Analysis of Inorganic Arsenic In Food Using X-Ray Fluorescence (XRF) Spectroscopy

Helen Lin
University of Massachusetts Amherst

Follow this and additional works at: https://scholarworks.umass.edu/masters_theses_2

Recommended Citation
https://doi.org/10.7275/28504747 https://scholarworks.umass.edu/masters_theses_2/1194

This Open Access Thesis is brought to you for free and open access by the Dissertations and Theses at ScholarWorks@UMass Amherst. It has been accepted for inclusion in Masters Theses by an authorized administrator of ScholarWorks@UMass Amherst. For more information, please contact scholarworks@library.umass.edu.
ANALYSIS OF INORGANIC ARSENIC IN FOOD USING X-RAY FLUORESCENCE (XRF) SPECTROSCOPY

A Thesis Presented

by

HELEN LIN

Submitted to the Graduate School of the University of Massachusetts Amherst in partial fulfillment of the requirements for the degree of

MASTER OF SCIENCE

May 2022

Department of Food Science
ANALYSIS OF INORGANIC ARSENIC IN FOOD USING X-RAY FLUORESCENCE (XRF) SPECTROSCOPY

A Thesis Presented

by

HELEN LIN

Approved as to style and content by:

Lili He, Chair

Hang Xiao, Member

Amanda Kinchla, Member

Lynne McLandsborough, Department Head
Food Science
DEDICATION

To my beloved family: my father who showed me utmost determination, my mother, and resilience from my sister Amy.
ACKNOWLEDGMENTS

I am more than happy to give thanks to those who have given me support and help throughout my six years of study in the Food Science department.

I am beyond grateful for my advisor Dr. Lili He for all the opportunities she provided for me: NSF Research Traineeship, lecturer for the Science of Food, and teaching assistant for Introductory Biology Lab which sustained and made my graduate study possible. Her patience and support have led me this far where I would not have been able to accomplish on my own. I would also like to thank the members of my committee members, Professor Amanda Kinchla and Hang Xiao for making this thesis possible.

I want to thank all my lab members for their support for the past three years. Thanks to Haochen Dai for his genuine and selfless advice on my research projects. Thanks to Caiping Jiang, Wanjun Gao, Shengnan Zhan for taking care of me while I was physically unwell.

I thank my friends Yunxi Zhang, Chengyun Li, Yuhan Sun, Wang Liao, Wenwei Liang for their unconditional love. I used to think of friends in terms of what they can offer me and the value they bring until I met them. Thanks for loving the sensitive, pessimistic, and insecure me. Thanks for being by my side since the day we met, supporting my reckless behavior even when you guys didn’t agree with me and help me get back on my feet. No matter how far apart we will be, I will always remember the love I was given.
ABSTRACT

ANALYSIS OF INORGANIC ARSENIC IN FOOD USING X-RAY FLUORESCENCE (XRF) SPECTROSCOPY

MAY 2022

HELEN LIN, B.S., UNIVERSITY OF MASSACHUSETTS AMHERST

M.S., UNIVERSITY OF MASSACHUSETTS AMHERST

Directed by: Dr. Lili He

Arsenic contamination in drinking water and foods is a prevalent concern across the world. Routine testing of inorganic arsenic ensures food safety but require a cost effective, rapid high throughput, and simple detection method. The objective of this work is to develop a green method using X-Ray fluorescence spectroscopy (XRF) to analyze inorganic arsenic (iAs) in food and their interaction with emerging food contaminants: microplastics and titanium dioxide nanoparticles. XRF measures the secondary X-ray that is characteristic to each element emitted by the sample.

In a prior study, we developed an approach that combines the Gutzeit method and elemental analysis using XRF for arsenic detection in food. This approach is based on a commercial mercury bromide strip to capture arsine gas. Concerning the high toxicity of mercury bromide, we explored the feasibility of using a greener chemical, silver nitrate, to replace mercury bromide. This would benefit the safety of the operating personnel and reduce chemical hazard impact on the environment. In addition, organic acids and zinc nanoparticles were explored for iAs detection. Optimization of various reagents were done to maximize the efficacy of iAs capture and detection. The result demonstrated the greener method has a lower of quantification (3.40 µg/L) compared to the original
method based on mercury bromide (16.2 µg/L) due to less elemental interferences in the XRF spectrum. The standard curves of water and apple juice were compared, no significant difference was found, suggesting matrix interference is minimal. The spiked apple juice with 0 to 133 µg/L iAs had a good recovery ranging from 85-99% with an average relative standard deviation below 20%, indicating decent reproducibility.

Other than iAs detection, we also explored the XRF to study the iAs and their interaction between microplastics and titanium dioxide nanoparticles, which are considered emerging contaminants of public concerns that may serve as vectors for pollutants and potentially enhances toxicity effects. We developed a screening method to quantify the adsorption under different conditions. The result showed iAs adsorption is highly dependent of particle size and surface morphology. In conclusion, this study demonstrates the feasibility and great potential of XRF quantification of inorganic arsenic in food matrices in a cost-effective and reliable manner and the capability of rapidly quantifying the interaction with emerging contaminants such as microplastics and titanium dioxide nanoparticles.
# TABLE OF CONTENTS

DEDICATION .................................................................................................................. iv

ACKNOWLEDGMENTS .................................................................................................. v

ABSTRACT .................................................................................................................... vi

TABLE OF CONTENTS .................................................................................................. viii

LIST OF TABLES ......................................................................................................... x

LIST OF FIGURES ....................................................................................................... xi

Chapter

1. **INTRODUCTION** .................................................................................................. 1

   1.1 Prevalence of arsenic contamination in the environment and the food system ......................... 1
   1.2 Arsenic toxicity and its impact on health .................................................................................. 2
   1.3 Inorganic arsenic (iAs) regulations ......................................................................................... 3
   1.4 Arsenic detection and quantification methods ......................................................................... 3
   1.5 X-ray Fluorescence Spectroscopy (XRF) ............................................................................ 4
   1.6 Goals and objectives of the study .......................................................................................... 5

2. **OPTIMIZATION OF THE GUTZEIT REACTION FOR XRF**

DETECTION .................................................................................................................. 6

   2.1 Introduction ..................................................................................................................... 6
       2.1.1 Principle of the Gutzeit reaction .................................................................................... 6
       2.1.2 Objectives of this study ............................................................................................... 6
   2.2 Materials and methods ...................................................................................................... 7
       2.2.1 Materials ..................................................................................................................... 7
       2.2.2 Standard curve establishment ..................................................................................... 7
       2.2.3 Inorganic arsenic determination in liquid samples ....................................................... 8
       2.2.4 X-ray fluorescence spectroscopy and data analysis ................................................... 8
   2.3 Results and discussion ..................................................................................................... 9
       2.3.1 iAs standard curve ..................................................................................................... 9
       2.3.2 Optimization of the Gutzeit reaction .......................................................................... 9
       2.3.3 Arsenic speciation .................................................................................................... 16
       2.3.4 Application of commercial arsenic test kit in red wine ............................................. 17
       2.3.5 Silver nitrate test disc ................................................................................................. 18
       2.3.6 Optimization based on silver nitrate test discs .......................................................... 19
3. EXAMINATION OF POLYSTYRENE MICROPLASTIC INTERACTION WITH IAS

3.1 Introduction.............................................................................................................28
  3.1.1 Microplastic source and concerns.................................................................28
  3.1.2 Adsorption of pollutants by microplastics ...................................................29
  3.1.3 Choice of microplastic......................................................................................30
  3.1.4 Titanium dioxide on iAs adsorption ..............................................................31
  3.1.5 Raman spectroscopy and application in microplastic analysis ......................31
  3.1.6 Objective of this study....................................................................................32
  3.2.1 Materials ......................................................................................................32
  3.2.2. Fenton aging treatment .............................................................................32
  3.2.3 Quantification of iAs adsorption .................................................................33
  3.2.4 Raman analysis of polystyrene microplastics .............................................33
  3.2.5 Scanning electron microscope (SEM) .........................................................33
  3.3 Results and discussion .....................................................................................33
    3.3.1 iAs adsorption on different vehicles .........................................................33
    3.3.2. iAs interaction with pristine polystyrene microplastics .........................35
    3.3.2 Pristine microplastic degradation using Fenton reagents .........................37
    3.3.3 Conclusion: ..............................................................................................39

CONCLUSION..............................................................................................................41

BIBLIOGRAPHY..............................................................................................................42
# List of Tables

<table>
<thead>
<tr>
<th>Table</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>Table 1. Limit of detection and quantification for mercury bromide and silver nitrate test discs.</td>
<td>25</td>
</tr>
<tr>
<td>Table 2. Recoveries and relative standard deviation of spike additions of iAs to apple juice. Mean concentrations reported as µg/L ± SD (n=3).</td>
<td>26</td>
</tr>
<tr>
<td>Table 3. iAs adsorption average and standard deviation of polystyrene microplastics (10 µm, 1 µm, 200 nm), polyethylene microplastics, titanium dioxide, and titanium dioxide nanopowder (15 nm).</td>
<td>35</td>
</tr>
</tbody>
</table>
LIST OF FIGURES

<table>
<thead>
<tr>
<th>Figure</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Figure 1.</td>
<td>Schematic figure of arsine gas generation and detection</td>
</tr>
<tr>
<td>Figure 2.</td>
<td>Correlation between XRF intensity and iAs concentration. Error bars are standard deviations (n=3).</td>
</tr>
<tr>
<td>Figure 3.</td>
<td>XRF peak intensities of arsenic at 15, 30, and 60 minutes. Error bars are standard deviation (n=3).</td>
</tr>
<tr>
<td>Figure 4.</td>
<td>XRF peak intensities for reactions with potassium iodide and without potassium iodide. Error bars are standard deviations (n=3).</td>
</tr>
<tr>
<td>Figure 5.</td>
<td>XRF peak intensities for reactions with potassium iodide, reactions and without potassium iodide, and reactions with potassium iodide substituted with water. Error bars are standard deviations (n=3).</td>
</tr>
<tr>
<td>Figure 6.</td>
<td>XRF peak intensities for reactions with tin chloride and without tin chloride. Error bars are standard deviations (n=3).</td>
</tr>
<tr>
<td>Figure 7.</td>
<td>XRF peak intensities for reactions with both potassium iodide tin chloride and without both potassium iodide tin chloride. Error bars are standard deviations (n=3).</td>
</tr>
<tr>
<td>Figure 8.</td>
<td>XRF intensities comparison between reactions: control, without KI, without SnCl2, and without both reagents. Error bars are standard deviations (n=3).</td>
</tr>
<tr>
<td>Figure 9.</td>
<td>XRF intensities comparison between control and reactions without wait time but extended reaction times at 30mins, 40 mins, and 45 mins. Error bars are standard deviations (n=3).</td>
</tr>
<tr>
<td>Figure 10.</td>
<td>(A)XRF intensities for reactions with different zinc powder size. (B) Comparison of XRF intensity and arsenic concentration on zinc size at &lt;150 μm, 40-60nm, and 80-100nm. Error bars are standard deviations (n=3).</td>
</tr>
<tr>
<td>Figure 11.</td>
<td>Comparison of XRF intensities between different organic acids. Error bars are standard deviations (n=3).</td>
</tr>
<tr>
<td>Figure 12.</td>
<td>Standard curve comparison mercury bromide and optimized conditions. Error bars are standard deviations (n=3). The significant difference between the curves were calculated by paired t-test (p &lt; 0.05).</td>
</tr>
</tbody>
</table>
Figure 13. XRF intensities for arsenic collected from reaction with inorganic and DMA both before and after washing with acetone. Error bars are standard deviations (n=3).......................... 17

Figure 14. (A) Arsenic concentration analysis comparison between XRF and commercial kit of red wine. (B) Red wine test strip color comparison to the color chart provided by the commercial test kit. .... 18

Figure 15. (A) XRF peak intensity of silver nitrate and mercury bromide blanks. (B) XRF peak intensity of silver nitrate and mercury bromide with 33 µg/L. Error bars are standard deviations (n = 3). Different letters for each column indicated a significant difference (p ≤ 0.05 one-way ANOVA). .......................................................... 19

Figure 16. (A) XRF spectrum of mercury bromide test strip with 133 µg/L iAs captured. (B) XRF spectrum of mercury bromide test strip with 0 µg/L iAs captured. ........................................................... 21

Figure 17. (A) XRF spectrum of silver nitrate test strip with 133 µg/L iAs captured. (B) XRF spectrum of mercury bromide test strip with 0 µg/L iAs captured. ........................................................... 22

Figure 18. (A) XRF peak intensities for 133 µg/L of arsenic collected with different silver nitrate concentrations. (B) XRF peak intensities for arsenic collected at different min reaction times. (C) XRF peak intensities for 133 µg/L of arsenic collected with different concentrations of sulfuric acid. (D) XRF peak intensities for 133 µg/L of arsenic collected with different amounts of zinc powder. Error bars are standard deviations (n = 3). Different letters for each column indicated a significant difference (p ≤ 0.05 one-way ANOVA). .......................................................... 24

Figure 19. Plot of XRF peak intensity as a function of iAs concentration for mercury bromide and silver nitrate test discs. Error bars are standard deviations (n = 3). Paired t test significant difference set at p ≤ 0.05. .......................................................... 25

Figure 20. Plot of XRF peak intensity as a function of iAs concentration for water and apple juice with silver nitrate test discs. Error bars are standard deviations (n = 3). Paired t test significant difference set at p ≤ 0.05. .......................................................... 26

Figure 23. Comparison of XRF intensities of pristine polystyrene microplastics (10 µm, 1 µm, 200 nm), degraded polyethylene microplastics, titanium dioxide, and titanium dioxide nanopowder
(15 nm) incubated with 20 µg/mL arsenic. Error bars are standard deviations (n=3). ................................................................. 35

Figure 21. A comparison of XRF intensities for 106 microplastics in 10 µg/mL arsenic solutions 1, 3, 5, and 7 days where control is microplastics in double distilled water. Error bars are standard deviations (n=3). ................................................................. 36

Figure 22. A comparison of XRF intensities for 106 microplastics in 10 µg/mL arsenic solutions at pH of 3, 6, and 9 for 1, 3, and 5 days where control is microplastics in double distilled water. ......................... 37

Figure 24. Comparison of XRF intensities of polystyrene microplastics and Fenton treated polystyrene microplastics incubated with 10 µg/mL iAs. Error bars are standard deviations (n=3). ................................. 38

Figure 25. SERS spectra of polystyrene microplastics and polystyrene microplastics treated with Fenton for 7 days. ........................................... 39

Figure 26. (A) SEM images of pristine polystyrene microplastics in water. (B) microplastics treated with Fenton’s reagent. Both solutions were placed under UV light (365 nm) for seven days. ................................. 39
Chapter 1

INTRODUCTION

1.1 Prevalence of arsenic contamination in the environment and the food system

Arsenic is a heavy metal with a long history of use as a poison. Pollution sources are from both anthropogenic and geogenic activities. Arsenic containing pesticides and herbicides accumulate in soil and water. Coal/ fossil fuel burning, volcanic eruptions and mining actives, leachate bring out arsenic from Earth’s crust (Nachman et al., 2017). Arsenic contamination in drinking water is prevalent concern affecting South and Southeastern Asian countries and the Americas. It is estimated over 150 million people in Bangladesh and India suffers from high levels of As from well water (Baghel, Singh, Pandey, & Sekhar, 2007). The transition from surface water to tube wells in Bangladesh for water source in hopes to reduce microbial contamination resulted in mass arsenic poisoning (Gundert-Remy et al., 2015). This tragedy raised attention of arsenic contamination worldwide.

Crops absorbs iAs from pesticide application and/or contaminated water used for irrigation. Rice, being a staple food product for over 2 billion people in Asian households is a notorious culprit for human arsenic intake (Ismail Hassan & Niaz, 2017). Arsenic enters rice through contaminated water used for irrigation and contaminated water used for cooking the rice. Rice takes up more inorganic arsenic comparing to other crops. Brown rice contains 80% more inorganic arsenic than white rice because iAs is mostly stored in the germ layer (Ismail Hassan & Niaz, 2017). Consequently, brown rice arsenic concentration is higher than white rice. Bioavailability of iAs in cooked rice is over 90% (Ismail Hassan & Niaz, 2017). Seafood contains the highest amount of total arsenic
(6,000 µg/kg wet weight) with mostly arsenobetaine (AsB) but undetectable iAs (Tyson, 2013). However, seaweed contains high arsenic concentration. Algae dietary supplements contains as high as 6134 µg/kg iAs (Gundert-Remy et al., 2015). Adults consume a variety of foods while infants are limited to baby food with ingredients that are known to have high concentrations of arsenic. Infants are exposed to arsenic three times more than adults (Tyson, 2013). Both high exposure and dosage leads to public health concerns.

1.2 Arsenic toxicity and its impact on health

The most toxic form of As is arsenite (iAs\textsubscript{III}) followed by arsenate (iAs\textsubscript{V}), then organic arsenic (oAs) species such as monomethylarsonate (MMA\textsubscript{V}), dimethylarsenate (DMA\textsubscript{V}), arsenobetaine (AsB), and arsenochline (AsC)(Tyson, 2013). oAs species are generally considered less and even nontoxic to humans as the DMA and MMA LD\textsubscript{50} = 890-10,600 mg/kg body weight while AsB LD\textsubscript{50} > 10,000 mg/kg body weight and is mostly excreted through urine (Luvonga, Rimmer, Yu, & Lee, 2020). Recent studies stated MMA\textsubscript{III} and DMA\textsubscript{III} is more toxic than iAs (Luvonga et al., 2020). For regulation purposes, this paper solely focuses on iAs detection and quantification.

Long term arsenic exposure results in diabetes, neurotoxicity, cardiovascular diseases, skin, lungs, bladder, and or kidney cancers (Bustaffa, Stoccoro, Bianchi, & Migliore, 2014). It causes long term adverse pregnancy outcomes such as stillbirths and spontaneous abortion. Infants exposed to arsenic during pregnancy and after birth are at greater risk of cancers mentioned previously (Upadhyay, Shukla, Yadav, & Srivastava, 2019). Arsenic species substitutes zinc and changes protein and more than 200 enzymes structures leading to loss of functions involved in DNA transcription, synthesis, and repair (Gundert-Remy et al., 2015; Ratnaike, 2003). Arsenic induce epigenetic
alterations, tumor promotion, chromosomal aberrations, and DNA strand breakage (Bustaffa et al., 2014; Gundert-Remy et al., 2015). Arsenic react with cells in the body by replacing phosphate in ATP synthesis, thus affecting ATP formation and leads to neurological and cardiovascular damages (Ismail Hassan & Niaz, 2017). A dose between 0.3 to 8 µg/kg body weight/day is estimated to have a 1% increase rate of cancers (Gundert-Remy et al., 2015).

1.3 Inorganic arsenic (iAs) regulations

The US EPA limits iAs concentration in drinking water at 10 µg/L. iAs species are not distinguished as iAsV reduces to iAsIII within cell. European Commission and US Food Drug Administration set guideline on arsenic levels in infant rice cereals at 100 µg/kg. The only country that has strict arsenic limit in food is China, with maximum contaminant level of iAs at 200 µg/kg. FDA should update standards and have strict limit for arsenic in different types of foods.

1.4 Arsenic detection and quantification methods

The Association of Official Agricultural Chemists’ (AOAC) standard method for arsenic quantification is by Inductively coupled plasma-mass spectrometry (ICP-MS). To do so, arsenic species must be separated because ICP-MS will detect all arsenic species while the law solely focuses on inorganic arsenic species. This could be accomplished by ion chromatography (IC) or high-performance liquid chromatography (HPLC). Though HPLC detection limit goes below µg/L, it requires trained personnel to perform experiments and the equipment is expensive. Despite cost reasons and technical performance, it is also time consuming. It would be inefficient and chaotic when large
number of samples require testing. A rapid detection method of iAs in water and foods is on demand. Current rapid detection methods are modification of the Gutzeit method acid zinc redox to generate arsine gas that is captured by mercury bromide test disc, resulting a yellow/brown stain (Eq. 1 and 2).

\[
\begin{align*}
\text{AsO}_3^{3-} + 3\text{Zn} + 9\text{H}^+ & \rightarrow \text{AsH}_3 + 3\text{Zn}^{2+} + 3\text{H}_2\text{O} & \text{(1)} \\
\text{AsH}_3 + 3\text{HgBr}_2 & \rightarrow 3\text{HBr} + \text{As(HgBr}_3) & \text{(2)}
\end{align*}
\]

The color is then matched to a calibration chart. The darker the color, higher the arsenic concentration in the sample. Firstly, distinguishing color by human eye is unreliable. Another limitation exists in matrix interference. For example, red wine contains sulfite compounds that will darken the color of the strip. The reading gives a much higher iAs concentration than there is in the sample. Though cotton balls soaked with lead acetate are used to block sulfite interference, the cotton ball density blocks arsine gas from forming a complex with mercury bromide. The current state of work has been using computer software to analyze RGB values of the test strips and is able to achieve a detection limit near 5 µg/L(Kearns & Edson, 2018).

1.5 X-ray Fluorescence Spectroscopy (XRF)

XRF is a non-destructive, rapid high throughput, elemental based detection, and quantification instrument. X-ray of sufficient energy knockout electrons from the sample. Once an electron emits, an electron from the outer shell fills the vacancy and releases energy as a fluorescent X-Ray. XRF measure and analyze the energy that is distinct to each element. The instrument can distinguish and quantify multiple elements simultaneously. The peak energy on the spectra tells the element identity while the height indicates concentration. A calibration standard must be established using this instrument.
The calibration sample matrix should be representative of samples that are being measured. Measured samples must have an elemental concentration within the calibration range.

In recent years, the food industry has been utilizing XRF to classify geological origin of tea (Rajapaksha et al., 2017) and coffee (Worku et al., 2019). Another application is determination of mineral profile in matrices like coco powder (Herreros-Chavez, Cervera, & Morales-Rubio, 2019), dry pet foods (Perring et al., 2017), milk and dairy products including infant milk powder (Herreros-Chavez, Morales-Rubio, & Cervera, 2019; Pashkova, 2009). XRF detection has advantages over colorimetric methods and inductively coupled plasma mass spectrometry (ICP-MS). Neither does it does not falsely quantify arsenic concentration like colorimetric method nor require chemicals for analysis like ICP-MS.

1.6 Goals and objectives of the study

A green chemistry detection method based on XRF will be developed for arsenic contamination in different matrices. This will be achieved by accomplishing the following:

Objective 1: Develop and optimize green chemistry approach for iAs quantification using XRF.

Objective 2: Detect and quantify iAs in food matrices.

Objective 3: Analyze the interaction between iAs and microplastics.
Chapter 2

OPTIMIZATION OF THE GUTZEIT REACTION FOR XRF DETECTION

2.1 Introduction

Chapter 1 discussed the importance of arsenic detection and ways to quantify arsenic in samples. Previous work done by He’s lab (Z. Zhang et al., 2021) has demonstrated the feasibility of iAs detection by XRF. The goal of this research was to develop a simple green test kit to detect arsenic in water and complex matrices. This was accomplished by using XRF to eliminate colorimetric interferences and unreliable results from the commercial test kit. The green chemistry approach makes in situ detection safer and is more sustainable.

2.1.1 Principle of the Gutzeit reaction

\[
\begin{align*}
H_3AsO_4 + 3I^- + 2H^+ & \rightarrow H_3AsO_3 + I_3^- + H_2O \quad (3) \\
H_3AsO_4 + Sn^{2+} + 2H^+ & \rightarrow H_3AsO_3 + Sn^{2+} + H_2O \quad (4) \\
AsO_3^{3-} + 3Zn + 9H^+ & \rightarrow AsH_3 \uparrow + 3Zn^{2+} + 3H_2O \quad (5) \\
AsH_3 + 3HgBr_2 & \rightarrow 3HBr + As(HgBr)_3 \text{ (yellow)} \quad (6)
\end{align*}
\]

Potassium iodide and tin chloride both facilitate the reduction of As(V) to As(III), which in turn speeds up the generation process of arsine gas (Eq. 3, 4). Zinc reduces arsenic trioxide to As\(^{3-}\), the acidic environment protonates As\(^{3-}\) to form arsine gas (Eq. 5). Mercury bromide captures arsine gas generated and forms a yellow to brown stain (Eq. 6).

2.1.2 Objectives of this study

The objectives of this study were to: (1) develop and optimize the Gutzeit method using XRF and (2) establish a green method for iAs quantification in liquid matrices.
2.2 Materials and methods

2.2.1 Materials

Zinc powder (Zn), tin (II) chloride (SnCl2), potassium iodide (KI), cacodylic acid (dimethylarsinic acid), oxalic acid, ascorbic acid, citric acid, and mercury bromide (HgBr2) test strips were purchased from Sigma-Aldrich (St. Louis, USA). Sulfuric acid was purchased from Fisher Scientific (Hampton, NH). Apple juice and red wine were purchased from local supermarkets. Double distilled water was used to dilute solutions. All arsenic stock solutions were diluted to 1 µg/mL. Arsenic detection commercial arsenic test kit (481396-W) was purchased from Industrial Test Systems (York City, SC).

2.2.2 Standard curve establishment

Calibration curve was established by serial dilution of 1 µg/mL Arsenic solution of 0.0, 0.2, 0.4, 1.0, 2.0 mL with DI water to achieve a final volume of 15mL for each standard. The dilution standard concentrations would be 0, 3.4, 6.7, 13.4, 33.3, 66.7, and 133.3 µg/L. 5 mL potassium iodide (KI, 20%), 220 µL tin chloride (SnCl2, 40% m/V), and 10 mL of sulfuric acid (H2SO4, 1.5M) were added and let sit for 15 mins. After that, zinc powder was added, and the flask was capped immediately with the gas outlet tube with a holder. The mercury bromide test disc was put in the holder to trap arsine gas. After 30 mins, the mercury bromide test disc was removed and scanned by XRF Epsilon 1, Marvel Panalytical (Figure 1). The best fit of the linear relationship between concentration and the area of the arsenic Kα line was established by least squares regression (Prism 8).
2.2.3 Inorganic arsenic determination in liquid samples

To detect unknown arsenic concentrations in liquid sample such as apple juice, the same procedure follows as mentioned in the previous section but with 15 mL of the sample instead.

2.2.4 X-ray fluorescence spectroscopy and data analysis

The mercury bromide test disc was placed in XRF. The $K_a$ peak was used for quantification. The XRF peak intensity value was plotted into the standard curve to obtain an arsenic reading.
2.3 Results and discussion

2.3.1. iAs standard curve

A standard curve (Figure 2) was established based on the protocol.

![Mercury Bromide Standard Curve](image)

Figure 2. Correlation between XRF intensity and iAs concentration. Error bars are standard deviations (n=3).

2.3.2 Optimization of the Gutzeit reaction

Firstly, XRF intensities at different reaction times were examined. This step is crucial as it gives information on when reaction plateaus, meaning most arsine gas has been collected. Reaction time of 30 minutes is sufficient to generate and collect arsine gas (Figure 3). The XRF intensity at 15 minutes is significantly lower than reaction times of 30, 60, and 75 minutes.
Figure 3. XRF peak intensities of arsenic at 15, 30, and 60 minutes. Error bars are standard deviation (n=3).

The second step is to determine the necessity of potassium iodide and tin chloride. Both are used to facilitate reduction of arsenate (As(V)) to arsenite (As(III)). The stock solution used was in the form of As(III). The two reagents were theoretically unnecessary to speed up reaction time. As seen in Figure 4, XRF intensity for reaction without potassium was higher than the control. To ensure this occurrence was independent of dilution effects, potassium iodide was substituted with water (Figure 5). This water substitution resulted in higher XRF peak intensity. Therefore, it is concluded potassium iodide lowered arsenic readings. One explanation could be that iodide hindered arsenic reading in XRF because it has a dense electron shell and XRF was not calibrated for iodine. It absorbed some of X-ray emitted to sample.
Figure 4. XRF peak intensities for reactions with potassium iodide and without potassium iodide. Error bars are standard deviations (n=3).

Figure 5. XRF peak intensities for reactions with potassium iodide, reactions and without potassium iodide, and reactions with potassium iodide substituted with water. Error bars are standard deviations (n=3).

Tin chloride on the other hand, is crucial for arsine gas generation. XRF intensity for reactions without tin chloride is about five times lower than the protocol (Figure 6). It is deemed essential for XRF analysis of inorganic arsenic. For the reaction labeled acid and zinc, reaction time was extended to 45 minutes because the 15-minute wait time was unapplicable, and all reactions should have a total of 45 minutes of reaction time. The XRF intensity is about five times lower than the control (Figure 7). XRF analysis cannot rely solely on the acid-zinc redox reaction to generate gas. The reaction must be
facilitated by tin chloride as shown in Figure 8, where reactions without potassium iodide, tin chloride, potassium iodide and tin chloride.

Figure 6. XRF peak intensities for reactions with tin chloride and without tin chloride. Error bars are standard deviations (n=3).

Figure 7. XRF peak intensities for reactions with both potassium iodide tin chloride and without both potassium iodide tin chloride. Error bars are standard deviations (n=3).

Figure 8. XRF intensities comparison between reactions: control, without KI, without SnCl2, and without both reagents. Error bars are standard deviations (n=3).
Comparing potassium iodide and tin chloride importance, it is decided potassium iodide could be neglected when detecting As(III). The XRF peak intensity is significantly higher when potassium iodide was excluded. The two reactions without tin chloride are about five times lower than the control and reaction without potassium iodide.

Theoretically, the 15-minute wait time is unnecessary because the stock solution is in the form of As(III). We were interested to see if the XRF intensity would change if the 15-minute wait time was removed and extended reaction time. Without wait time and a total reaction time at 30 mins, XRF intensities were lower than the control group. However, the XRF intensities were higher than the control group with reaction time of 45 minutes (Figure 9).

![Figure 9. XRF intensities comparison between control and reactions without wait time but extended reaction times at 30mins, 40 mins, and 45 mins. Error bars are standard deviations (n=3).](image)

With the idea of nanotechnology, we hypothesized zinc nanopowder can increase reaction efficiency because of larger surface area. Zinc powder with sizes less than 150 μm, 80-100 nm, and 40-60 nm were used to capture 133 μg/L of arsine gas. Zinc nanopowder (40-60nm) yielded a significant difference (Figure 10A). At 15 min reaction time, zinc nanopowder reached the chemical reaction plateau while zinc powder (<150
μm) was still reacting. As seen in Figure 10B, the different zinc powders display no difference at low concentrations. However, the slopes are significantly different (p = 0.0052). An explanation is the increased surface area of zinc nanopowder speeds up chemical reactions and as a result reaches reaction equilibrium faster. However, zinc nanopowder was not included in the optimization process because it is ten times more expensive than zinc powder <150 μm. Cost was taken into consideration while designing this method for in situ detection. Thus, this data is included to demonstrate how nanotechnology improves reaction efficiency.

![Figure 10. (A)XRF intensities for reactions with different zinc powder size. (B) Comparison of XRF intensity and arsenic concentration on zinc size at <150 μm, 40-60nm, and 80-100nm. Error bars are standard deviations (n=3).](image)

Acid is another variable that was investigated for the purpose of green chemistry. Sulfuric acid is a strong acid with a very low pH that is difficult to dispose in situ.

Therefore, different green acids were tested in hopes to achieve a similar XRF intensity of sulfuric acid. The sole purpose of acid in a redox reaction is oxidizing agent. Thus, higher reduction potentials of the acids were preferred. Three different acids were tested: ascorbic acid, citric acid, and oxalic acid. The acids were chosen for use at their maximum solubility because other work demonstrated success in using those reagents (Baghel et al., 2007). Oxalic acid was observed to achieve the highest intensity compared
to the two other green acids (Figure 11). However, the XRF intensity for oxalic acid is half of sulfuric acid. Then, a combination of oxalic acid and ascorbic were tested and it yielded similar XRF intensity as the sulfuric acid. The data was difficult to replicate afterwards, so it was also excluded from the final optimization.

![Figure 11. Comparison of XRF intensities between different organic acids. Error bars are standard deviations (n=3).](image)

Two curves were established using the mercury bromide test discs (Figure 12). One was before optimization and the other optimized under previously mentioned conditions. The two curves are statistically significant ($p = 0.0014$), meaning the optimized mercury bromide curve has a higher XRF intensity when the iAs concentration is the same, making detection at lower concentrations feasible.
2.3.3 Arsenic speciation

Organic arsenic species must be distinguished from inorganic arsenic species during XRF analysis because current regulations are only focused on inorganic arsenic. It was unexpected that DMA were also collected onto the test discs, shown in Figure 13. This could be the volatile reaction product gas adhered onto the strips. XRF does not distinguish inorganic arsenic and organic arsenic but total arsenic concentration. This yields a high reading for inorganic arsenic though it was a DMA solution. An interesting note on the strips with DMA was colorless. Organic arsenic does not react with mercury bromide to form color. It was easily removed from the strips by rinsing with acetone. Inorganic arsenic remains on the strip and is not significantly affected by acetone wash. This method is powerful in terms of quantifying both species.
Figure 13. XRF intensities for arsenic collected from reaction with inorganic and DMA both before and after washing with acetone. Error bars are standard deviations (n=3).

2.3.4 Application of commercial arsenic test kit in red wine

We compared the results of our proposed method and a commercial test kit for red wine samples (Figure 14A). Sulfites in red wine interferes with colorimetric methods because hydrogen sulfide darkens the color of the test strip (Figure 14B). The red wine sample analyzed by XRF gives a concentration of 4.6 μg/L of arsenic while the test kit gives an arsenic concentration of about 30 μg/L. This experiment was later validated with ICP-MS with total arsenic concentration at 6.7 μg/L.
2.3.5 Silver nitrate test disc

A substitute for mercury bromide in arsenic analysis is silver nitrate. Though an acute and chronic hazard to the aquatic environment, skin corrosion, and more expensive compared to mercury bromide, it is less dangerous to the operating personnel. Many studies demonstrated the feasibility of silver nitrate as an effective capture substitute. With that concept in mind, we would like to see the compatibility of silver nitrate using XRF analysis. The objective of this study is to replace mercury bromide with silver nitrate for reaction safety, stability, and cleaner XRF spectrum background. In this study, we further optimized the Gutzeit reaction based on previous work and established a green method for in situ iAs quantification. This method is applied to apple juice samples to demonstrate applicability to different liquid matrices. Arsine gas in contact with silver nitrate gives a brownish grey colored compound on the test disc (Eq. 9).

\[
\begin{align*}
H_3\text{AsO}_4 + Sn^{2+} + 2H^+ & \rightarrow H_3\text{AsO}_3 + Sn^{2+} + H_2O \\
\text{AsO}_3^{3-} + 3Zn + 9H^+ & \rightarrow \text{AsH}_3 \uparrow + 3Zn^{2+} + 3 H_2O \\
\text{AsH}_3 + 3 \text{AgNO}_3 & \rightarrow 3\text{HNO}_3 + \text{AsAg}_3 \text{(grey)}
\end{align*}
\]
2.3.6 Optimization based on silver nitrate test discs

We compared the XRF intensities of silver nitrate and mercury bromide blank (Figure 15A) and arsenic concentration at 33 µg/L (Figure 15B). The blank of silver nitrate had a lower XRF intensity reading comparing to the mercury bromide blank. When arsenic concentration is at 33 µg/L, the two XRF intensities are not statistically significant. Though silver nitrate test disc intensity for iAs is significantly lower, this does not mean there is a lower amount of iAs. Quantification is dependent on the standard curve. This demonstrates silver nitrate is just as effective as mercury bromide at capturing arsine gas.

![Blank comparison](image)

**Figure 15.** (A) XRF peak intensity of silver nitrate and mercury bromide blanks. (B) XRF peak intensity of silver nitrate and mercury bromide with 33 µg/L. Error bars are standard deviations (n = 3). Different letters for each column indicated a significant difference ($p \leq 0.05$ one-way ANOVA).

XRF Spectrum analysis is important when there are high concentrations of multiple elements. The overlapping fluorescent lines could lead to the misidentification of peaks. Arsenic Kα line was chosen for the identification because the peak is significant and easily distinguished from other peaks. Figure 16A shows a XRF spectra of mercury bromide strip with 133 µg/L iAs, where arsenic Kβ line is overlapped with the mercury peak. Figure 16B is a blank mercury bromide strip and the arsenic Kα deconvolution is
shifted. Silver nitrate is free of these two issues as seen in Figure 17A (silver nitrate test strip with 133 µg/L iAs) and Figure 17B (blank silver nitrate test strip). Silver nitrate has a lower blank reading due to cleaner spectra and less elements were present to alter deconvolution.
Figure 16. (A) XRF spectrum of mercury bromide test strip with 133 µg/L iAs captured. (B) XRF spectrum of mercury bromide test strip with 0 µg/L iAs captured.
The first optimization was done on silver nitrate test disc. The concentration of silver nitrate should be enough to efficiently capture arsine gas in sample but not excessive for disposal purposes. In Figure 18A, 0.01 M silver nitrate showed no significant difference compared to 1 M of silver nitrate. 0.01 M silver nitrate will not be saturated with 133 µg/L iAs and it is important to avoid having the test disc to be the
limiting factor. Therefore, future experiments will utilize 0.01 M silver nitrate. Arsenic concentration of 133 µg/L was used for experiment optimization to ensure there are enough reagents to react with the iAs in unknown samples.

Reaction time was then optimized. It was observed the 35 minutes of reaction time was not statistically significant from 60 minutes (Figure 18B). Therefore, the following experiments will be performed at 35 minutes.

Sulfuric acid was used to generate redox reaction. 3 M sulfuric acid was used in our previous work. For green chemistry purposes, we decreased the acid concentration. It is observed 1 M was just as effective as 3 M in our study (Figure 18C). Referring to Figure 18D, 0.8 g of zinc powder has high variation and XRF intensity is significantly lower than 1, 2, and 3 g of zinc powder. This indicated the reaction was ongoing and 0.8 g of zinc was insufficient for reaction completion. 1 g of zinc powder is not statistically significant from 3 g of zinc powder. We deemed 1 g of zinc is sufficient to react with the concentration of acid used.
2.3.7 Comparison of mercury bromide and silver nitrate

The two standard curves were established from 0 to 33 µg/L. The LOD and LOQ for mercury bromide is 4.86 µg/L and 16.2 µg/L, respectively (Table 1). As for silver nitrate, the LOD and LOQ are 1.02 µg/L and 3.40 µg/L, respectively. Silver nitrate has LOD and LOQ below 10 µg/L (the maximum allowable concentration in water set by FDA and WHO), which is better than mercury bromide because its LOQ exceeded the limit. The t test between the two slopes were calculated and p value is 0.0327, indicating the two reagents used for iAs capture is different. In Figure 19, there is evident difference
at low concentrations until there is an overlap at 16.67 µg/L. Less deviation is observed in silver nitrate, which is the reason for lower LOD and LOQ.

Table 1. Limit of detection and quantification for mercury bromide and silver nitrate test discs.

<table>
<thead>
<tr>
<th></th>
<th>Mercury bromide</th>
<th>Silver nitrate</th>
</tr>
</thead>
<tbody>
<tr>
<td>Limit of detection</td>
<td>4.86</td>
<td>1.02</td>
</tr>
<tr>
<td>Limit of quantification</td>
<td>16.2</td>
<td>3.4</td>
</tr>
</tbody>
</table>

Figure 19. Plot of XRF peak intensity as a function of iAs concentration for mercury bromide and silver nitrate test discs. Error bars are standard deviations (n = 3). Paired t test significant difference set at p ≤ 0.05.

2.3.8 Apple juice spike and recovery analysis

Using the optimized reaction conditions and a switch to silver nitrate test discs, standard curves for water and apple juice were established (Figure 20). At concentrations above 16.67 µg/L, the XRF intensity for apple juice is lower than the intensity of water. However, the two curves were not significantly different (p < 0.0862), indicating this quantification method is applicable to apple juice matrix. Table 1 shows apple juice iAs recovery and relative standard deviation from 0-133 µg/L. The recovery was high (98% and above) for concentrations lower than 16.67 µg/L. Accurate quantification at around 10 µg/L is crucial because it is the set limit of iAs in beverages. The relative standard
deviation (RSD) represents reproducibility, it varied from 7.4-24.5%. Though RSD is high for 10 and 16.67 µg/L, the average RSD is reasonable,

An iAs concentration was found to be at 2.25 µg/L in unspiked apple juice using the method proposed in this study. This value is very close to the iAs concentration of 2.7 µg/L from previous work. (Z. Zhang et al., 2021) Zhang’s result was validated by ICP-MS, where total arsenic concentration is 4.60 µg/L.

Matrix comparison between water and juice

![Figure 20. Plot of XRF peak intensity as a function of iAs concentration for water and apple juice with silver nitrate test discs. Error bars are standard deviations (n = 3). Paired t test significant difference set at p ≤ 0.05.]

Table 2. Recoveries and relative standard deviation of spike additions of iAs to apple juice. Mean concentrations reported as µg/L ± SD (n=3).

<table>
<thead>
<tr>
<th>Concentration added (µg/L)</th>
<th>Concentration found (µg/L)</th>
<th>Recovery (%)</th>
<th>RSD (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.0</td>
<td>2.92</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>6.7</td>
<td>6.6 ± 1.1</td>
<td>98</td>
<td>17.5</td>
</tr>
<tr>
<td>10</td>
<td>10.2 ± 0.4</td>
<td>98</td>
<td>21.7</td>
</tr>
<tr>
<td>16.7</td>
<td>17.1 ± 2.4</td>
<td>98</td>
<td>24.5</td>
</tr>
<tr>
<td>33.3</td>
<td>39.2 ± 0.72</td>
<td>85</td>
<td>7.4</td>
</tr>
<tr>
<td>66.6</td>
<td>61.5 ± 8.4</td>
<td>92</td>
<td>16.8</td>
</tr>
<tr>
<td>133.3</td>
<td>134.4 ± 15.6</td>
<td>99</td>
<td>13.2</td>
</tr>
</tbody>
</table>
2.4 Conclusion

An iAs quantification method was established by arsine gas capture using the Gutzeit method and XRF for analysis. Both mercury bromide and silver nitrate can be used to capture arsine gas after optimization. Numerous conditions were investigated to increase reaction efficiency for As(III) detection. Standard curves were established as reference for future sample measurements. This method has the potential to become the newest rapid *in situ* iAs detection worldwide for its accuracy and ease of use. After the successful method application to apple juice and red wine, future work can include iAs quantification in solid food samples such as rice and seaweed.
Chapter 3

EXAMINATION OF POLYSTYRENE MICROPLASTIC INTERACTION WITH INORGANIC ARSENIC

3.1 Introduction

3.1.1 Microplastic source and concerns

Plastic brought forth technology advancement and convenience and earned itself an irreplaceable and fundamental position. The US Environmental Protection Agency (US EPA) reported 35.7 million tons of plastic were produced in 2018 waste in the United States alone. In the same year, the landfill in the United States received 27 million tons of plastic. Accumulation of plastics in the environment leads to fragmentation known as microplastics, defined as plastic less than 5 mm in size (Lang et al., 2020). Primary plastics are intentional manufacture uses in exfoliating beads for personal care and detergents. Secondary microplastics are plastic degraded through ultraviolet radiation, thermal treatment, weathering, oxidation, and biodegradation.

Concerns regarding microplastics are their ubiquitous presence, longevity, and potential toxicities. With its great functionality and durability comes a huge environmental concern. The service time of plastic could be as short as minutes, but its entire lifespan could be hundreds of years and have long-lasting impact on the environment. Microplastic threatens agricultural sustainability and food security on a global scale (Kwon et al., 2014; Sun et al., 2021). The distinctive nature of microplastics manufacture and morphology, chemicals used for enhancement in performance, durability, and degradation in plastics will leach into the surrounding environment. For
example, bisphenol A, flame retardants, stabilizers, and pigments. Microplastics migrate through the ecosystem through landfill leachate and improper disposal. Industrial discharge such as synthetic textiles releases microplastics into river streams used for irrigation or released into the ocean. Contamination of the food supply happens through prey misidentification and or unintentional consumption, then through the phenomenon of trophic transfer. Microplastics end up in wastewater treatment plants (WWTP) and concentrate in water and sewage sludge which are used for watering crops and fertilizers on agricultural soil. Seafood such as bivalves, shellfish, and fish exposed to microplastics showed adverse physiological and behavioral changes such as internal abrasions and blockages, reproductive dysfunctions, feeding efficiencies (Martinez-Tavera et al., 2021). Human microplastic consumptions are reportedly sourced from drinking water, salt, seafood, and crops grown in contaminated soil and water (Ding et al., 2020; Parker et al., 2020). Concern arises when microplastics were detected in human stool samples (N. Zhang, Li, He, Zhang, & Ma, 2021). Ingested microplastics cause physical harm by accumulation in the gastrointestinal tract and translocate across the gut to secondary organs (Wright & Kelly, 2017). The adverse effects of microplastics in humans are not fully revealed, Investigation must take place because of the uncertainty of microplastic impact on human health.

3.1.2 Adsorption of pollutants by microplastics

Weathered microplastics occur naturally in the environment as mentioned previously. Small particles have large surface area, allowing for the colonization of pathogenic bacteria and the adsorption of toxic pollutants such as heavy metals and hydrophobic organic pollutants such as DDT (Engler, 2012; Lin, Kuo, & Lo, 2021).
Microplastics were reported to increase the bioavailability and accumulation of the coexisting contaminants and exacerbate toxicity (Lu, Qiao, An, & Zhang, 2018). We will study microplastics adsorbed with arsenic because of its prevalence and our expertise on arsenic detection.

3.1.3 Choice of microplastic

Polystyrene has a wide range of applications in food packaging, disposable cutlery, medical products, laboratory ware, electronics, and toys. It is the most common type of microplastics found in freshwater and polystyrene can account for over 60% of all microplastics at sampling sites. (Ding et al., 2020; Mani et al., 2019; Wagner et al., 2014) Polystyrene will be studied in this project because it has been found in paddy fields, sewage sludge, and water sources that may be used for irrigation (Dong, Gao, Song, & Qiu, 2020; Li et al., 2018) The polystyrene microplastics should be deliberately aged to mimic real-life conditions such as oxidation, mechanical forces, and ultraviolet degradation. The aging process will change both the chemical and physical properties of plastics, altering surface area and morphology, hydrophobicity, particle size, and reactivity. This will be accomplished using Fenton reagents as it occurs naturally in the environment, and it is more effective than treatments with hydrogen peroxide only which generally used for the aging process. As for microplastic size, 10 µm polystyrene microspheres will be used because 10-500 µm microplastic are often found in sewage sludge (EL Hayany et al., 2020).
3.1.4 Titanium dioxide on iAs adsorption

Titanium dioxide is a chemically inert white pigment often used in paint, sunscreens, personal care, and foods. Titanium dioxide is a great photocatalyst for organic and inorganic compounds because it is safe and an inexpensive adsorbent (Bang, Patel, Lippincott, & Meng, 2005). Many studies have used titanium dioxide to adsorb iAs (López Paraguay, Cortes, Pérez-Robles, & Alarcón-Herrera, 2014). Similar to microplastics, iAs adsorption is dependent on the vector particle size and surface area (Gupta et al., 2013). Titanium dioxide iAs adsorption will be investigated in this study.

3.1.5 Raman spectroscopy and application in microplastic analysis

Raman spectroscopy is a rapid and non-destructive analytical technique. An excitation laser is shone at the sample and results in light scattering. When incident light frequency equals the frequency of light being scattered, it is known as Rayleigh scattering. The light scattered with a frequency different from the incident light is known as Raman (inelastic) scattering. Raman spectroscopy provides spectra with information on molecular fingerprints. Qualitative analysis is achieved through Raman shift wavenumbers as different molecules have their characteristic peaks, thus identifying sample composition. Quantitative analysis is given by the intensity of the characteristic peaks. Raman spectroscopy has an advantage over Fourier Transform Infrared Spectroscopy (FT-IR) and pyrolysis GC/MS, which are the current standards used for microplastic detection and identification. Raman spectroscopy has an established use over the past few years for microplastic detection down to 1 µm where the previously mentioned instruments’ capacity is above 20 µm. Raman spectroscopy successfully detected microplastics in various types of samples including water and wine (Prata et al.,
2020), seafood (Akhbarizadeh et al., 2020; Ghosal, Chen, Wagner, Wang, & Wall, 2018; Vinay Kumar, Löschel, Imhof, Löder, & Laforsch, 2021), marine sediments (Liu et al., 2020), and sewage sludge (Li et al., 2018).

3.1.6 Objective of this study

The objective of this study was to examine iAs uptake by polystyrene microplastics by varying conditions such as concentration, storage days, pH, and degraded microplastics.

3.2.1 Materials

Commercial polystyrene microspheres were purchased from Degradex (Hopkinton, MA) with stock solution concentration of $1.65 \times 10^7$ for 10 µm and is diluted to desired concentrations with double distilled water. Fenton reagent was prepared by iron (II) sulfate heptahydrate (ACS reagent ≥99%) from Sigma Aldrich (St. Louis, USA) and 30% hydrogen peroxide ($\text{H}_2\text{O}_2$) from Lab Alley (Austin, TX). Sulfuric acid and sodium hydroxide were purchased from Fisher Scientific (Fair Lawn, NJ). Polyethylene was purchased from Cospheric (Goleta, CA). Food grade titanium dioxide (anatase, 223 nm) was purchased from FLAVORS and COLOR (Diamond Bar, CA). Titanium dioxide nanopowder (anatase, 99.5%, 15 nm) was purchased from US Research Nanomaterials Inc. (Houston, TX).

3.2.2. Fenton aging treatment

The polystyrene microplastics were treated with 0.3 mL 30% $\text{H}_2\text{O}_2$ in 19.4 mL of double distilled water with a pH of 4.0 along with 0.3 mL 200 mM Fe$^{2+}$ for seven days.
During the seven days, the solution was exposed to UV light on a rotary shaker. The microplastic was then filtered and rinsed with sulfuric acid.

### 3.2.3 Quantification of iAs adsorption

The stock solution of iAs was diluted to desired concentrations of 1 mL in 1.5 mL centrifuge tubes and incubated with polystyrene microplastics or titanium dioxide. The solutions were placed on a rotary shaker. After 24 hours, the solution will be centrifuged. Supernatant was diluted to 10 mL for XRF analysis of iAs concentration.

### 3.2.4 Raman analysis of polystyrene microplastics

The PVDF filter is placed in the DXRxi Raman microscope (Thermo Fisher Scientific, Madison, WI). The laser setting was 780 nm wavelength to minimize fluorescence interference, 50 mm slit width for 2 seconds, and 20× confocal microscope objective lens. The collected spectrum is then analyzed using OMNIC™ software (version 9.1). At least ten spectra were selected from each sample.

### 3.2.5 Scanning electron microscope (SEM)

Polystyrene microplastic morphology was observed by SEM. The PVDF filter was sputter coated with gold for 2 minutes. Then, the coated filter was placed in the scanning electron microscope (Quanta200 FEG; FEI; USA).

### 3.3 Results and discussion

### 3.3.1 iAs adsorption on different vehicles
Different vehicles for iAs adsorption were tested. As shown in figure 23, polystyrene microplastics with three different sizes: 10 µm, 1 µm, 200 nm, degraded polyethylene microplastics, titanium dioxide (E171), and titanium dioxide nanopowder were compared for their iAs adsorption. For 10 µm polystyrene microplastics, it displayed no statistically significant difference compared to the control where microplastics were present, both showed lack of iAs adsorption. With decreasing size in polystyrene microplastics of 1 µm and 200 nm, iAs XRF intensities decreased significantly. Polyethylene was grinded into microplastics (less than 5 mm diameter), and it shows higher adsorption of iAs than the three sizes polystyrene microplastics. Food grade titanium dioxide and titanium dioxide nanopowder of 15 nm showed statistically significant changes in iAs adsorption compared to all microplastics. With a decrease in titanium dioxide size, a higher iAs adsorption was observed.

Particle size impacts iAs adsorption because of difference in surface area. Hence the observation of higher iAs adsorption in smaller particles for polystyrene microplastics and titanium dioxide. Surface morphology of the particle also impacts iAs adsorption. Polyethylene less than 5 mm had more iAs adsorption despite being much larger in particle size compared to all pristine polystyrene microplastics. This is contributed by the mechanical grinding process causing a rough and porous surface, allowing more iAs interaction. Table 2 shows XRF average and standard deviations of measured iAs in the supernatant. A higher XRF intensity indicates more unbound iAs in the solution.
Figure 21. Comparison of XRF intensities of pristine polystyrene microplastics (10 µm, 1 µm, 200 nm), degraded polyethylene microplastics, titanium dioxide, and titanium dioxide nanopowder (15 nm) incubated with 20 µg/mL arsenic. Error bars are standard deviations (n=3).

Table 3. iAs adsorption average and standard deviation of polystyrene microplastics (10 µm, 1 µm, 200 nm), polyethylene microplastics, titanium dioxide, and titanium dioxide nanopowder (15 nm).

<table>
<thead>
<tr>
<th></th>
<th>Average XRF intensities (cps)</th>
<th>Standard deviation (cps)</th>
</tr>
</thead>
<tbody>
<tr>
<td>iAs</td>
<td>98.25</td>
<td>9.56</td>
</tr>
<tr>
<td>Polystyrene microplastics (10 µm)</td>
<td>96.42</td>
<td>9.15</td>
</tr>
<tr>
<td>Polystyrene microplastics (1 µm)</td>
<td>92.87</td>
<td>2.85</td>
</tr>
<tr>
<td>Polystyrene microplastics (200 nm)</td>
<td>94.16</td>
<td>4.92</td>
</tr>
<tr>
<td>Polyethylene microplastics (&lt;5 mm)</td>
<td>88.90</td>
<td>5.51</td>
</tr>
<tr>
<td>Food grade titanium dioxide</td>
<td>77.06</td>
<td>2.16</td>
</tr>
<tr>
<td>Titanium dioxide nanopowder (15 nm)</td>
<td>9.11</td>
<td>4.12</td>
</tr>
</tbody>
</table>

3.3.2. iAs interaction with pristine polystyrene microplastics

Polystyrene microplastics (10 µm) were diluted to $1.65 \times 10^6$ and incubated with 10mL of 10 µg/mL iAs. The solutions were placed on a rocking shaker at room...
temperature. The solution was filtered onto PVDF filter using a syringe filtration at different time points, in this case 1, 3, 5, and 7 days. Polystyrene microplastics along with adsorbed iAs were captured by the filter. The filtrate collected were placed in XRF for iAs analysis. Polystyrene microplastic adsorption of iAs were shown in Figure 21. The storage times did not change iAs adsorption compared to the control, where microplastics were replaced with water. Several factors contribute to this observation as listed: sorption behavior is dependent on pH, the state of microplastic (whether it is pristine or aged), the concentration of both microplastics and iAs should be adjusted to see more evident changes in adsorption.

![Graph](image-url)

**Figure 22.** A comparison of XRF intensities for 106 microplastics in 10 µg/mL arsenic solutions 1, 3, 5, and 7 days where control is microplastics in double distilled water. Error bars are standard deviations (n=3).

The following experiment was essentially identical to the previous but polystyrene microplastics and iAs solution were stored in different pH. Referring to Figure 22, polystyrene microplastics and iAs stored in pH 3, 6, and 9 did not show a
difference in iAs concentration. Adsorption of iAs in pristine microplastics is minimal due to weak electrostatic interactions.

![Arsenic and Microplastic Incubated at Different pH levels](image)

**Figure 23.** A comparison of XRF intensities for 106 microplastics in 10 µg/mL arsenic solutions at pH of 3, 6, and 9 for 1, 3, and 5 days where control is microplastics in double distilled water.

3.3.2 Pristine microplastic degradation using Fenton reagents

The lack of interaction with iAs and pristine polystyrene microplastics, degradation of microplastics was thought to increase interaction due to a change in porosity. According to literature, polystyrene microplastics treated with Fenton reagent should degraded. The degraded polystyrene surface should be porous, allowing arsenic adsorption. Pristine microplastics and Fenton treated microplastics were incubated with 10 µg/mL iAs for 24 hours. The measured iAs levels are shown in Figure 24. Interestingly, statistically significance for the two types of microplastics were unobserved.
This observation is rather interesting because the microplastic concentration used is less than the referenced literatures. This led to an estimation that microplastics were not degraded by the Fenton reagent. Raman analysis of the two microplastics were collected in Figure 25. Fenton treated microplastics’ intensity is much lower than microplastic alone though the concentrations were the same. SEM images were taken to inspect the surface morphology of the microplastics. Figure 26A was microplastics stored in water while Figure 26B is the microplastics treated with Fenton. Both figures had little specs on the microplastics, the identity of the specs remain uncertain.
Figure 25. SERS spectra of polystyrene microplastics and polystyrene microplastics treated with Fenton for 7 days.

Figure 26. (A) SEM images of pristine polystyrene microplastics in water. (B) microplastics treated with Fenton’s reagent. Both solutions were placed under UV light (365 nm) for seven days.

3.3.3 Conclusion:

The 10 µm polystyrene microplastics incubated with 10 and 20 µg/mL iAs solution did not show adsorption for iAs throughout 1, 3, 5, and 7 days of storage under pH of 3, 6, and 9. Significant changes in iAs adsorption was observed when polystyrene
microplastics of smaller diameters were used. Microplastic size is a factor affecting iAs adsorption. Another factor is the morphology of the microplastic. From the SEM and Raman analysis, Fenton reagent was ineffective for polystyrene microplastics. To verify if this method allows for iAs adsorption, polyethylene microplastics less than 5 mm made in the lab, titanium dioxide, and titanium dioxide nanoparticles were used in place of pristine polystyrene microplastics. The results showed statistically significance of iAs concentration, indicating iAs adsorption using this method is feasible.
CHAPTER 4

CONCLUSION

The methodology development of iAs detection in water and apple was successful through optimization of experimental conditions. Mercury bromide and silver nitrate were both effective for the capture of arsine gas, the latter more suitable for XRF analysis because of less elemental interferences. The method proposed is more accurate and precise when quantifying low levels of arsenic compared to commercial test kits. Not only did the green chemistry approach overcome the concern of low reaction efficiencies, also safer for the operating personnel. The two advantages evidently proved itself worthy to become the newest in situ detection method for iAs.

In addition, the adsorption of iAs on microplastics and titanium dioxide as vehicles were examined. Both vehicle size and morphology are critical to iAs adsorption. This is a powerful way to characterize the concentration relationship of iAs and microplastics or titanium dioxide because it is rapid. Further research can use different sized microplastics or titanium dioxide and measure adsorption of various heavy metals of concern such as cadmium, lead, mercury, and chromium.
BIBLIOGRAPHY


