool, named the Scientific Reasoning Tool (SRT), was developed during the fall of 2014 and implemented in spring 2015. The redesign of the CHEM 3030L Instrumental Analysis Laboratory Course began in fall 2014 and involved the revision and addition of laboratory experiments as well as expansion of laboratory report guidelines and materials. Course redesign is an iterative process and thus, is ongoing for the CHEM 3030L course.
4.1.2 Development of Scientific Reasoning Tool

The development of the SRT began with several objectives in mind. The design of the tool needed to be simple, straightforward, and require limited time to complete so that it would not be a burden for students. In addition, the tool needed to correspond with the critical parts of the scientific method as carried out in a laboratory experiment. Finally, the tool needed to mirror the laboratory report guidelines and laboratory report rubric to provide alignment with course learning objectives.

The basis of any laboratory report or scientific article is argumentation. Stephen Toulmin, a twentieth-century British philosopher developed a framework for argumentation which is more commonly known as the Toulmin Argument Pattern (TAP). Much of the work on argumentation and assessing scientific reasoning has focused on the TAP or a modified version of the TAP. One way to strengthen students’ argumentation skills is by teaching a structure for argument then the students apply the structure which has been published as part of the IDEAS project and McNeill’s Claim, Evidence, Reasoning (C-E-R) framework. McNeill’s C-E-R framework provides a simplified and more accessible version of TAP and provides the basic structure of an argument. McNeill’s definitions of claim, evidence, and reasoning can be seen in Table 4.2.
McNeill’s definitions of Claim, Evidence, Reasoning

<table>
<thead>
<tr>
<th>Claim</th>
<th>An assertion or conclusion that answers the original question.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Evidence</td>
<td>Scientific data that supports the claim; the data needs to be appropriate and sufficient to support the claim.</td>
</tr>
<tr>
<td>Reasoning</td>
<td>A justification that links the claim and evidence and shows why the data counts as evidence to support the claim by using the appropriate and sufficient scientific principles.</td>
</tr>
</tbody>
</table>

Table 4.2 McNeill’s definitions of Claim, Evidence, and Reasoning.

Much of McNeill’s work has focused on scientific argumentation and explanations in K-12 education, particularly grades 5-8. This comes as no surprise with the introduction of the Next Generation Science Standards (NGSS) and the emphasis on engaging in scientific argumentation and explanation. While McNeill’s C-E-R framework is quite simple, entire chemistry laboratory courses have been redesigned to involve collaborative scientific argumentation, such as Joi Phelps Walker’s recent instructional model called Argument-Driven Inquiry (ADI). Due to the constraints of the CHEM3030L course, a tool that required minimal time and effort was necessary. The simplicity of the C-E-R framework was appealing for this course, but we felt that creating a tool with a few guiding questions in addition to the C-E-R would strengthen the connection to the laboratory report.
To guide students in hypothesis formation as well as connecting the experiment to scientific context, two additional guiding questions were added “What are the research questions you are trying to answer in the lab?” and “Why is this lab important? (Think about the bigger picture/beyond this course)”. These two questions directly related to the abstract, introduction, and conclusion sections of the laboratory report. In addition, these questions support the course learning outcomes. After consultation with several faculty and graduate students, the SRT, which can be found in Figure 4.2 was created as a one-page worksheet.

### 4.2 Materials and Methods

#### 4.2.1 Experiment Design

The formal lab report guidelines, laboratory report rubric, and SRT were incorporated into the course starting in the spring 2015 semester. During the spring semester, a total of 120 students were enrolled in CHEM 3030L in five sections, two of which used the SRT. Before the semester started, I reviewed the materials with the instructors and TAs of the course as well as the data collection procedures. Within the first few weeks of the semester, before the students started the instrumental experiments, I provided 20-30 minutes of training and practice with the students that would be using the SRT for the instrumental experiments. During the spring 2015 semester, laboratory experiment rubrics and SRT’s were photocopied, collected, and stored in a locked filing cabinet. Digital data was stored on a password protected computer behind a firewall (UC’s network). Identifiable information was removed from all student work.

As the spring 2015 semester progressed, it was clear that there was not a consistent level of assessment using the laboratory report rubrics. This concern was raised by the instructors as well as some of the TAs. This may have been due to the range of TA assessment experience. As a result, laboratory report rubrics,
SRTs, and ICP-MS reports were collected during the spring 2016 semester using a similar data collection protocol which allowed for external review of student work. Similar training and practice was administered to the sections that would use the SRT.

During the spring 2016 semester, 90 students were enrolled in four sections of CHEM 3030L. During the summer 2016 semester, 11 students were enrolled in one section of CHEM 3030L. Students were required to work in groups of three within the lab period and write group lab reports. Two sections in the spring 2016 semester used the SRT for a total of 50 students and the remaining students in the two spring sections and one summer section did not use the SRT for a total of 51 students. In addition, student course grades reflected their group lab report grades, peer assessment scores, and attendance. Laboratory report rubrics, SRTs, and ICP-MS laboratory reports were collected and the reports were evaluated by Dr. Christa Currie, Associate Professor and Chair of the Department of Chemistry at Mount St. Joseph University and myself. A rubric, referred to as the SRT Impact Rubric, based on McNeill’s Scientific Explanation Rubric was developed with the help of Dr. Kathy Koenig of the Department of Physics at the University of Cincinnati to evaluate the reports. Seven ICP-MS laboratory reports and capstone laboratory reports using ICP-MS from the spring 2015 semester were used as a training set. The two raters were in 87.5% agreement for the training set which was deemed sufficient by the evaluators. In total, 24 ICP-MS laboratory reports were collected during the spring and summer 2016 semesters. One of the ICP-MS laboratory reports had to be discarded because the photocopy was not complete. A total of 13 ICP-MS laboratory reports were collected from the two sections that used the SRT. A total of 10 ICP-MS laboratory reports were collected from the three sections that did not use the SRT. Two additional data collection methods were employed including student surveys targeting attitudes and confidence in writing laboratory reports and using the SRT as well as instructor interviews to collect their view on the impact of the laboratory report materials and SRT.
4.2.2 Materials

The SRT incorporated two guiding questions as well as a graphic organizer for the claim, evidence, reasoning portion of the tool. A similar handout with explanations was also created to provide directions to students and facilitate the connection to the laboratory reports. In order to practice using the tool, a sample experiment and data was developed. These SRT materials can be seen in Figures 4.2, 4.3, and 4.4.
Scientific Reasoning Tool
University of Cincinnati – Chemistry Department

What are the research questions you are trying to answer in the lab?

Why is this lab important? (Think about the bigger picture/beyond this course.)

Evidence/Data ➔ Reasoning ➔ ➔ ➔ ➔ Claim

Figure 4.2 Scientific Reasoning Tool (SRT)
**Scientific Reasoning Tool**

University of Cincinnati – Chemistry Department

What are the research questions you are trying to answer in the lab?

**STEP 1: Start here!** This section will help you formulate the research questions you are trying to answer. This is similar to purpose, keep in mind you may have more than one question. This will help you collect your thoughts as you create your hypothesis which you will answer with your claim (see below).

Why is this lab important? (Think about the bigger picture/beyond this course.)

**STEP 2:** This section will help you make a connection between the content (the experiment) and the context (the greater world). This will also help you when you write sections of your lab report!

<table>
<thead>
<tr>
<th>Evidence/Data</th>
<th>Reasoning</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>STEP 4:</strong> Evidence includes scientific data from the experiment, as well as journal articles, textbooks, etc. The data needs to be relevant to and sufficiently support the proposed claim. Data does not only have to be numbers, observations and background information also count!</td>
<td><strong>STEP 5:</strong> Reasoning provides justification that links the evidence and the claim. It explains why the data counts as evidence to support the claim using appropriate logic. This is the hard part. It may take you a minute to put into words why the evidence supports the claim.</td>
</tr>
</tbody>
</table>

**Claim**

**STEP 3:** Fill this one in before evidence and reasoning! The claim can be seen as the answer to your hypothesis. It is the assertion or conclusion that addresses the original research question.

---

Figure 4.3 Scientific Reasoning Tool with directions
Sam wants to see if plants really do grow better in sunlight before he starts his seeds indoors in the winter for his garden in the spring. He uses 3 plants of the same type and size in 3 locations. Plant A is placed on a table in the center of the room which has daylight fluorescent bulbs in the ceiling lights, Plant B is placed inside the cabinet, and Plant C was placed near window sill. After 5 days, two of which were big snow storms, Sam measures the growth of each plant and documents it in the table below.

<table>
<thead>
<tr>
<th>Sam's Plant Growth Experiment</th>
<th>Height on Day 1</th>
<th>Height on Day 5</th>
</tr>
</thead>
<tbody>
<tr>
<td>Plant A</td>
<td>12.9 cm</td>
<td>15.6 cm</td>
</tr>
<tr>
<td>Plant B</td>
<td>12.1 cm</td>
<td>13.6 cm</td>
</tr>
<tr>
<td>Plant C</td>
<td>11.9 cm</td>
<td>16.0 cm</td>
</tr>
</tbody>
</table>

What are the research questions you are trying to answer in the lab?

Why is this lab important? (Think about the bigger picture/beyond this course.)

![Scientific Reasoning Tool](image.png)

Figure 4.4 Scientific Reasoning Tool with example experiment and data
The laboratory report rubric was developed as an expansion of criteria typically used for laboratory reports in CHEM 3030L. The laboratory report rubric was designed in alignment with the updated laboratory report guidelines. A conscious effort was made to make the laboratory report rubric efficient for grading by the TAs. Shading and bold lines create visual distinction between sections of the laboratory report. Criteria were divided into sections of the laboratory to increase grading efficiency and organization. In addition, descriptions for each criteria and point deductions were included. During the development of the laboratory report rubric, several faculty and graduate students in the Department of Chemistry at the University of Cincinnati provided feedback on several revisions.
<table>
<thead>
<tr>
<th>Component</th>
<th>Points</th>
<th>Deductions</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Format</td>
<td>/2</td>
<td>-0.5 pt for each missing item</td>
<td>Names, date, title is descriptive/distinct/succinct, headings present/proper grammar</td>
</tr>
<tr>
<td>Objective/Purpose</td>
<td>/3</td>
<td>-1 pt needs improv.</td>
<td>Objective clearly and succinctly describes the purpose of the experiment</td>
</tr>
<tr>
<td>Summary</td>
<td>/3</td>
<td>-1.5 pts needs improv.</td>
<td>Clear and brief summary of how the experiment was conducted, key results with uncertainty</td>
</tr>
<tr>
<td>Hypothesis</td>
<td>/2</td>
<td>-1 pt needs improv.</td>
<td>Testable hypotheses, if... then..., states if results support or reject hypothesis</td>
</tr>
<tr>
<td>Background</td>
<td>/3</td>
<td>-1.5 pts needs improv.</td>
<td>Shows evidence of research and addresses important points, relates to context, what do we know already?</td>
</tr>
<tr>
<td>Context</td>
<td>/3</td>
<td>-1.5 pts needs improv.</td>
<td>Demonstrates clear understanding of the “big picture”. Why is this question important/useful?</td>
</tr>
<tr>
<td>Hypothesis</td>
<td>/2</td>
<td>-1 pt needs improv.</td>
<td>Clearly stated, testable hypotheses, if... then...</td>
</tr>
<tr>
<td>Materials and Methods</td>
<td>/4</td>
<td>-1.5 pts not complete</td>
<td>Detailed list of all materials, concentrations, instruments, etc.</td>
</tr>
<tr>
<td>Procedure</td>
<td>/3</td>
<td>-1.5 pts needs improv. or not in narrative</td>
<td>Clear, complete, narrative form. Written so that someone could repeat experiment</td>
</tr>
<tr>
<td>Data/Calculations</td>
<td>/3</td>
<td>-2 pts some errors</td>
<td>Clear, complete, graphs and charts have correct formatting, correct sig figs and units, correct statistical analysis (if applicable), example calculations, identifies which data may be faulty (if applicable)</td>
</tr>
<tr>
<td>Results</td>
<td>/2</td>
<td>-1 pt needs improv.</td>
<td>Identifies what was determined by the experiment, relates to hypothesis</td>
</tr>
<tr>
<td>Significance of results</td>
<td>/4</td>
<td>-2 pts needs improv.</td>
<td>Logical explanation of results, alternative explanations are considered and eliminated in persuasive discussion</td>
</tr>
<tr>
<td>Error and faulty data</td>
<td>/4</td>
<td>-4 pts missing</td>
<td>Addresses human error and experimental error, how the errors affected results, conflicting data is addressed</td>
</tr>
<tr>
<td>Improvement</td>
<td>/4</td>
<td>-4 pts missing</td>
<td>Improvement related to human error and improvement related to experimental error/experimental design</td>
</tr>
<tr>
<td>Importance of experiment</td>
<td>/4</td>
<td>-4 pts missing</td>
<td>Big picture idea, relation to other courses, goes beyond the classroom</td>
</tr>
<tr>
<td>Conclusion</td>
<td>/4</td>
<td>-2 pts needs improv.</td>
<td>Brief summary of how the experiment was conducted</td>
</tr>
<tr>
<td>Summary of results</td>
<td>/4</td>
<td>-2 pts needs improv.</td>
<td>Brief summary of results</td>
</tr>
<tr>
<td>Hypothesis</td>
<td>/2</td>
<td>-2 pts needs improv.</td>
<td>Testable hypotheses, if... then..., explain how results support or reject hypothesis</td>
</tr>
<tr>
<td>Proper citation</td>
<td>/2</td>
<td>-1 pt needs errors or incorrect</td>
<td>ACS formatting</td>
</tr>
<tr>
<td>Raw data, spectra</td>
<td>/2</td>
<td>-2 pts missing</td>
<td>Includes ALL raw data</td>
</tr>
</tbody>
</table>

Total: /60

Comments:

Figure 4.5 Laboratory report rubric for instrumental laboratory experiments in CHEM 3030L.
Initially, a comparison of the lab reports using the laboratory report rubric was conducted by the two raters but was not deemed sufficient as an assessment of the impact of the SRT. The laboratory report rubric criteria reflect items included in the SRT such as objective, hypothesis, claim, evidence, and reasoning. Additional criteria such as the materials, procedure, and error are also part of the laboratory report rubric and do not necessarily correlate with argumentation skills. As a result, the SRT Impact Rubric was created (see Figure 4.5) and is modeled after McNeill’s rubric\textsuperscript{82} with input from Dr. Kathy Koenig. The SRT Impact Rubric was not used by the instructors or TAs within the course during the spring 2016 and summer 2016 when the data was collected. Information specific to the ICP-MS experiment was included in the SRT Impact Rubric to provide clarity in the evaluation of student work.
### Figure 4.6: SRT Impact Rubric

The Scientific Reasoning Tool (SRT) was developed to assess the impact of SRT on laboratory report quality. The rubric consists of criteria labeled A through H, each with performance levels of Missing, Inadequate, Needs Improvement, Complete, and Perfect. Numerical values of 0, 1, 2, and 3 are assigned to these levels.

<table>
<thead>
<tr>
<th>Criteria</th>
<th>Description</th>
<th>Complete</th>
<th>Needs Improvement</th>
<th>Inadequate</th>
<th>Missing</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>Assesses the clarity and organization of the report.</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>B</td>
<td>Evaluates the appropriateness of the data and analysis.</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C</td>
<td>Checks for the presence of any errors or omissions.</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>D</td>
<td>Verifies the accuracy of calculations and conclusions.</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>E</td>
<td>Assesses the relevance of the report to the scientific context.</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>F</td>
<td>Compares the report to similar reports in the literature.</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>G</td>
<td>Reviews the conclusions and recommendations.</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>H</td>
<td>Evaluates the overall impact of the report on scientific reasoning.</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Performance levels are assigned numerical values: Complete = 3, Needs Improvement = 2, Inadequate = 1, Missing = 0.
Two short, paper-based surveys (see Figure 4.6) were given to students in early February 2016 and late March 2016 to determine if the attitudes and confidence in writing lab reports and using the SRT (if applicable) changed over time. A Likert scale was used to measure student attitudes and confidence. To quantitatively compare survey results, “Extremely confident/useful” was rated a 5 and “Not confident/useful at all” was rated a 1. In March, the same questions were presented and students were also encouraged to provide written feedback on the course. Unfortunately, student responses were not tracked between the two survey dates and thus the population of students who took the survey in January is not the exact population of students who took the survey in March.
After the summer 2016 semester, instructor interviews were conducted to collect information about the course in the past, their views on the impact of the laboratory report rubric, SRT, and group reports. The interview questions also included aspects of the course such as group laboratory reports versus individual
laboratory reports and students coming to the second class meeting of each week to work on laboratory reports. While the responses gathered from these particular questions are relevant to the course redesign, they will not be discussed in this work. The interview responses as a whole provide important information on instructor perceptions of course and will be used to improve the course in future semesters.

<table>
<thead>
<tr>
<th>CHEM 3030L Faculty interviews</th>
<th>Faculty member:________________________</th>
</tr>
</thead>
</table>

**General**
1. When did you start teaching 3030L?
2. (if before 2014) How was student work assessed?/How were the lab reports graded?/Were there any guidelines for grading?

**Rubrics**
3. What has been the impact of using the rubric and the lab report guidelines in this course?
4. How have TA’s responded to using the rubric?
5. Have students given you feedback about the rubric?

**Scientific Reasoning Tool**
6. What has been the impact of using the Scientific Reasoning Tool in this course?
7. Do you think using the Scientific Reasoning Tool has led to higher quality lab reports?
8. Do you think the Scientific Reasoning Tool has helped students work together in groups?
9. How have TA’s responded using the tool?
10. Have students given you feedback about using the tool?

**Group reports and individual reports**
11. Do you see a difference in lab report quality between the individual and group lab reports?
12. What has been the impact of the peer assessment in 2016?
13. What has been the impact of students coming in the 2nd day of the week?
14. Have students given you feedback about coming in the 2nd day of the week?

**Figure 4.8** CHEM 3030L Faculty Interview Questions
4.3 Results and Discussion

4.3.1 Laboratory Reports

A consensus estimate of interrater reliability was used to compare the two raters because it was assumed that they should agree on how to use the rubric to score the student work. A percent-agreement statistic was chosen due to comparing only two raters. Between the two raters there was 85.9% agreement for the 23 ICP-MS laboratory reports. Of the disagreements, 81% differed in one performance level and the evaluators determined that this was sufficient for an initial study. The detailed nature of the rubric to provide clarity likely resulted in the strong agreement between raters. Quotations of student work that represent select criteria for the SRT Impact Rubric are given below.

**Criteria A:** Is able to identify research questions/objective/purpose in lab report

(Complete – 3) *Objectives for this lab were to determine the concentration of arsenic in sinus wash and tap water using ICP-MS; and to compare the results of the Helium collision cell versus no gas technique of ICP-MS.*

The students clearly stated the purpose of the experiment and included both sample types, the analyte of interest, and the two collision modes.

(Needs Improvement – 2) *The purpose of this experiment was to determine the concentration of Arsenic in tap water and sinus wash by utilizing and ICP-MS with an internal calibration of 1ppb Germanium.*

The students did not include relevant aspects of the experiment such as the two collision modes, but included irrelevant information regarding the internal standard.
The objective of the ‘analysis of arsenic in sinus wash and tap water through inductively coupled mass spectroscopy’ was to learn how to use the ICPMS by analyzing two different samples. The first of which was a sinus wash. The second sample was tap water.

The students’ purpose of the lab was extremely general and only stated the instrument used and the two sample types.

Criteria B: Is able to put the experiment in context and state why the experiment is important/useful.

Mass spectrometry is practical in the field of toxicology. With large-scale production and utility systems, over time there becomes a risk over time that contaminants enter which could cause major harm to exposed groups. These groups are especially vulnerable if there are no procedures in place to prevent contamination of products and the public water supply...

Arsenic is a metalloid that is useful in industry for the strengthening of copper and lead alloys. Trace quantities are good for the diet, but it just takes an amount over 10 ppb for it to be considered toxic. Arsenic contamination in groundwater affects millions of people around the world every year. This makes it crucial that there is a reliable detection method in place to test arsenic to ensure that the drinking water is safe.

The students mentioned how industry operations can lead to contaminated water which could cause harm to humans. They also mentioned how arsenic is used in industry, but can be toxic at low concentration. The connection was then made between arsenic in the groundwater and having reliable methods to ensure drinking water safety.

An Agilent 7700 instrument, used for this experiment, uses argon to create plasma. With the presence of chlorine in some samples, like the salt present in sinus wash and chlorine in tap water, there is the potential for ArCl⁺ to be formed. ArCl⁺ ions can interfere with measurements of analyte ions with the same mass to charge ratio. Arsenic has the same mass-
to-charge ratio of ArCl+ and because of this there is interference. Interference can be corrected by removing the source of the interference.

The students focused on interference removal for ArCl+ for samples that contain chlorine, but did not expand to include examples of other samples that may contain chlorine or other interferences that could be removed.

(Inadequate – 1) In general, ICP-MS is used for determining a sample based upon the elements that reside within it. It is particularly useful with regard to rare-earth elements. It has a high throughput, fantastic detection limits, ability to handle simple and complex matrix materials, and isotopic output.

*Arsenic is a carcinogen, so determining the arsenic concentration in tap water is something of extreme importance, and displays why purifying water is important.*

Both of these student groups made very generalized statements about the context of the experiment and did not provide specific examples of how the instrument or experiment could be used outside of the classroom.

**Criteria C:** Is able to identify **hypothesis** (hypotheses) tested in the experiment

(Complete – 3) *If the sinus wash and tap water are safe for consumer use, then the concentration of Arsenic as determined by the ICP-MS with helium collision cell will be below the EPA regulation of 10ppb.*

The students referred to both sample types, specified the use of the helium collision, and made the connection to federal regulations.
It is hypothesized that more Arsenic will be detected in the Sinus Wash than the Tap Water.

The students included both sample types and the analyte of interest, but there is no mention of the instrument that would be used to detect the arsenic.

The students hypothesized that if the ICP-MS was used in conjunction with 12 known solutions of arsenic, then the students would be able to use the calibration curve created to determine the unknown arsenic concentration in the sinus wash solution.

This hypothesis refers to instrument capabilities and not the output of the experiment.

**Criteria H:** Is able to provide adequate reasoning or justification for how evidence supports claim(s).

The sinus wash samples had an average arsenic concentration of 31.042 µg/L or ppb with a standard deviation of 1.471 ppb without using the collision cell. The use of Helium in the collision cell dropped the Arsenic amount down to an average of 0.921 ppb with a standard deviation of 0.142 ppb. This drop is due to the ArCl ions that fool the detector when the collision cell is not used. The amount of salts able to form this is much higher in the sinus wash than the tap water. The tap water is much closer together between using the collision cell and helium gas and without using the collision cell. The average Arsenic in tap water without the use of the collision cell is 1.329 ppb with a standard deviation of 0.229 ppb. Using the collision cell with helium gas yielded an average Arsenic amount of 0.932 ppb with a standard deviation of 0.052 ppb. These levels show that the amount of Arsenic is well below the limit allowed by the EPA in drinking water. This experiment shows the presence of ArCl ions can interfere and give false positive data for Arsenic and the use of Helium gas in the collision cell can mitigate ion interferences to give a more accurate detection.
The students provided explanation as to why the concentration of arsenic decreased when the collision cell as used. In addition, they compared the amount of salts in the sinus wash and the tap water to explain why the tap water concentrations were similar using the two modes.

(Needs improvement – 2) *With the actual results gathered, it was found that on average the sinus wash had a concentration of arsenic of 4.36 ppb, and that the tap water had an average of .011 ppb. Both of these values are below the maximum permissible concentration of arsenic stipulated by the EPA, which is 10 ppb. It certainly makes sense for a public resource such as tap water to have such low levels of a toxic compound like arsenic.*

The students did state that their results were below the federal regulations, but provided a generalized statement justifying why the level of arsenic in was low because it is from a public resource. The students did not provide reasoning for the sinus wash sample to connect their evidence to their claim.

(Inadequate – 1) *The first hypothesis was if unknown tap water and sinus rinse samples are run through the ICP-MS, the amount of arsenic in both solutions will be lower than 10 ppb. This was the case, as the concentration of arsenic in the sinus wash and tap water samples were 5.31 ppb and 0.24 ppb, respectively.*

The students simply stated the hypothesis and some of the evidence, but did not make any attempt to justify how the data support their claim.

While the sample size is rather small, this initial study provides interesting results regarding the impact of the SRT that will impact future use of the tool. Upon initial comparison, students that used the SRT scored at or higher than the students who did not use the SRT using the SRT Impact Rubric as shown in Figure 4.9 and Table 4.3. While these results are not statistically different, it suggests that students who used the SRT scored as well if not better than their peers.
The students in the two groups performed comparably in stating the research question or objective and reporting their evidence and using the SRT did not impede students’ ability to write laboratory reports. It
is not particularly surprising that students scored equally for these criteria because they do not require scientific explanation or argument which was the focus of the tool. Stating a testable hypothesis with independent and dependent variables as well as restating the hypothesis, claim, and reasoning showed a moderate, yet not statistically significant, increase for students who used the SRT. The students who used the SRT had to compose their thoughts for the SRT and may have refined their language or explanations while writing the lab report. These results may indicate that the SRT acted as a scaffold that had a positive effect on the students’ work. For the context of the experiment, students that used the SRT scored almost three quarters of a point higher than the students who did not use the SRT, but the results were not statistically different as seen in Table 4.3. This finding was a bit surprising at first because it is clearly stated in the laboratory report rubric and mentioned throughout the course, but the results suggest that students who used the SRT included the context of the experiment more often and with greater depth. Likely, the act of articulating the context of the experiment on the SRT not only prompted students to include it in their laboratory reports, but also prompted students to include more details in this section.
<table>
<thead>
<tr>
<th>SRT Impact</th>
<th>SRT Average</th>
<th>SRT Standard Deviation</th>
<th>No SRT Average</th>
<th>No SRT Standard Deviation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Research/Objective</td>
<td>2.08</td>
<td>0.41</td>
<td>2.05</td>
<td>0.60</td>
</tr>
<tr>
<td>Context</td>
<td>1.67</td>
<td>1.13</td>
<td>0.95</td>
<td>0.89</td>
</tr>
<tr>
<td>Hypothesis</td>
<td>2.08</td>
<td>0.78</td>
<td>1.75</td>
<td>1.16</td>
</tr>
<tr>
<td>IV and DV</td>
<td>1.88</td>
<td>0.61</td>
<td>1.50</td>
<td>1.10</td>
</tr>
<tr>
<td>Restate Hypothesis</td>
<td>2.13</td>
<td>1.12</td>
<td>1.75</td>
<td>1.48</td>
</tr>
<tr>
<td>Claim</td>
<td>2.21</td>
<td>0.41</td>
<td>2.00</td>
<td>0.97</td>
</tr>
<tr>
<td>Evidence</td>
<td>1.96</td>
<td>0.55</td>
<td>1.95</td>
<td>0.60</td>
</tr>
<tr>
<td>Reasoning</td>
<td>1.96</td>
<td>0.55</td>
<td>1.70</td>
<td>0.80</td>
</tr>
</tbody>
</table>

**Table 4.3** Results from the SRT Impact Rubric. Students were scored from 0 to 3. Reports with the SRT (n=13) and without the SRT (n=10). IV – Independent variable, DV – dependent variable

While this was an initial study, the results from the SRT Impact Rubric provide insight into changes that can be made in the CHEM 3030L course that may improve course outcomes. Most importantly, language from the SRT Impact Rubric can be integrated into the laboratory report rubric to clarify expectations and assessment. Student surveys and instructor interviews provide the student and instructor perspective and attitudes towards the tool which are a valuable source of feedback for the course redesign process.
4.3.2 Student Survey Results

The majority of the students took the student surveys in January and March. For the group of students who used the SRT, 27 out of 50 responded in January and 41 out of 50 responded in March. For the group of students who did not use the SRT, 39 out of 40 students responded in January and 33 out of 40 responded in March. A limitation of this initial study is the lack of tracking student responses between the two survey dates. In future studies, student survey responses will be coded and linked to student majors to compare the same population of students from the beginning of the semester to the end of the semester and to determine if there is a difference between chemistry and chemical engineering majors. Within this initial study, we expected that students would report that they were very confident in their writing abilities and connecting the experiment to the context of the greater world. This is supported by the results for Questions 1 and 2 from the two student groups as seen in Figure 4.8. Both the students who were using the SRT and the control group had virtually the same average, 3.85 ± 0.91 and 3.82 ± 1.00 respectively when asked about their confidence in writing formal laboratory reports. Both groups had a slight increase in confidence across the semester. Although not statistically significant, the results suggest that students may have gained confidence in writing formal laboratory reports over the course of the semester. When asked about their ability to explain the importance of the experiment beyond the course, again the results were very similar for both groups and shows that they were very confident, but the group that used the SRT had a slight increase from January to March and the group that did not use the SRT actually exhibited a slight decrease, but it was negligible, only 0.03 points. While these results are not statistically significant, it may suggest that students who used the SRT gained confidence in their ability to put the experiment in context over the course of the semester.
Figure 4.10 Student survey results from January and March 2016 comparing sections that used the SRT (50 students in spring 2016) and did not use the SRT (40 in spring 2016). SRT Jan n=27, SRT Mar n=39, no SRT Jan n=41, no SRT Mar n=33. Student results were not tracked between the two survey dates and thus, the population surveyed in January is not the exact same population surveyed in March. Error bars represent one standard deviation. Q1-Q4 refer to the 4 questions shown in Figure 4.7

We also expected that students would be very confident in using the SRT due to the design of the tool, but we also expected there would be some resistance in using the tool. This is reflected in the results from
Questions 3 and 4 as seen in Figure 4.10. There was virtually no change from January to March which was slightly unexpected, but may be due to the small number of students surveyed. One would expect that over time, students would become even more confident in using the tool. While we expected that students would be slightly resistant to the SRT, we did not expect that they would report that it was only somewhat useful and that their feelings remained unchanged. These results can be further explained by the freehand comments students made on the surveys in March. Twelve students provided written feedback. Student comments on the SRT were also almost all negative, but provided some constructive criticism including the following comments.

“The SRT was not a helpful exercise when compared to the natural process of research as understood at our age.”

“The scientific reasoning tool did not help with writing lab reports. The grading rubric is most useful.”

“The scientific reasoning tool is somewhat helpful with outline the information, but it seems very elementary at this point in our education. I feel like this technique would be better in the general chem labs. More often than not our group fills out the sheet right before we turn in our lab and it doesn't assist us in writing our reports.”

“Reasoning tool is okay. It helps you understand the goal. The results section doesn't seem useful.”

“I do not believe the scientific reasoning tool is very beneficial to lab and should be avoided”

“The scientific reasoning tool serves as a good introduction to the scientific method. I am not in middle school. I am a 5th year engineering student.”
The few positive comments about the SRT referred to its use in group reports and guiding student thoughts while working on the experiment and laboratory report as shown below. Comments from students to the TAs or instructors during class were mostly negative or neutral. Very few students expressed positive attitudes towards the tool.

“Seems more useful in group reports”

“Reasoning tool is helpful. It helps guiding student thoughts.”

The SRT may have been perceived as extra work and not an essential component of the laboratory report. The SRT was not graded for points, which may have been less motivating. In addition, the strong connection between the SRT and the laboratory reports may not have been stressed during class by the TAs and the instructors. Incorporating the part of the SRT into the actual laboratory manual or changing the format of the SRT may have provided more continuity between the laboratory experiment and the SRT. In addition, further clarifying language to link the parts of the SRT to the laboratory report may provide further alignment.

4.3.3 Instructor Survey Results

The three instructors who taught the CHEM 3030L Instrumental Analysis Laboratory Course in Spring 2016 were interviewed to gain more information about the history of the course as well as the impact of the laboratory report rubrics, SRT, and other components of the course. In terms of the laboratory report rubrics, the faculty felt that it made the TAs job easier and there were fewer complaints from students about grading, especially between sections. They did not feel that it created more consistency among TAs because some
took their jobs more seriously than others even with the rubric, causing inflated grades. The instructors also reported that some of the TAs were very resistant at first, but later they liked using the rubric. The other TAs were neutral about the rubric. They also reported that there were a few students who resisted the rubric at first and said it was too detailed, but then accepted it.

When asked about the impact of the SRT, the instructors expected that it would lead to higher quality laboratory reports. One instructor thought that the tool makes the process of scientific thinking more obvious, even if they have been doing it and they were not aware. In addition, the instructor felt that it helped bring different types of students to the same level with something common to work on. Another instructor thought that it may have an impact in other years, but for spring 2016, they thought that the TAs cared very little and did not know the theory behind the instruments, so the TAs set the tone for the course and did not value the SRT. The instructors did not report any student feedback about the tool and also mentioned that the TAs were neutral and did not complain about the tool.

The instructor interviews are extremely valuable because it shows that the success of the SRT may partially be a result of the instructor and TA motivation and to what degree they value the tool. Further conversations with the instructors and TAs before the semester starts about the importance of talking about the SRT, the rubric, and the laboratory report guidelines may lead to them valuing the SRT more which would show the students that they should also value the tool.
4.4 Conclusion

The redesign of the CHEM 3030L Instrumental Analysis Course in fall 2014 involved several changes to laboratory experiments, course materials, and assessment strategies. The SRT was developed and implemented in half of the sections during spring and summer 2016 to determine how it impacted scientific argumentation and writing skills within student laboratory reports corresponding to the achievement of course learning outcomes. ICP-MS laboratory reports were evaluated by two raters, external to the course, using a rubric designed to assess the impact of the tool. In addition, students were surveyed twice during the semester to measure their attitudes and confidence in writing lab reports and using the tool (if applicable). Instructors were also interviewed to provide historical context to the course and perceptions on the impact of changes made in the course. The results of this initial study suggest that while students did not necessarily enjoy using the SRT and some found it too simple or “elementary”, it may have had a slight positive impact on their scientific argumentation skills as demonstrated in their ICP-MS laboratory reports. This directly supports students in achieving the learning outcomes, in particular using chemical literature and demonstrating understanding of the principles and applications of instrumentation.
Chapter 5: Conclusions and Future Directions

5.1 Conclusions

This body of work demonstrates how complex modern instrumentation such as ICP-MS can be used not only in research laboratories, but also with undergraduate researchers and students. The metalloproteomics research described in this work in Chapter 2 was partially completed by undergraduates. They not only prepared samples, but learned the theory behind ICP-MS, ran the instrument, performed data analysis, and helped design experiments which may not be common in research laboratories. The ICP-MS experiment described in Chapter 3 is the first published undergraduate ICP-MS experiment in chemistry, to our knowledge. The hope is that other colleges and universities will implement this experiment or a variation of the experiment in undergraduate laboratory courses or use the sample data set as a way to teach students about the fundamentals of ICP-MS. Finally, part of the undergraduate chemistry curriculum is dedicated to students’ ability to communicate and engage in scientific discourse and utilize their knowledge of scientific principles and instrumentation through application. The SRT was created to support course learning outcomes in an effort to increase students’ scientific argumentation and writing skills within the new ICP-MS laboratory experiment.

The addition of new instrumentation is changing the nature of scientific research and industry, and colleges and universities must adapt to prepare students for their future careers. By exposing students to ICP-MS through undergraduate research and coursework, they become more prepared for the workforce. Many industrial positions for routine trace metal analysis are held by scientists with bachelor’s degrees. Whether students pursue a career in trace metal analysis or not, exposure and use of the instrumentation provides them with greater understanding of potential applications they may experience in their careers.
5.2 Future Directions

5.2.1 Metalloproteomics analysis of *Histoplasma capsulatum*

The results of the cell lysis work described in Chapter 2 will be continued with *H. capsulatum* to identify metalloproteins, particularly zinc metalloproteins. Proteins in the cell lysates will be tryptically digested and analyzed by LC-MS/MS to provide peptide fragmentation information. A species-specific protein database and metalloprotein annotation server (MPAS), constructed from the transcriptome of *H. capsulatum*, will be used for the protein identification process, as it adds valuable metal-ion binding information. The MPAS along with the ICP-MS data provides further support for metalloprotein identification.

In addition, multi-dimensional separation using SEC and Anion Exchange Chromatography prior to analysis by LC-MS/MS as well as solution based Iso Electric Focusing (IEF) allows for relative quantification of metalloproteins as well as quantification of metals corresponding to the proteins by ICP-MS. The comparison of Zn metalloproteins in *H. capsulatum* grown under varying conditions can also provide protein targets that are most affected by Zn stress. These targets can be further studied to develop a means to disrupt the Zn pathway, leading to a targeted therapeutic.

In addition, the process used to develop a suitable cell lysis method for metalloproteomics can also be used for other organisms, especially other microbes. Little research has been published on optimizing cell lysis methods for metalloproteomics. A wide range of organisms need to be studied to determine which methods may be applied to certain groups of organisms such as yeast, plants, fish, etc. In addition, new cell lysis techniques can also be developed to address the challenges of metalloproteomics, particularly
metalloprotein preservation. Currently, a similar process is being applied to bean roots as well as fish flesh for metalloprotein identification studies related to mercury uptake and storage in aquaponics systems.

5.2.2 CHEM 3030L Instrumental Analysis Course Redesign

Course redesign is an iterative process and a major component of course redesign is critical reflection. Through the work described in Chapter 3 or 4, changes to the Instrumental Analysis Course have been made and assessed. Several changes to the course will be made for the spring 2017 semester involving course materials as well as assessment strategies. The laboratory report rubric will be revised to include clarifying language for different performance levels as well as stronger alignment between the SRT and the laboratory report rubric. The SRT will be reformatted and used in half of the sections of the course and the laboratory reports will be assessed to determine the impact on students’ scientific argumentation and writing skills. Additional changes to create more consistent and representative assessment include assigning one TA per experiment for grading across all sections. Students will also submit laboratory reports electronically which will allow for the use of anonymous grading as well as electronic rubrics. These changes will lead to increased grading efficiency and transparency between sections of the course. Further improvements to our study design include further tracking of student responses between laboratory reports and survey data. In addition, student work and survey responses will be linked to chemistry and chemical engineering majors to determine how the SRT impacts the two populations. The student survey will also be expanded to include open ended response questions and students will be surveyed closer to the beginning of the semester.
5.2.3 Alternative ICP-MS Experiments

Due to insurance concerns at smaller institutions and the use of highly toxic substances, such as arsenic, additional ICP-MS laboratory experiments need to be developed. The American Chemical Society does provide a Chemical Educators Legal Liability policy to protect undergraduate teachers but the cost may not be covered by the school where they teach. Arsenic was chosen for the original experiment due to the environmental regulations and argon chloride interference. Additional experiments will be developed to analyze less toxic elements such as copper, iron, selenium, or titanium which all have a number of inferences and found in many consumer goods and throughout the environment.

At the time of submission, Chapter 2 has been submitted to Analytical and Bioanalytical Chemistry and is under review. Chapter 3 has been published in the Journal of Chemical Education. The work of Chapter 4 is ongoing and will be published within the next few years.
References

25. Brown, D. A.; Cook, R. A., Role of metal cofactors in enzyme regulation. Differences in the regulatory properties of the Escherichia coli nicotinamide adenine dinucleotide phosphate specific malic enzyme, depending on whether magnesium ion or manganese ion serves as divalent cation. Biochemistry 1981, 20 (9), 2503-2512.


58. Papanayotou, I.; Sun, B.; Roth, A. F.; Davis, N. G., Protein aggregation induced during glass bead lysis of yeast. *Yeast* 2010, 27 (10), 801-816.
A comparison of consensus, consistency, and measurement approaches to estimating interrater reliability

Practical Assessment, Research, and Evaluation

Stemler, Steven E

2004

9(4)

Journal Article

2004, 9 (4)
Appendix A: Quick Guide for Agilent 7700

- Turn the **gases on** (large valve on tanks) Argon and Helium.
- Turn chiller on
- Put the waste tubing (light yellow) and sample tubing on the peripump.
  - Make sure both pieces of tubing are round (there are 2 sections on each piece of tubing you can use) If both sections are flat, tie a knot in the tubing and throw it out. Extra tubing is in the drawer labeled ICPMS tubing.
  - Make sure that the waste comes from the instrument, goes around the peripump and out to the waste container.
  - Make sure that the sample tubing goes from the autosampler, around the peripump, and into the instrument. Lock the peripump.
- ICP-MS software, ICP-MS Instrument Control, click instrument control
  - Make sure you see the autosampler. If you don’t you can add it in sample introduction.
- Autosampler:
  - Make sure the autosampler is on
  - Uncover tray, uncover solutions
  - Make sure the DDI water and 2% nitric acid solutions are full
  - Move autosampler probe to position 1 (DI water)
    - Click the big autosampler button, and click position 1, Go to. The probe/needle will move to the DI bottle
  - Make sure the rinse port DI water bottle is full
  - Make sure the rinse port waste bottle is empty (can go down sink)
  - Double check that the ICPMS waste bottle on the floor is not full (must be neutralized with acid waste
☐ Turn on the ICP-MS plasma, click the small black arrow next to plasma, click plasma on

☐ Make sure the ignition sequence box is UNCHECKED

☐ WAIT 20 minutes for the ICP-MS is warmed up

☐ While the instrument is warming up, find the group of students that is working on the ICPMS and have them make their batch on the instrument. A batch name will be the date and initials. Example: Anna ran on Jan 14th 2015, so my batch name is 011415_AMD

☐ Creating a batch (while the instrument is warming up for 20 minutes)

☐ Click on the black arrow next to batch, new batch folder

☐ Make sure the batch is saved in 3030L Spring 2015 folder

☐ Create from an Existing batch

☐ Click Select

☐ Click the 3030L ICPMS Experiment batch (located inside the 3030L Spring 2015 folder), click open, make sure all boxes are checked, click ok

☐ Put in batch name (Ex: 011415_AMD)

☐ Click Create

☐ Tuning the instrument after 20 minutes of warming up – batch tune, no gas mode and He mode

☐ Fill a vial with tune solution, put it in position 3 of the autosampler

☐ Move the autosampler probe to position 3, wait until the solution gets into the instrument (you will see the air bubble moving through the tubing)

☐ Inside the batch, you will see 3 tabs: Acquisition Method, Data Analysis Method, and Sample List

☐ There is a Tune tab under Acquisition Method (you will see no gas mode and He mode)

☐ Make sure No gas mode is checked

☐ Click send to ICP-MS (this will send the parameters to the instrument)
- Click Autotune (if may ask if the probe is in tune solution it should be in position 3, then click ok)
- When the autotune is done, it will produce a tune report which will be saved in the batch folder. You may encourage students to put this in their report.
- Now click on the He mode
- Click send to ICP-MS (this will send the parameters to the instrument)
- Click Autotune (if may ask if the probe is in tune solution it should be in position 3, then click ok)
- When the autotune is done, it will produce a tune report which will be saved in the batch folder. The counts for \( m/z \) 59 should be at least 5000, if the instrument is trying to tune and counts are really low (1000 or less), the sample tubing may be too stretched out, switch to the other section of tubing. If both are stretched out, replace the tubing. New tubing is in the drawer labeled ICPMS tubing. Tie a knot in the old tubing and throw it away.
- **YOU NEED TO RINSE THE PROBE after you tune**
  - Click on autosampler, click rinse port (keep it there for 15 seconds)
  - Move autosampler probe to DI water (position 1) for 20 seconds
  - Move autosampler probe to 2% nitric (position 2) for 20 seconds
  - You can leave the probe in DI water (position 1) while the students finish making solutions. Just make sure it is full of DI water!
- **BE SURE TO CLOSE THE TUNE SOLUTION BOTTLE TO LIMIT CONTAMINATION!**
- After students have exported their data:
  - Move the autosampler position to home
  - Undo the peripump tubing
  - Click the small black arrow next to plasma, click plasma off
- Turn off the chiller
- Close the gas tanks
Appendix B: Experiment Manual

University of Cincinnati CHEM 3030L
Experiment 11: Inductively Coupled Plasma – Mass Spectrometry
Determination of Arsenic in Sinus Wash and Tap Water

Introduction

In this lab, you will analyze commercially available sinus wash as well as tap water (the latter which you can supply → be sure to note the source of the water) for the amount of arsenic. You will be required to do a little bit of research and find out if any regulations exist for arsenic in water. The instrument we will employ for this analysis, the Inductively Coupled Plasma-Mass Spectrometer (ICP-MS), couples an inductively coupled plasma ionization source with a mass spectrometer for ion separation and detection. This particular instrument, the Agilent 7700 is equipped with a collision cell. Such an apparatus is used to eliminate certain polyatomic interferences. For example, arsenic is monoisotopic at mass 75. The ICP-MS uses argon gas to generate the plasma. As some samples may contain a high level of chlorine, there exists the potential for the formation of $^{40}\text{Ar}^{35}\text{Cl}^+$ in the argon plasma, which would mask low levels of arsenic. In order to eliminate this interference, the collision cell can be used by pressurizing it with helium gas. When the sample passes through the collision cell, the helium collision gas is more likely to collide with the larger diameter $\text{ArCl}^+$ ion, lowering its kinetic energy so that it cannot proceed to the detector.

Background


ICP-MS: Ch.21, Internal Standard: Ch. 5
Hypothesis

Construct your hypothesis/hypotheses before the lab period. Try to relate your hypothesis to the instrument, regulations, and the samples. Create your hypotheses as “if…, then…” statements.

1.

2.

3.

Experimental

Supplies

16 x 10-mL volumetric flasks with stoppers
3 x 10-ml beakers, one for concentrated acid, one for the germanium internal standard (40 ppb) and one for the arsenic standard (100 ppb)
2 x 50mL beakers for the sinus wash and tap water samples
1 x 150 mL beaker for DDI for diluting
17 x acid washed ICP-MS autosampler vials
Eppendorf pipets (200μL and 1mL, *Note: One 200μL pipet has been designated for nitric acid)
Pipet tips (200μL and 1mL)

Equipment

Agilent 7700 Inductively Coupled Plasma-Mass Spectrometer

Reagents

Nitric acid
100 ppb Arsenic standard
40 ppb Germanium standard
Sinus wash-supplied by TA’s
Tap water-supplied by your group
Sample preparations

When using the ICP-MS, matrix matching is crucial for a proper analysis. All standards and samples need to have a final nitric acid concentration of 2% by volume (20 mL of concentrated (70%) nitric acid per liter of solution). *IMPORTANT NOTE* When pipetting concentrated nitric acid, use the pipet designated for nitric acid! We do not want to corrode the new pipets! In addition, you need to add germanium internal standard so that each standard and sample has a final concentration of 1 ppb Ge.

1. Prepare your sinus wash samples first in quadruplicate. Pipet 8mL of sinus wash into each of your 4 volumetric flasks (S1, S2, S3, and S4) using the 1mL Eppendorf pipet. Add the appropriate amount of Ge internal standard, nitric acid (using the nitric acid pipet), and dilute to the mark with DI water. You will notice that the solution appears cloudy which is caused by a reaction with the acid that produces a gas. Consider the ingredients in the sinus wash which may cause this reaction. You may want to discuss this in your report. After you secure the caps on the flasks, you want to mix them well every few minutes and vent the flask to reduce the amount of gas in solution.

2. Prepare your tap water samples in a similar manner. Pipet 8mL of your tap water sample into each of your 4 volumetric flasks (T1, T2, T3, and T4) using the 1mL Eppendorf pipet. Add the appropriate amount of Ge internal standard, nitric acid (using the nitric acid pipet), and dilute to the mark with DI water.

3. Prepare a calibration curve using DI water. Add the appropriate amount of Ge internal standard, nitric acid (using the nitric acid pipet), and dilute to the mark with DI water.

All the flasks and caps should already be labeled so you won’t waste time matching them up!
**Calibration curve:**

<table>
<thead>
<tr>
<th>As Concentration (ppb)</th>
<th>Volume of 100ppb As stock (mL)</th>
<th>Volume of 40ppb Ge internal standard stock (mL)</th>
<th>Volume of concentrated nitric acid (mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0ppb</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.2ppb</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.5ppb</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1ppb</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2ppb</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>5ppb</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>10ppb</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>25ppb</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

4. Transfer your samples and standards into labeled acid washed ICP-MS autosampler vials. Split your 0ppb standard into two separate vials, 0ppb A and 0ppb B. Place the vials in positions 1 through 17 on the autosampler tray next to the ICP-MS.

**Experimental Procedure**

1. The TA will turn on the ICP-MS and tune the instrument at the beginning of lab. Everyone in your lab group should observe/help out!
2. Create a new batch by clicking the small black arrow next to batch. Make sure your batch will be saved in the 3030L Spring 2015 folder. Create the batch from an existing batch called 3030L ICPMS Experiment batch (located inside the 3030L Spring 2015 folder), click open, make sure all boxes are checked, click ok. *IMPORTANT NOTE* Name your batch in the following manner: date (mmddyy_your initials). Example, Anna ran the instrument on Jan 14, 2015, she named her batch 011415_AMD. This will avoid confusion when you save your data by providing a unique identifier. Finally click Create. There are three tabs in the batch: Acquisition mode (contains acquisition parameters and tune), Data Analysis Method (contains levels of your calibration curve), and Sample List. Click on sample list. Make sure that the sequence starts with two 0ppb samples (A and B) followed by the rest of your calibration curve and samples. Make sure that the samples correspond to the correct position on the autosampler. Also, assure that the correct levels are in place for your calibration curve (0ppb is Level 1, 0.2ppb is Level 2, etc). Finally, add the data files by using your batch name followed by a number. Example: 011415_AMD_01, 011415_AMD_02, 011415_AMD_03. When you have verified that all parts of the batch are correct, click save batch, then click add to queue to start the run. While the instrument is running, make sure your calibration curve is linear. Once you determine that your data looks okay by verifying with the TA, you can dispose of the calibration standards and samples as well as any leftover nitric acid and stock solutions in the acid waste bottle and rinse all the glass with DI water and place it back in the experiment box.

As your standards and samples are being analyzed consider the following:

- The linearity of your calibration curve.
• Evaluate the results both with and without the use of the collision cell. Compare the sinus wash to the tap water in both modes (no gas mode and He mode).

• Consider the % recovery of the internal standard. (You can view this graph by clicking the Internal Standard Stability Graph button in the lower left section of the page). You should export this graphic as a jpeg to include in your report (right click, export, export graphic).

3. Clean up your workspace and all your glassware. You can dispose of the calibration standards and samples as well as any leftover nitric acid and stock solutions in the acid waste bottle and rinse all the glass with DI water and place it back in the experiment box. **You do not need to remove the sharpie labels from items going in the experiment box.**

4. After the analysis is complete, go into the data analysis software and remove the first sample that was run (0ppbA) by checking the box to the left of the sample, then reprocess the data. Remove any outlier points on the calibration curve (you can click on the point on the graph to remove it) and reprocess the data. Right click on the chart and export table. Save it as an excel file in your batch folder and on a flash drive. Again, you also want to save the internal standard stability graphic as well.

**Data Analysis/Calculations**

• Consider your $R^2$ value of your calibration curve. Is it reasonable?
• Compare the results obtained for arsenic in sinus wash and tap water obtained both with and without collision cell. Compare these levels with each other and with regulatory levels set by the EPA (be sure to cite your source!) Discuss.

• This quantitation was done with an internal standard. What are the advantages of this technique?

• What results would the concentration be with an external calibration?

• The samples were run in quadruplicate. What is the purpose of this? What is an advantage of running quadruplicates instead of triplicates?

• Calculate the average arsenic value of the samples (taking into account the dilution), as well as the standard deviation and % RSD (relative standard deviation) for each sample type.

• Include a copy of all instrument printouts, raw data, sample calculations and laboratory notebook pages relevant to these experiments in your report.
Appendix C: Instructor Notes for ICP-MS Experiment

Instrumentation and data analysis

It is recommended that the TA/instructor review the principles of the ICP-MS instrument prior to the course. A short description can be found in Ch. 21 of Harris, D.C., Quantitative Chemical Analysis, 9th Edition, 2016. The inductively coupled plasma is a high temperature plasma discharge used to generate positively-charged ions and represents a very efficient way in which to ionize before mass spectral analysis. In the extreme conditions of an inductively coupled plasma, the sample aerosol is desolvated, atomized, and ionized once the smallest droplets of the aerosol produced by the nebulizer are brought from the Peltier cooled spray chamber to the argon plasma. Ions formed are guided through the collision cell and separated by mass-to-charge by a quadrupole mass filter and quantified by their naturally abundant isotopes with an electron multiplier detector. ICP-MS is susceptible to the formation of polyatomic ions in the high energy plasma and these may mask analytes of interest if the masses overlap as a result of the poor mass to charge separation of the quadrupole mass filter.

It is important to note that the nickel cones, spray chamber, and torch should be monitored for salt buildup. Additionally, students’ calculations for acid concentrations should be checked prior analysis to prevent undue damage to the cones as well as irreproducible results.

While an autosampler is not necessary for this experiment, many students have not had the opportunity to use an autosampler. As our instrument was equipped with an autosampler, we regarded this as an opportunity to discuss how an autosampler works including the necessary cleaning steps between samples.
The raw data presented by the ICP-MS is in two forms: counts per second (cps) and ppb. It is valuable, but not necessary to discuss the difference between the two, but the students should be aware of how the software creates the calibration curve and calculates the concentration of each sample. In addition, students need to calculate a dilution factor to determine the concentration of arsenic in the original sample. In designing this experiment, a small dilution factor was desired due to the low arsenic concentration in the samples. Practicing calculating dilution factors as well as discussing why dilution factors are necessary is a helpful exercise.

It may be useful to review data analysis and basic statistical analysis before students conduct the experiment. Such concepts include: dilution calculations, average, standard deviation, percent relative standard deviation, and Grubb’s test.

**Constructing a Hypothesis**

Many students struggle to create a hypothesis for an experiment if the objective of the experiment is not to find a right or wrong answer, but to discover something that was previously unknown. Constructing a hypothesis as an “if… then…” statement can help students focus their thoughts. Several hypotheses can be constructed for this experiment with some more obvious than others. The most obvious hypothesis is that if the ICP-MS is run in He mode and no gas mode by way of argon plasma ionization for the quantitation of arsenic in sinus wash and tap water, no gas mode will produce an inflated signal for arsenic due to the ArCl+ interference of arsenic at \( m/z \) 75. Students can create a more robust hypothesis by adding that the higher chlorine content in sinus wash will show a larger interference effect in the signal between no gas mode and He mode. Another basic hypothesis is that if the tap water is from a public water system, then the concentration of arsenic is expected to be below the EPA limit of 10 ppb As. Students can be challenged
when asked to write a hypothesis that compares the tap water and sinus wash and struggle to come up with a reason why one has a higher amount of arsenic. A careful look at the packaging can help students construct this third hypothesis. Sinus wash is typically made with distilled water to limit bacteria growth. Distilled water should have a lower level of metals than tap water. If the sinus wash is properly prepared with distilled water, then it will contain less arsenic than tap water which contains low levels of metals.

It is good teaching practice to have students construct the hypotheses before the lab period, as many students try to write a hypothesis after the data has been collected. This can also provide an opportunity to discuss how scientists construct hypotheses based on prior knowledge and follow the scientific method to conduct experiments. The lab manual for this experiment has been altered for future use by including a hypothesis section to be completed before the lab period. In addition, students should not be discouraged if their hypothesis is incorrect. This provides an opportunity to discuss how the data can be explained and provides an opening for students to suggest further experiments to verify a new hypothesis.

**Sample Preparation**

Typical undergraduate experiments require a certain level of care during sample preparation. Due to the ability to detect analytes at very low levels, small amounts of contamination can be detected by the ICP-MS. Many students have not worked in such an environment where even putting a cap face down on the bench can cause contamination. A review of proper handling of chemicals and sample preparation may be beneficial before the experiment in order to limit contamination and dust particles which can easily clog the nebulizer.
Sinus wash may contain sodium bicarbonate which forms minute gas bubbles causing the sample to look cloudy when nitric acid is added. The experiment manual was designed so that students prepare the sinus wash samples first. It can be useful to alert students that the samples will look different when acid is added. The sinus wash packing may give students a clue as to what is happening in the sample. The samples can be degassed to some degree by inverting the flasks and venting.

This lab requires the repeated use of a piston-stroke pipet. If students are unable to use a pipet properly and fully depress the plunger, it is very obvious within the first few minutes of sample preparation because incorrectly pipetting 8mL of solution will fill a 10mL volumetric flask above the mark. It may be helpful to review pipetting technique before the experiment. For the purposes of our experiment, one 200 μL pipet was designated for use with nitric acid. It should be noted that there are pipets on the market with plastic coated parts to limit acid corrosion.

**Choice of Matrix**

As stated, one of the primary goals of this laboratory experiment was to illustrate the use of a collision cell when determining an element prone to polyatomic interferences. The ArCl\(^+\) polyatomic ion is a notorious problem in the analysis of arsenic in high chlorine matrices. We therefore sought an everyday sample that might be analyzed for arsenic, but that also contained a high chlorine composition. Those we investigated in the development of this laboratory protocol included club soda, mineral water, various energy drinks, pool water, tap water and two “fizzing” over the counter medications. We found that sinus wash (purchased at the local drugstore) gave the most dramatic results and required little sample preparation. Although we chose this matrix for our experiment, others may find alternate sample matrices of interest.
Connection to other experiments

The Instrumental Analysis Laboratory course at the University of Cincinnati offers eight hands on analytical experiments. Individual classes are capped at 24 students; students are asked to work in groups of three and over a period of eight weeks and each group spends a week (two 3.5 hour laboratory periods) on each analytical technique. Another of the laboratory experiments at this university utilizes a Flame Atomic Absorption Spectrometer to analyze for Zn in specialty cough drops. As different groups are performing each of these two elemental analyses, the TAs/instructors are asked to conversationally point out the strengths and limitations of each technique. Because FAAS is based on the absorption of a specific wavelength of light, it is not a simultaneous multi-elemental technique. Different elements can only be analyzed on this instrument once the appropriate lamp is placed and detection limits are found to be in the part per billion (ppb) range. In contrast, the ICP-MS offers multi-element capability often times at sub part per trillion (ppt) limits of detection and because it is based on atomic mass, identification is more definitive. These advantages come, of course, with a much higher initial and operating cost.

Materials Required

Nitric acid CAS # 7697-37-2

ICP-MS Arsenic Standard CAS # 7440-38-2

ICP-MS Germanium Standard CAS # 7440-56-4

Ultra High Purity Argon (>99.999% Purity)

Ultra High Purity He (>99.999% Purity)

Sinus Wash – saline based, made with distilled water
### Instrument Conditions

<table>
<thead>
<tr>
<th>Parameter</th>
<th>No gas mode</th>
<th>He mode</th>
</tr>
</thead>
<tbody>
<tr>
<td>RF Power</td>
<td>1550 W</td>
<td>1550 W</td>
</tr>
<tr>
<td>Carrier Gas</td>
<td>1.05 L/min</td>
<td>1.05 L/min</td>
</tr>
<tr>
<td>Makeup Gas</td>
<td>0.12 L/min</td>
<td>0.12 L/min</td>
</tr>
<tr>
<td>Sample Depth</td>
<td>8.0 mm</td>
<td>8.0 mm</td>
</tr>
<tr>
<td>Nebulizer Pump</td>
<td>0.1 rps</td>
<td>0.1 rps</td>
</tr>
<tr>
<td>S/C Temp</td>
<td>2 °C</td>
<td>2 °C</td>
</tr>
<tr>
<td>OctP Bias</td>
<td>-8.0 V</td>
<td>-21.0 V</td>
</tr>
<tr>
<td>He flow</td>
<td>0.0 mL/min</td>
<td>3.0 mL/min</td>
</tr>
</tbody>
</table>
Appendix D: Fall 2012 Lab Report Guidelines

Cover Page/Abstract (5 pts)
Your report should contain a cover page with the experiment title, your name, your lab partner’s names, your group letter and the date the experiment was performed. In addition, a one paragraph abstract summarizing the experiments done should be included.

Background (10 pts)
Provide a brief (1 page) description of the techniques used in the experiment and why the technique is important.

Experimental (5 pts)
Provide a description of the experimental procedure used in the study. Do not copy the experimental procedure verbatim from the lab manual. Rather, use your own words to explain how the experiments were performed.

Data (5 pts)
Raw numerical data, as well as any observations, instrument parameters, chemical information, etc. This must be separate from the Procedure section. Computer filenames and identities should be recorded here as well. If several chromatograms, etc. are collected for a given experiment, only one or two example spectra need be included in the notebook. A data table summarizing all data must be included for the complete data set.

Data Analysis/Results (10 pts)
Data tables are given in experimental instructions. Present an *organized* example of your calculations, with brief explanations provided when necessary. Include any plots and graphs and a table of summary data. Don't forget relevant statistical analysis and consideration of significant figures!

**Discussion and Conclusion (10 pts)**

This section is weighed most heavily when grading. Include a discussion of the significance of your results in terms of meaning, accuracy, precision and error analysis. Include any insights you feel are important as well as appropriate references.

**Additional Content (5 pts)**

Include copies of your notebook pages that are relevant for this experiment.

**References:**

Any books or journal articles cited should be indicated at the proper point in the text and should be included in the bibliography at the end of the report using proper presentation of literature citations.

**Questions:**

Answer any questions given on the experiment instruction sheet.

**Other reminders:**

- Be Prepared! Come to lab with an understanding of the experiment and with the Title and Procedure sections of your notebook complete and the Data section ready to go.
- Keep an updated Table of Contents for your notebook.
- Use only permanent ink in your notebook, correct errors by drawing a single line through the incorrect entry and initial the correction.
• The use of spreadsheets for data analysis and presentation is not only acceptable but also encouraged.

• Whenever inserting a computer generated plot or spreadsheet, affix a copy to both the original and carbon copy page in your notebook.

• Sign and date the lower right-hand corner of each page as it is used.

• Include in the Data section the brand, name, serial number and settings of all instruments as well as lot numbers and suppliers of all chemicals.
Appendix E: Formal Laboratory Report Guidelines

This guide provides a more detailed description of how to write a formal lab report. Your lab report should read as a seamless document. The only section that will be similar for you and your lab partners is the data section. All other sections are your own and should be written as such.

A hard copy of your lab report will be turned in. Electronic copies will not be accepted. The reports must be typed and all graphs/charts must be created on a computer. You must staple a blank copy of the rubric to the back of the lab report. Failure to include the rubric and/or failure to staple the lab report will result in points deducted.

Language and Style:

A laboratory report is an account of something that you have done, and should be written in the PAST tense, using a PASSIVE voice. Share all the necessary information as concisely as possible so that the reader could repeat your experiment and get the same results you did.

- Passive voice: Use passive voice instead of active voice.
  - Passive: Acetone (10 ml) was added to the mixture drop-wise.
  - Active: I added (10 ml) of acetone, one drop at a time.

- Past tense: When describing something that you have already done as part of the experiment, it is a past event so you should use the past tense.
  - Past tense: The temperature of the mixture was recorded after one hour.
  - Present tense: An increase in temperature occurs in one hour.
Format:

Your title should be distinctive and succinct, not Lab 2. Each section described below represents a section of your lab report. Each section should have the appropriate heading.

1. Abstract: (200-400 words)

This provides a summary of your report and your main conclusions. The abstracts will consist of a sentence or two of introduction which includes the objectives of your experiment, a description of the investigation to be conducted, methods, and rationale for the hypothesis you have made. It should be understandable without reference to the main text.

2. Introduction: (1-3 paragraphs)

This includes a brief overview of the experiment and emphasizes the significance of the work (why?) as well as the approach (how?). This section will end with your hypothesis. The hypothesis testable and differs from a guess in that it is based upon prior knowledge or evidence. It should be supported by previously developed evidence and/or concepts. The hypothesis should be the statement that drives your laboratory investigation. It can be useful to form your hypothesis in the form of an “if… then…” statement. If (hypothesis), and (method), then (expectation).
3. Materials and Methods:

This includes a list of the materials and equipment used in the lab. Be very specific and include quantities, concentrations, instrument type/brand/model, etc. Also include a detailed account of the basic procedure you followed and techniques you used in narrative form. (DO NOT COPY AND PASTE THE PROCEDURE). State any hazards that were encountered during the lab and precautions that were taken to avoid such hazards. Someone should be able to pick up your lab report and duplicate your work!

4. Data (Results and Calculations):

Here, you present your data in a way that is easy to read. Create a data table as appropriate for all observations and measurements. If you include a graph of data, make sure it has appropriate titles and labels. Show the work for any required calculations (example of each type of calculation) as well as appropriate units and significant figures. You can also include calculations derived from your data, including averages, highs and lows, percent error, percent yield, etc.

Do not hide or eliminate suspected faulty data (which can also include observations) but present it and note that you think it is faulty data. Later, in your discussion, you may explain why you have decided not to use suspected errors in your analysis. Good scientists present the data they obtain even when it is suspected to be faulty. They explain why they feel they are in error in the discussion of their results later. This is why a true experiment has many trials and much peer review occurs before results are accepted by the scientific community at large.
Data may be qualitative (observational without numbers) or quantitative (with numbers) or both. Often qualitative data may be used to support or explain discrepancies in quantitative data in your conclusion.

You must specify what was determined by the experiment at the end of this section. This can be done in a few sentences.

Note: Data and results refer to calculated data, raw data and spectra will be part of the supplementary materials

5. Discussion:

Here is where you discuss the significance of your results. You should summarize the important patterns, trends, or pieces of your data. You can also talk about faulty data, errors (both human error and experimental error). Discuss how you would do the lab differently next time. This is the section where you can be creative and talk about the story of this lab. Why is it an interesting story? Why is it important?

6. Conclusion

Summarize the important procedures and result(s) of the lab. Here is where you provide a summary of results and the corresponding the reasoning that supports or rejects your hypothesis. There should be a detailed discussion of why the hypothesis should be accepted or refuted (rejected). If there is a deviation from the expected results, that should also be discussed here.
A good conclusion does not merely restates the results or the procedure...it should tie together the entire experiment. Someone should be able to read your abstract, then your conclusion and know the purpose of your experiment, what you did, what you found, and how it relates to your hypothesis.

7. References

All sources of information used in your understanding and writing of the report MUST be listed in this section in the appropriate format. (Numbered references corresponding to superscripts in report) This should include the textbook (if one was used). Use the ACS format for all citations

Here’s a helpful website for the ACS style [http://library.williams.edu/citing/styles/acs.php](http://library.williams.edu/citing/styles/acs.php)

8. Supplementary material

Include all raw data, spectra, etc.

Rubric:

Attach a blank copy of the rubric to the back of your report and staple it all together. A missing rubric or lack of staple will result is points deducted.
**Plagiarism:**

Reports containing plagiarism will result in a grade of zero for the report and it will be reported to the instructor, the second offense will be reported to the Director of Undergraduate Studies for the Chemistry Department for review.

Selections from [http://www.uc.edu/conduct/Academic_Integrity.html](http://www.uc.edu/conduct/Academic_Integrity.html)

Plagiarism is an extremely serious violation of academic integrity. The Student Code of Conduct defines plagiarism as:

1. Submitting another's published or unpublished work, in whole, in part, or in paraphrase, as one's own without fully and properly crediting the author with footnotes, citations, or bibliographical reference.

2. Submitting as one's own, original work, material obtained from an individual or agency without reference to the person or agency as the source of material.

3. Submitting as one's own, original work, material that has been produced through unacknowledged collaboration with others without release in writing from collaborators.
Plagiarism can occur in myriad of forms and media. Although most commonly associated with writing, all types of scholarly work, including computer code, music, scientific data and analysis, and electronic publications can be plagiarized. The aim of this section is to help students and faculty deal with the complex and important issue of plagiarism on campus.

**Paraphrasing**

Like a direct quotation, a paraphrase is the use of another's ideas to enhance one's own work. For this reason, a paraphrase, just like a quotation, must be cited. In a paraphrase, however, the author rewrites in his or her own words the ideas taken from the source. Therefore, a paraphrase is not set within quotation marks. So, while the ideas may be borrowed, the borrower's writing must be entirely original; merely changing a few words or rearranging words or sentences is not paraphrasing. Even if properly cited, a paraphrase that is too similar to the writing of the original is plagiarism.

Good writers often signal paraphrases through clauses such as "Werner Sollors, in Beyond Ethnicity, argues that..." Such constructions avoid excessive reliance on quotations, which can clog writing, and demonstrate that the writer has thoroughly digested the source author's argument. A full citation, of course, is still required. When done properly, a paraphrase is usually much more concise than the original and always has a different sentence structure and word choice. Yet no matter how different from the original, a paraphrase must always be cited, because its content is not original to the author of the paraphrase.