EVALUATION OF MICELLE-MEDIATED PRECONCENTRATION METHODS FOR DETERMINING LEAD IN DRINKING WATER USING FLAME ATOMIC ABSORPTION SPECTROMETRY

A Thesis Presented

by

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Table of Contents

List of Figures

List of Tables

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1.0 Abstract

Low concentrations of lead contamination in drinking water can have irreversible health effects such as impaired brain development and anemia. Infants and young children are the most vulnerable to lead contamination and the chronic health issues it can cause. This research investigated the use of micelle-mediated preconcentration methods that allow for the detection of low lead concentrations in aqueous solutions using Flame Atomic Absorption Spectroscopy (FAAS). Specifically, it compared the efficacy of the chelating agents Ammonium Pyrrolidinedithiocabamate (ADPC), 8-Hydroxyquinoline (Oxine), 1-(2-pyridylazo)-2-napthol (PAN) and 1-(2-thiazolylazo)-2-napthol (TAN) in conjunction with the surfactant Triton X-114 (TX-114). The goal of this project was to evaluate each chelating agent in order to determine which method resulted in the greatest increase in absorbance. This work indicates that using TX-114 with TAN offers the most promising pre-concentration method, which will hopefully allow for the future quantitation of low lead concentrations in drinking water samples using FAAS.

2.0 Introduction and Background
2.1 History of Lead in Boston Public Drinking Water

Harmful levels of lead in drinking water has been a recurring issue for Massachusetts in recent years, especially in schools. In 2016 the water fountains of several Boston public schools were turned off due to the lead concentrations that exceeded the Environmental Protection Agency (EPA) action limit of 15 parts per billion. While no concentration of lead in water is considered safe the EPA action limit is the concentration at which remediation is required. Among the schools affected were the prestigious Boston Latin School, and several elementary schools.[1] The most recent study conducted during the 2017/2018 school year showed that of the 162 schools tested across Massachusetts, nearly one third had faucets or drinking fountains with lead concentrations above the action limit.[2] It is Boston's aging plumbing infrastructure which likely contributes to the lead contamination observed among these schools. Consequently, other public water sources such as water fountains in parks and libraries, and water in private residences can potentially have high lead concentrations as well. Due to the health risks lead in drinking water can pose, public awareness of this issue and an inexpensive method to test lead concentrations are necessary. This research evaluated different preconcentration methods that would allow detection of low lead concentrations in drinking water samples using Flame Atomic Absorption Spectroscopy (FAAS). FAAS is an analytical method which, is less expensive and more readily accessible than the most sensitive standard methods of water testing.

2.2 Health Effects from Exposure to Lead

Lead (Pb) is a naturally occurring metal which is deemed dangerous to humans at low concentrations. Exposure to lead has been shown to cause a variety of health issues including neurological, renal, cardiovascular, immunological, reproductive, developmental issues, and in

extreme cases death.[3] The individuals at the highest risk for lead poisoning are children, pregnant women, and the elderly, therefore much of the efforts to decrease human contact with Pb focus on these populations.[3] Currently, the most common method for monitoring human Pb exposure is taking blood samples and determining the Pb concentration present. While no concentration of Pb in the blood is considered safe, the current United States Center for Disease Control (CDC) focus is on lowering blood Pb levels in children to below 5 micrograms per deciliter (μg/dL).[3] As of 2017, in Massachusetts 2% of the population of children ages 9-47 months had Pb levels equal to or above 5 μg/dL.[4]

2.3 Lead in Drinking Water

One common route of lead exposure for humans is drinking water. This water can be contaminated with lead at the site of the natural water source, during the water filtration processes or, most commonly, by passing through older lead lined pipes which are known to corrode over time.

Pb levels in drinking water are reported in micrograms per liter (μg/L). 1μg/L can also be expressed as one part per billion (ppb), which is the term that will be used throughout this paper. As previously stated the EPA action limit for lead in drinking water is 15 ppb.[5] Similarly the United Nations' World Health Organization (WHO) recommends that the maximum concentration of Pb in water should not exceed 10 ppb.[6] However the American Academy of Pediatrics insists that lead levels in school drinking water should be lower than 1 ppb in order to prevent lead poisoning among children.[7]

Water treatment and distribution plants in the United States are constantly monitoring for lead contamination and do not release the water if it tests above the EPA action limit. This means

in order for contamination to occur the lead must be introduced on the water's path between the distribution center and the faucet. Which is frequently caused by older pipes within individual buildings which are leaching lead. This makes monitoring for lead levels in tap water the responsibility of the building owner. Currently in Massachusetts the only accurate way to know if an individual's household water supply is lead free is for the homeowner to pay $20-$40 and deliver a water sample to a Massachusetts Department of Environmental Protection certified laboratory, of which there is only two in the greater Boston area.[8]

2.4 Drinking Water Analytical Methods

According to the EPA code of federal regulations Title 40 (Protection of the Environment), subsection §141.23 (Inorganic chemical sampling and analytical requirements), currently the only acceptable methods to test for lead levels in drinking water are Graphite Furnace Atomic Absorption Spectrometry, ICP-Mass Spectrometry, or Differential Pulse Anodic Stripping Voltammetry.[6,9] These methods are required because of their low limit of detections (LOD), which allow for the detection of lead levels under 10 ppb. However, as shown below in Table 1, these methods require expensive analytical instrumentation that limit the number of laboratories, which are capable of lead testing; significantly increasing the price of testing.

Analytical Instrument	Instrument Estimated Cost	Pb Dynamic Range	Pb LOD (Limit of Detection)
Flame Atomic Absorption Spectrometer (Agilent 240FS AA)	$45,000-$61,000[10]	1000 to 10,000 ppb without preconcentration[9]	10 ppb[11]
Graphite Furnace Atomic Absorption Spectrometer (Agilent 240Z AA)	$56,000-$76,000[10]	5 to 100 ppb[9]	0.04 ppb[11]
ICP-Mass spectrometry (Agilent 7900 ICP-MS)	$196,000-$236,000[10]	$0.1 - 1.0 \times 10^{-5}$ ppb[11]	0.002 ppb [12]
Differential Pulse Anodic Stripping Voltammetry (Metrohm 969 Portable VA)	$10,900[13]	1.5-15 ppb[14]	0.2 ppb[15]

Table 1. Cost and LOD comparison of various Pb analytical instruments

As Table 1 shows, FAAS offers a method that has a good balance of ease of use and cost. However, a comparison of sensitivity of the instruments show FAAS to be lacking. Unfortunately, the current water sampling methods for FAAS do not provide a low enough LOD to detect lead in the 10-15 ppb range. In an attempt to decease the lead LOD of FAAS several studies have focused on developing a preconcentration method for water samples. This research will evaluate several of these micelle-mediated lead preconcentration methods to recommend a method for the future study of Boston public drinking water.

2.5 Micelle-Mediated Separation of Metal Ions from Aqueous Solutions

Originally introduced in 1976, micelle-mediated separation followed by cloud-point extraction is a method that can be used to preconcentrate and determine low levels of metals in aqueous solutions.[5,16–22] This method uses surfactant molecules, which consist of a hydrophilic head and hydrophobic tail as shown below in Figure 1. Once the concentration of surfactant in solution reaches the critical micelle concentration (CMC) the surfactant monomers begin to self-assemble in to a variety of aggregates including the simplest, the spherical micelle as shown in Figure 2.[20] This newly formed aggregate has an internal hydrophobic region, which can be used for the separation of metal ions that have formed a hydrophobic complex with a chelating agent.

Figure 1. Surfactant monomer
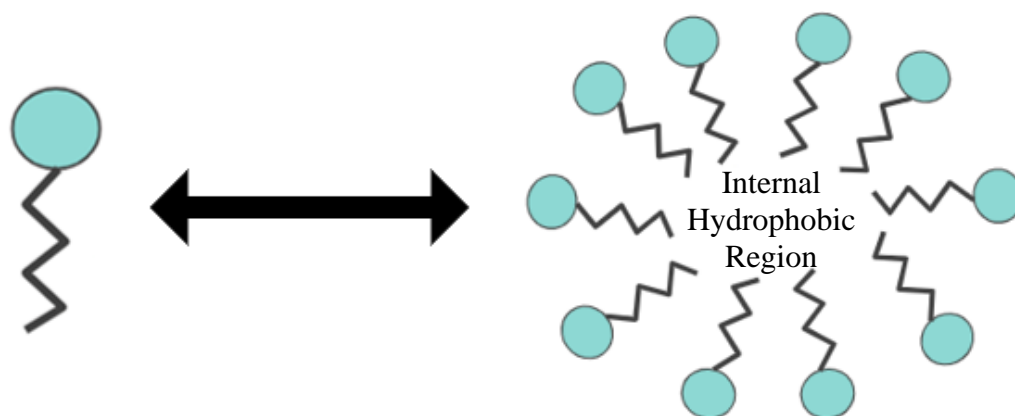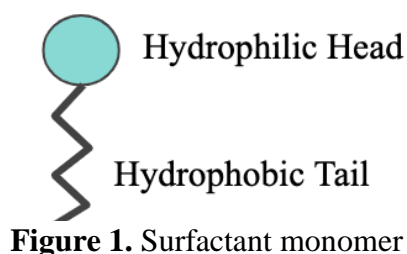
Figure 2. Once the concentration of the surfactant reaches the "critical micelle concentration" micelle aggregates begin to form – a reversible process that is encouraged by heating.

2.6 Cloud-Point Extraction

Cloud-point extraction (CPE) is a method of separating and then removing micelles (with the metal complex inside) from an aqueous solution. When aqueous solutions with non-ionic or amphoteric surfactants are heated the micelles become less soluble. At the critical "cloud point" temperature two distinct liquid phases separate from each other. An organic phase containing the micelle and analyte separates from the aqueous phase containing everything else in the original solution. These two phases can be isolated through centrifugation, and then the aqueous layer can be discarded leaving only the organic layer containing the micelles and analyte. If the phases are not separated, then the cloud point phenomenon is reversible, and can be reunified into one homogenous solution.[23] An overview of the entire micelle-mediated cloud-point extraction method for metals is shown below in Figure 3.
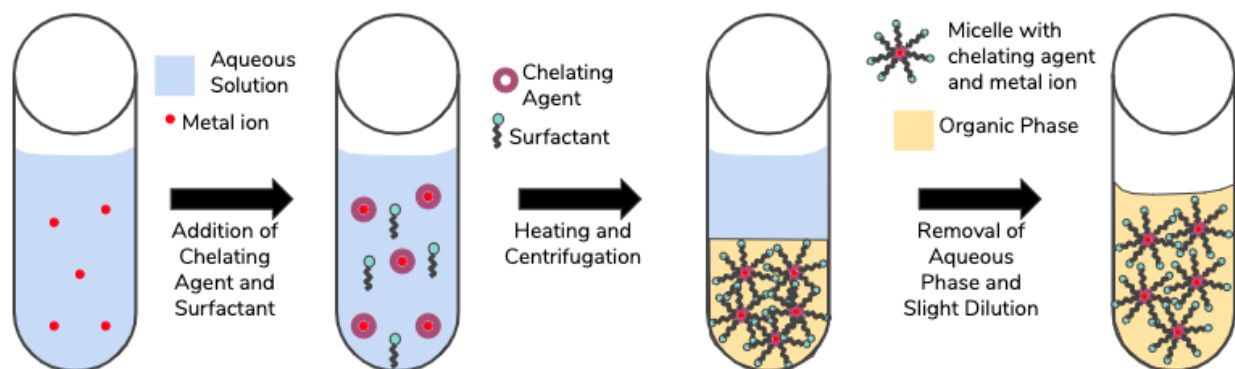
Figure 3. Diagram of the micelle-mediated cloud point extraction method

There are several parameters that can be modified to optimize CPE including the specific chelating agent, surfactant type and concentration, solution pH, incubation temperature, and centrifugation time. For extraction of inorganic molecules, the chelating agent selected should form a strong bond with the analyte and should be at a concentration that does not leave any free analyte uncomplexed. The surfactant species should be chosen based on its cloud point temperature, the viscosity of the final organic phase, and should be at the optimum concentration,

which traps all of the chelated analyte without being in excess, which unnecessarily decreases the final concentration factor, which is defined as a sample's concentrated absorbance divided by its expected not preconcentrated absorbance. These extractions also have an optimum pH range where the chelating agent is in the right degree of ionization to form a complex with the analyte. Incubation time, the duration of time that the sample is heated for, and temperature are also vitally important. The solution must reach the surfactant's cloud point temperature to separate the micelles and have enough time for the kinetically controlled chelation reaction and subsequent diffusion into the micelle to occur. A final aspect that will affect the final concentration factor is the centrifugation time and speed. The centrifugation is what accelerates the phase separation, separating the micelles from the aqueous phase to produce the most concentrated final product as possible.[22]

2.7 Lead Preconcentration Using Micelles

As previously stated, there have been several articles published on the use of micelle-mediated cloud-point extraction of metals to increase the LOD of FAAS[5,18,21]. All of these studies follow the same methodical steps as shown in Figure 3, however, they differ in the chelating agents used, the solution pH, and the surfactant used. For example, the experimental design proposed by Giokas, Paleologos and Karayannis, which was the inspiration for this research project, used ammonium pyrrolidinedithiocarbamate (ADPC) as the chelating agent and Triton X-114 (TX-114) as the surfactant (the structures of which is shown below in Figures 4 and 5) with a solution pH of 4.0. TX-114 was selected as the surfactant because of its low cloud point temperature, the high viscosity of the final organic phase, cost-effectiveness, and low health risks associated with

TX-114. Using this method their students were able to detect lead levels in wastewater as low as 5.0 ppb with a percent relative standard deviation of 3.2.[21]
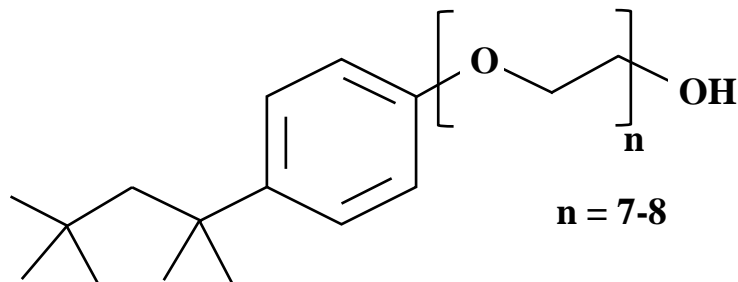
Figure 4. Structure of Triton X-114
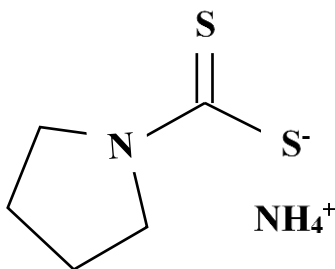
Figure 5. Structure of ammonium pyrrolidinedithiocarbamate

Mohammadi, et al, selected 1-(2-pyridylazo)-2-napthol (PAN), shown in Figure 6, as the chelating agent and Triton X-114 as the surfactant, at a pH of 6. They also utilized a micelle extraction method known as salting-out, which involves the addition of sodium chloride to aid in the separation of the micelles from the aqueous layer. They were able to achieve a LOD of 5.27 ppb, a concentration factor of 30, and a relative standard deviation of 1.6% with these conditions.[16]
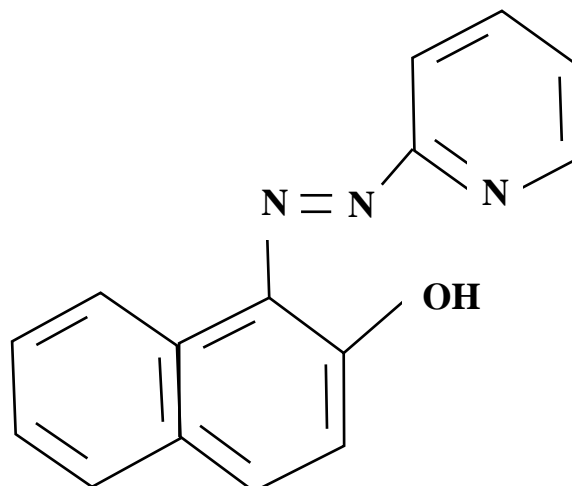
Figure 6. Structure of 1-(2-pyridylazo)-2-napthol

Naeemullah, et al used 8-Hydroxyquinoline (Oxine) as a chelating agent, shown in Figure 7, and again used Triton X-114 as their surfactant. Their research demonstrated the optimum pH for lead recovery to be 7.0 with which they achieved an LOD of 0.44 ppb and an concentration factor of 50.[5]
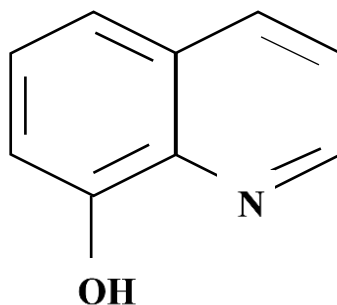
Figure 7. Structure of 8-hydroxyquinoline

Chen and Teo [19] experimented with a combination of Triton X-114 and 1-(2-thiazolylazo)-2-napthol (TAN), shown in Figure 8, for the micelle-mediated cloud point extraction and subsequent FAAS analysis of cadmium, copper, lead and zinc in water samples. A pH study was

conducted and determined the optimum pH for this system to be between 7.0 to 9.0. This article

reported achieving a lead limit of detection of 1.1 ppb, and a relative standard deviation of 3.5%

for a 20 ppb sample. A summary of these experimental conditions is shown below in Table 2.
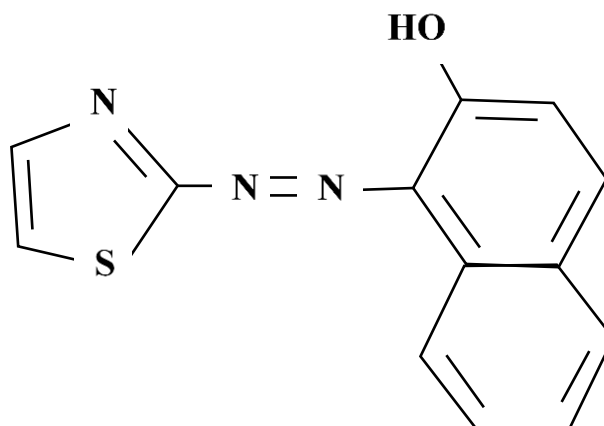
Figure 8. Structure of 1-(2-thiazolylazo)-2-napthol

Chelating Agent	Surfactant	Solution pH	Incubation Temperature (°C)	Additional Factors	LOD or Lowest Reported Detectable Concentration	Concentration Factor	Reference
ADPC	Triton X-114	4.0	60	—	Lowest Reported- 5.0 ppb	—	(21)
Oxine	Triton X-114	7.0	50	—	LOD- 0.44 ppb	50	(5)
PAN	Triton X-114	6.0	40	3.5% NaCl	Lowest Reported- 5.27 ppb	30	(18)
TAN	Triton X-114	7.0-9.0	40	—	LOD- 1.1 ppb	—	(19)

Table 2. Comparison of published lead preconcentration experimental conditions.

3.0 Experimental

3.1 Instrumental Details

All experiments in this study were conducted using an Agilent 240FS AA atomic absorption/flame emission spectrometer equipped with deuterium background correction and a lead hollow cathode lamp. While the specific instrumental method parameters were varied from experiment to experiment, optimum results were obtained using a wavelength of 217 nm, a slit width of 1 nm, using 4 sample replicates each of 1 second, with a measurement mode of integration, 7-point smoothing, and a manually plotted linear calibration curve. As shown below in Figure 9 the Agilent 240FS AA is a sensitive instrument capable accurately analyzing lead concentrations based on absorbance. However, under typical not preconcentrated conditions the standard deviation error range makes it impossible to accurately analyze low concentration samples, which is why preconcentration methods are necessary.
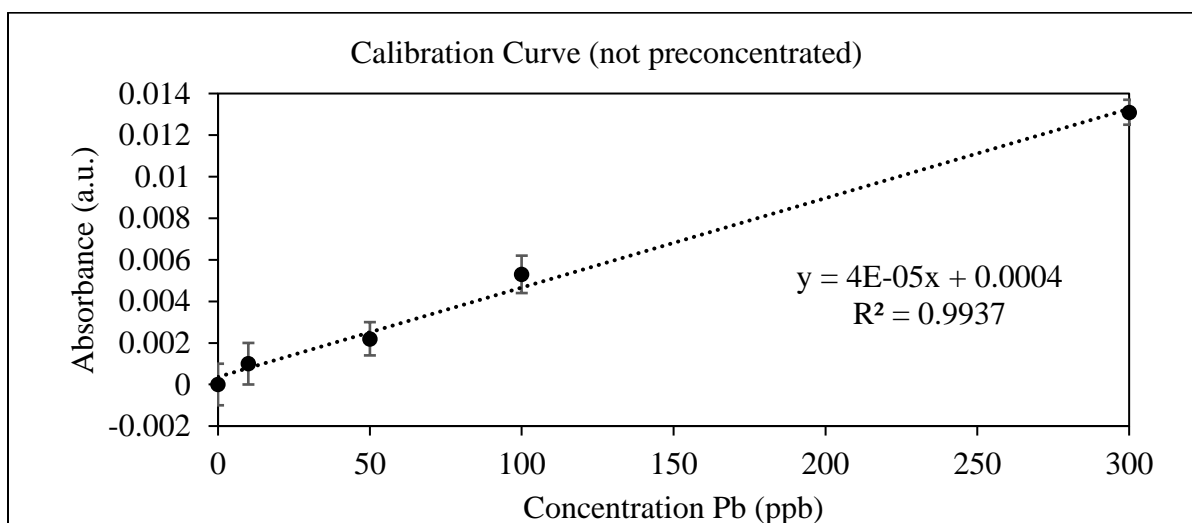
Figure 9. FAAS calibration curve of not preconcentrated samples taken during PAN method experimentation.

The pH data for all of the experiments was collected using a Fischer Scientific Accument AB-15 pH meter calibrated with standard buffers of pH 2, 4, 7 and 10.

3.2 Importance of RSD for LOD Determination

The overall goal of these preconcentration experiments was to find a method that would decrease the limit of detection (LOD) of the FAAS low enough to detect 10-15 ppb Pb. The LOD of an instrument is dependent on the intensity of the signal in addition to the standard deviation between replicate in each sample. LOD is defined as:[24]

$$LOD = \frac{3 \times \text{standard deviation of low concentration sample}}{\text{slope of calibration curve}}$$

Micelle-mediated preconcentration methods increase the signal (the absorbance) of the samples by decreasing the overall volume, which in turn increases the relative concentration. The standard deviation of these sample analyzed with FAAS are determined by the variation between smaller replicates. Standard FAAS methods aspirate each sample for 15 seconds, analyzing 3 replicates that are each 5 seconds long. It then generates an average absorbance for that sample along with a standard deviation (SD) and relative standard deviation (RSD). When preforming FAAS some elements have multiple wavelengths that can be used for analysis, each wavelength comes with a different signal intensity and RSD. For lead there are two wavelengths, 217 nm which is typically used and 283.3 nm. At 217 nm a standard 5 ppm Pb sample should result in an absorbance of 0.20 a.u. At 283.3 nm 5 ppm has an absorbance of 0.1 a.u. but has a decreased RSD compared to 217 nm because there are fewer chemical interferences at 283.3 nm. This sparked a set of side experiments within this research to develop a sampling method for small volumes that generates RSD and SD data, along with a study of 217 nm versus 283.3 nm to determine which wavelength decreased the LOD the most with micellular preconcentration.

3.3 The Problem of Sampling Small Volumes with FAAS

The first set of exploratory experiments were conducted to determine the best practice for aspirating the small volumes of sample (0.5 to 2 mL) that remain after preconcentration into the spectrometer. Usually the capillary tube is inserted into the sample and the sample begins aspirating into the flame for about 5 seconds before data collection is initiated on the computer. This order of sample introduction works best when there is an excess of sample present (greater than 10 mL), allowing more than 15 seconds for data collection per sample. For optimum signal amplitude and lowest signal variability, the FAAS requires that samples are aspirated at a rate of 6 to 10 mL/min.

However, the preconcentrated samples have volumes typically only 0.5 to 2 mL, volumes that are completely aspirated into the flame in a matter of seconds. Agilent, the instrument's manufacturer, was consulted regarding this issue and they could not provide any advice, they believed that sampling such small volumes would be very problematic for the FAAS. Through experimentation, the best order of sample introduction for small volumes was to have no read delay in the FAAS method, to line up the capillary tube with the sample (without inserting it), and then inserting the tube and waiting about half a second before selecting the sample in the software. This procedure allowed for typically 5 seconds of data collection, and the method was set to collect a new data replicate every second. After every run the spectrometer generates a graph for each sample of the signal versus sampling time and this was used to determine if any replicates needed to be rejected because the sample ran out. Shown below are several examples of the spectrometer's graph of absorbance signal vs. time. Figure 10 shows an example of a sample analysis where all five replicates of one second each can be used to calculate average absorbance and the relative standard deviation, since there is no delay in the signal or drop off in signal at the end. In

comparison, Figure 11 is an example in which the data collection was initiated too early and the

sample had not yet reached the flame. Finally, Figure 12 is an example of premature signal drop

off due to aspirating all the sample before the end of the final replicate.
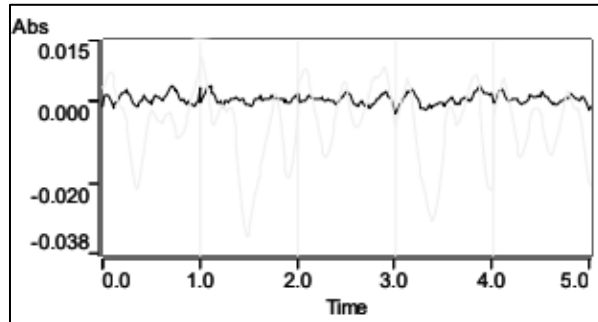
Figure 10. FAAS generated graph of signal versus time with a consistent signal across all 5
replicates.
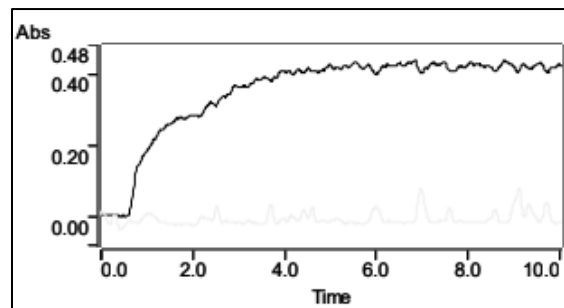
Figure 11. FAAS generated graph of signal versus time with a 4 second delay between sample
selection and a consistent signal.
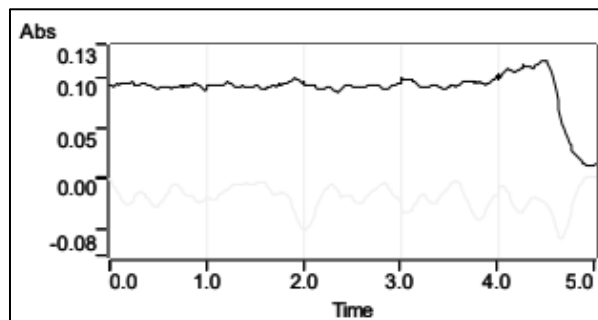
Figure 12. FAAS generated graph in which the sample was exhausted before the end of the fifth
replicate sampling time.

3.4 General Preconcentration Method

All the preconcentration experiments followed the same general five steps as outlined below in Figure 13. First, the chelating agent and TX-114 were added to the water sample and the pH of the solution was adjusted. The solution was then heated to form the micelles before it was separated into centrifuge tubes and centrifuged to separate the micelles from the aqueous phase. The aqueous phase was then decanted, and nitric acid was added to slightly dilute the micelle layer. Sections 3.4 through 3.8 of this paper will describe how this general preconcentration method was modified for the use of four different chelating agents (ADPC, PAN, Oxine, and TAN).

Chelating agent
Tx-114
pH adjustment

Aqueous Phase

Concentrated
Organic Phase

Step 1.   Step 2.   Step 3.   Step 4.   Step 5.
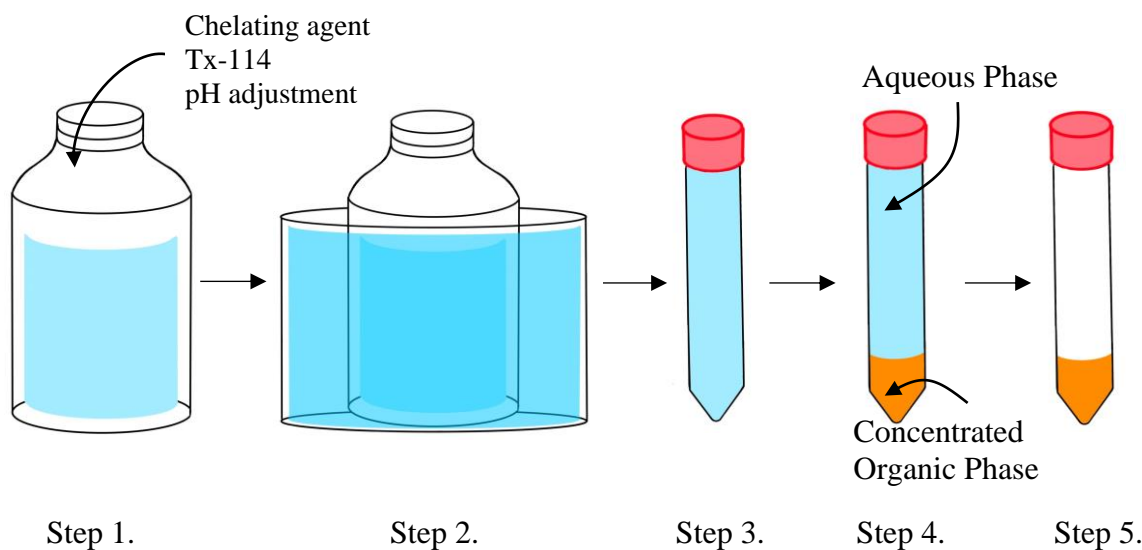
Figure 13. Outline of the general 5 step preconcentration method. Alterations were made for specific chelating agents according to methods described in the literature. Step 1, addition of chelating agent and TX-114 to water sample. Step 2, heating the solution. Step 4, cooling of the solution in ice. Step 5, decanting the aqueous phase and slight dilution.

3.5 ADPC Method

Pyrrolidinedithiocarbamic acid ammonium salt (ADPC) was the first chelating agent that was studied in this research based on the procedure of Giokas, Paleologos and Karayannis.[21] After several optimization experiments the finalized procedure for this thesis was to take 50 mL of the

water sample, add 1.5 mL of 10% ADPC, and 500 µL of 100% TX-114, sonicating after each addition. The pH of this solution was then adjusted to 4.0 using dilutions of NaOH and HCl. The pH modified solution was then heated in a 60 °C water bath for 10 minutes to initiate cloud point formation. The solution was then divided between 4, 15 mL centrifuge tubes and centrifuged for 10 minutes at 3500 rpm to separate the organic micelle layer from the aqueous phase. The tubes were then cooled in ice for 5 minutes to solidify the organic layer before inverting the tubes to decant off the aqueous phases into one 50 mL centrifuge tube. All the organic layers were then transferred to one glass 5 mL conical vial, which was placed into an approximately 118°C oven for 15 minutes to remove any residual water. The vial was cooled at room temperature for 15 minutes before adding 750 µL of methanolic solution of concentrated nitric acid and vortexed to combine. The resulting solution was filtered through a 0.22 µm aqueous filter before being aspirated into the FAAS. Analysis were conducted at both 217 nm and 283.3 nm. A detailed procedure and list of chemical solutions can be found in Appendix A.

This finalized procedure was a result of several optimization studies, which evaluated how changing different method parameters impacted the final concentration factor. Experiments were conducted using varying amounts of APDC, a solution pH of 4.0 versus 7.0, and the impact of filtering the final solution before aspirating into the spectrometer. The results of these experiments are discussed in results section 4.1.

3.6 PAN Method

1-(2-pyridylazo)-2-napthol (PAN) was the second chelating agent evaluated in this research based on the method proposed by Mohammadi et al. [18] For this thesis research the modified procedure was to take 12 mL of a water sample in a 50 mL centrifuge tube and add 100

μL of 0.01 M PAN, 1 mL of 0.3% TX-114, 1 mL of 0.2 M Acetic Acid buffer, and 1 mL of 3.5% NaCl solution, sonicating after adding each solution. The pH of the resulting solution was then adjusted to 6.0 with dilute NaOH or HCl and transferred to a 15 mL centrifuge tube. The tube was then heated in a 40 °C water bath for 15 minutes to initiate cloud point formation. The tube was then centrifuged for 5 minutes at 3,000 rpm to separate the organic and aqueous phases. It was then placed in an ice bath for 5 minutes to solidify the organic layer before pipetting off the aqueous phase into another 15 mL centrifuge tube. Finally, 500 μL of 0.5 M HNO_3 was added to the organic phase and vortexed to combine before aspirating directly into the spectrometer. Analyses were conducted at both 217 nm and 283.3 nm. A detailed procedure and list of materials can be found in Appendix B. Three experiments were conducting using this PAN procedure and are discussed in the section 4.2.

3.7 Oxine Method

8-Hydroxyquinoline (Oxine) was the third chelating agent that was investigated using an adapted version of the procedure published in the Journal of Analytical Methods in Chemistry by Naeemullah et al.[5] For this thesis the modified procedure was to take 25 mL of water sample in a 50 mL centrifuge tube and add 20 μL of the Oxine solution, sonicate, and add 1.25 mL of the 10% TX-114. The pH was then adjusted to 7.0 with 0.1 M NaOH and 0.1 M HCl. The solution was divided between 2, 15 mL centrifuge tubes, which were heated in a 50°C water bath containing a stir bar for 10 minutes. The tubes were centrifuged for 10 minutes at 3,500 rpm before cooling in an ice bath for 10 minutes. The tubes were both inverted to decant the aqueous phases into one 50 mL centrifuge tube. Finally, 100 μL of 0.1 M HNO_3 in Ethanol was added to both organic phases which were vortexed and then combined into one of the 15 mL tubes and aspirated directly into

the spectrometer and analyzed at 217 nm. A detailed procedure and list of materials can be found in Appendix C. Only one experiment was conducted using Oxine as the complexing agent, and the results are discussed in section 4.3.

3.8 TAN Method

1-(2-thiazolylazo)-2-napthol (TAN) was the fourth chelating agent investigated for this research based on the article by Chen and Teo.[19] For this thesis research the method was modified as follows: to 50 mL of water sample in a 125 mL glass bottle 1.0 mL of a solution of 2.6% Triton X-114 and $1x10^{-3}$ M TAN was added and sonicated. 1.0 mL of a 0.1 M $Na_2B_4O_7 \cdot 7H_2O$ pH 8.6 buffer was added before adjusting the pH of the final solution to 8.6 using 1 M HCl and 1 M NaOH and sonicating for a second time. The bottle was placed into a 40°C water bath for 15 minutes with a stir bar in the water going at 350 rpm. The solution was divided among 4, 15 mL plastic centrifuge tubes and centrifuged at 5,000 rpm for 15 minutes after which the tubes were placed in ice for 10 minutes. After the organic layer thickened the centrifuge tubes were inverted to decant the aqueous phases into one 50 mL centrifuge tube. Finally, 50 µL of a 0.1 M HNO_3 solution was added to each tube and vortexed before the solutions were all combined into one tube which was aspirated directly into the FAAS and analyzed at 217 nm. A detailed description of the procedure and list of materials can be found in Appendix D. One preliminary study was done using this method along with a full preconcentration test to determine the accuracy of the method against the results from Cambridge Water Department, a local water testing facility. The results of both of these experiments are discussed in results section 4.4.

3.9 Cambridge Water Quality Lab Tests

The final experimental portion of this research was independent analysis by the Cambridge Water Department's (CWD) Water Quality Lab of samples, which were also preconcentrated and analyzed as described in Section 3.8. Four samples (a method blank, 5 ppb, 10 ppb, and 20 ppb) were sent out to the Water Quality Lab for blind analysis using their residential water sample analysis method that implores Inductively Coupled Plasma-Mass Spectrometry. Aliquots from these 4 samples were also preconcentrated and analyzed using the TAN method described above. This experiment was conducted to identify the percent difference in the lead concentrations reported by these methods to get an idea of how the TAN method compares to the EPA's standard drinking water analysis methods.

4.0 Results

All of the method studies were evaluated based on the Concentration Factors (CF) they

achieved.

$$\text{Concentration Factor} = \frac{\text{Abs of preconcentrated sample}}{\text{Expected Abs based on a not preconcentrated calibration curve}}$$

For each preconcentrated sample both the organic and aqueous phases were analyzed using

FAAS. The aqueous phase was tested to calculate if any lead remained in the aqueous phase. In

the following Figures "standards" are the samples used to generate the FAAS calibration curve.

"Unknown" samples are defined as the samples aspirated into the FAAS with the intent of

calculating their concentration. A "corrected" absorbance refers to a sample that has had the

absorbance of a method blank subtracted. The "concentrated" phase is the organic layer that

remained after CPE, containing the micelles and chelated complex. Finally, the "aqueous" phase

is the top layer after CPE that contains the water without the micelles.

An example of these calculations is a 50 ppb sample preconcentrated with TAN. The

average absorbance for this preconcentrated sample was 0.09265 a.u., and concentrated method

blanks for this run had an average absorbance of 0.00234 a.u. The "corrected" absorbance for this

sample is therefore 0.09265-0.00234 or, 0.09031. To find the concentration factor this corrected

absorbance is divided by the absorbance of a not preconcentrated 50 ppb sample estimated from

the calibration curve (0.0031 a.u.). The expression 0.09031÷0.0031 results in a concentration

factor of 29.13 for this sample, meaning the absorbance has increased by a factor of 29.

4.1 ADPC Results

Several experiments were conducted using ADPC as the chelating agent. The first

successful ADPC preconcentration following the literature procedure resulted in a maximum

concentration factor of 16.9 with a 250 ppb Pb sample. Figure 14 illustrates the increase in absorbances that were achieved through preconcentration in this experiment. These samples were analyzed with FAAS as "unknown" samples after a not preconcentrated calibration curve was established. In Figure 14, the "not preconcentrated" values are the absorbances expected for that ppb based on a not preconcentrated calibration curve. The "concentrated" samples have been preconcentrated with the ADPC method. The difference between the "concentrated" and "not preconcentrated" absorbances is a visual representation of the concentration factor, how the signal has increased. This study showed that with 2 mL of 10% (w/v) ADPC and a pH of 4.0 the concentration factor increased as the concentration of lead increased with a linear range of 0 to approximately 750 ppb Pb.
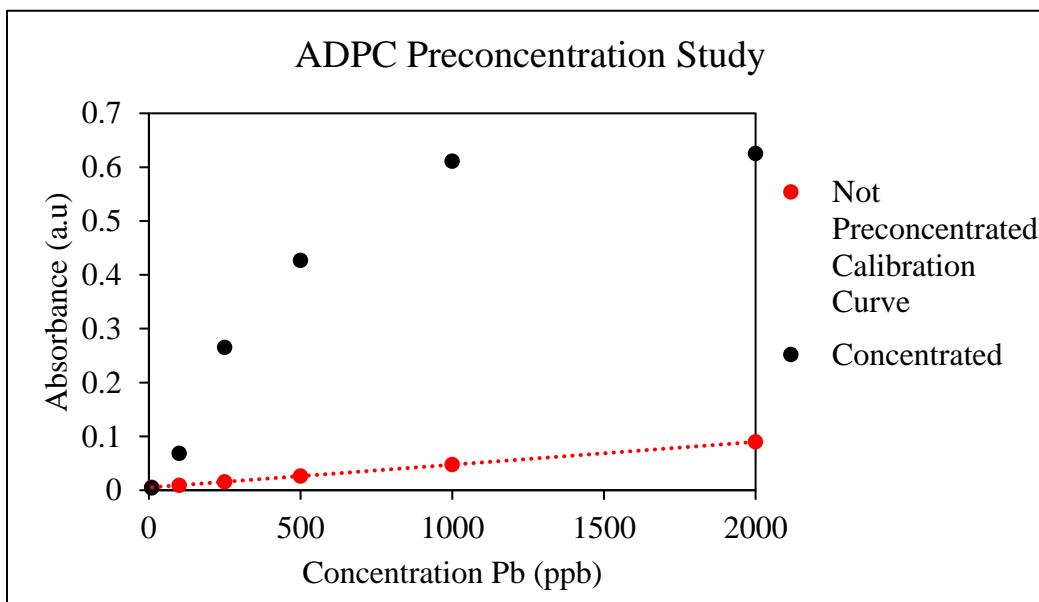
Figure 14. Graph of sample concentration versus absorbance for lead samples preconcentrated with ADPC as a chelating agent. Both the actual preconcentrated absorbance and theoretical not preconcentrated absorbance are plotted.

A second ADPC experiment was conducted to determine the effect of APDC concentration on absorbance, to determine the effect of the APDC to Pb ratio on preconcentration effectiveness. Three solutions of 100 ppb Pb were preconcentrated using 2 mL, 1 mL and 0.5 mL of 10% (w/v) ADPC at a pH of 7.0. As shown in Figure 15 the optimum concentration of ADPC initially added to the water sample is 0.008 moles. Additionally, this figure shows that relatively no lead was left behind in the aqueous phases of these samples.
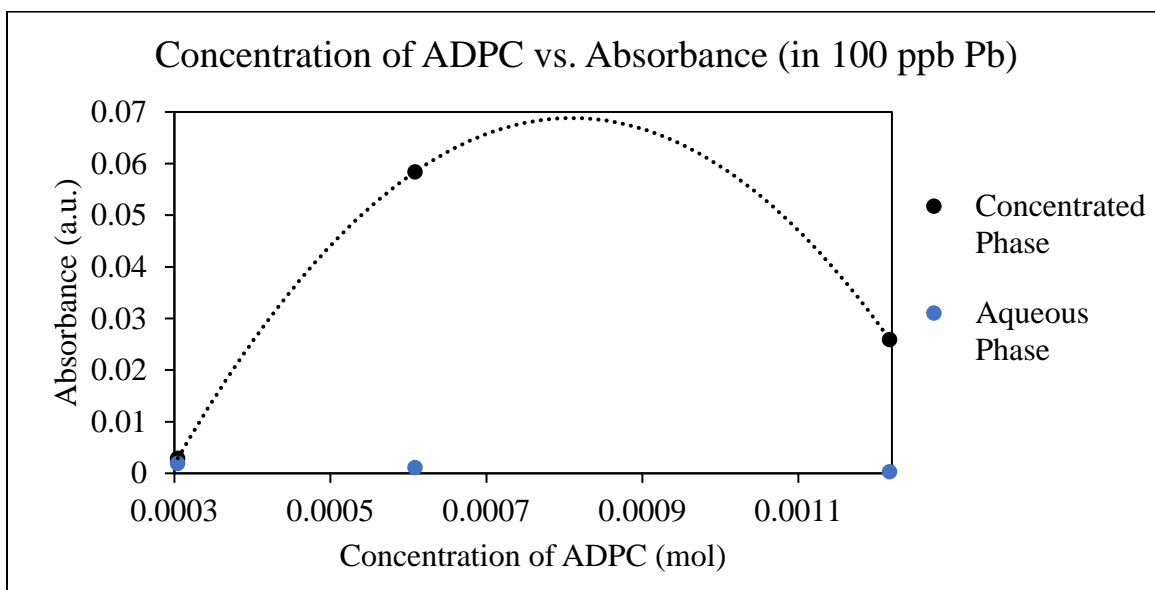
Figure 15. Graph of absorbance versus moles of ADPC added for 100 ppb lead samples. Both the absorbances of the concentrated and aqueous phases are plotted.

Based on all the ADPC optimization studies, the ideal preconcentration conditions for ADPC preconcentration are: 1.5 mL of 10% (w/v) ADPC, 0.5 mL of 100% TX-114, a pH of 4.0, and analysis at 283.3 nm. Under those conditions, a maximum concentration factor of 31.47 was achieved for the final 100 ppb sample and a CF of 13.67 for the final 10 ppb sample. The two main issues with this method were the significant amount of time required (up to an hour per sample), and the frequency of capillary tube blockage. Graphs of the results from all ADPC experiments can be found in Appendix E.

4.2 PAN Results

Of the three experiments conducted using PAN as the chelating agent, only one resulted in a successful preconcentration. Working with PAN proved to be extremely difficult, mainly because it would not generate a viscous concentrated phase after centrifugation and cooling. Red flakes of PAN would not settle to the bottom of the centrifuge tube, which made it nearly impossible to pipette of the aqueous phase. The highest concentration factor achieved with PAN was only 1.3 with a solution of 50 ppb lead. However, that was not reproducible, as shown in Figure 16, where the other two replicates from that experiment exhibited a <u>decrease</u> in absorbance after preconcentration. Results from all the PAN experiments can be found in Appendix F.
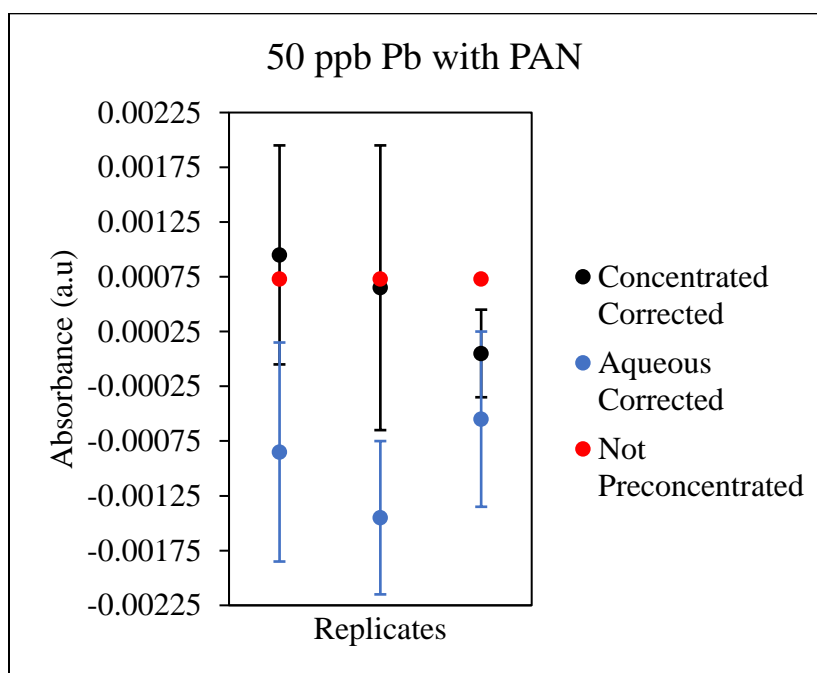
Figure 16. Graph of the concentrated and aqueous phases corrected absorbances (actual absorbance – method blank) for replicate 50 ppb Pb samples preconcentrated using PAN. The expected absorbance for 50 ppb Pb based on a not preconcentrated calibration curve is also shown.

4.3 Oxine Results

Only one preconcentration experiment was conducted using Oxine as the chelating agent because of severe operational problems with the FAAS; the capillary tube repeatedly clogged resulting in inconsistent sample uptake, along with negative results from the first experiment. As shown below, in Figure 17, in this experiment three replicates of 50 ppb Pb all showed a <u>decrease</u> in absorbance after preconcentration with Oxine. The absorbance of the aqueous phases are not reported because of the capillary tube clog. This analysis was conducted at the same time as the final PAN experiment and therefore they use the same standard curve, which can be found in Appendix F under "Results from FAAS run on 2-7-20".
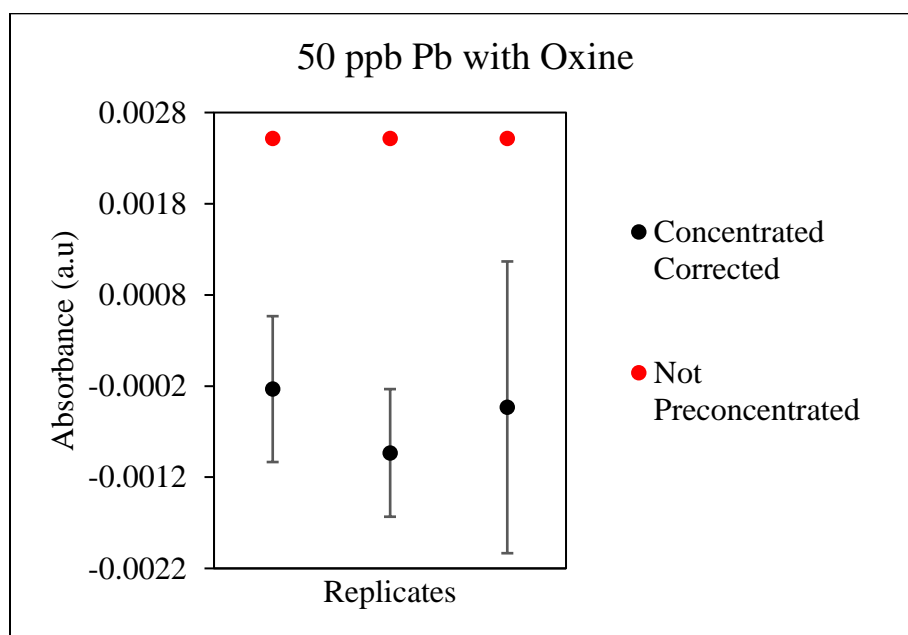
Figure 17. Graph of the concentrated phase corrected absorbance for replicate 50 ppb Pb samples preconcentrated using Oxine. The expected absorbance for 50 ppb Pb based on a not preconcentrated calibration curve is also shown.

4.4 TAN Results

Two experiments were conducted using TAN as the chelating agent. The first was an evaluation of the concentration factor achieved using the literature method on three replicate

samples of 50 ppb Pb. As shown below, in Figure 18, the absorbance of all three preconcentrated samples was significantly higher than the expected not preconcentrated absorbances. They also all fell within a range of 0.0199 a.u. from each other, with replicates 1 and 3 only varying by 0.00485 a.u.. The maximum concentration factor (achieved with replicate 3) was 30.69.
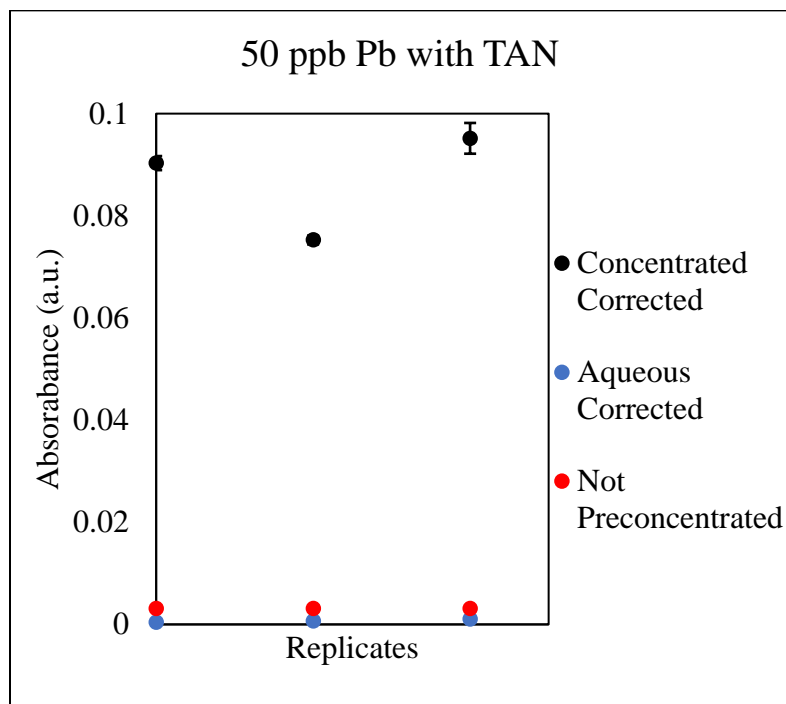
Figure 18. Graph of the concentrated and aqueous phase corrected absorbances for replicate 50 ppb Pb samples. The expected absorbance for 50 ppb Pb based on a not preconcentrated calibration curve is also shown.

A second TAN study was conducted in collaboration with Cambridge Water Department's (CWD) Water Quality Lab to determine if this TAN preconcentration method could be used to accurately determine the concentration of lead in an "unknown" sample. For this experiment a preconcentrated calibration curve was collected and the absorbances of several "unknown" samples (5 ppb Pb, 10 ppb Pb, and 20 ppb Pb) were used to determine their concentration using the calibration curve. The same (not preconcentrated) samples were also sent to CWD for analysis. CWD's lab uses an ICP-Mass spectrometer to test their samples (without preconcentration). Figure 19, shows the results of this study, there was a 4.7% difference between the results for the 5 ppb

sample, a 33.1% difference with 10 ppb, and a 17. 1% difference with 20 ppb. Interestingly the

CWD results also showed 2.021 ppb Pb in the sample of Simmons's DI water. The FAAS method

blank (consisting of just preconcentrated DI water) also showed an elevated absorbance and was

removed from the calibration curve. The full results from both TAN studies can be found in

Appendix G.
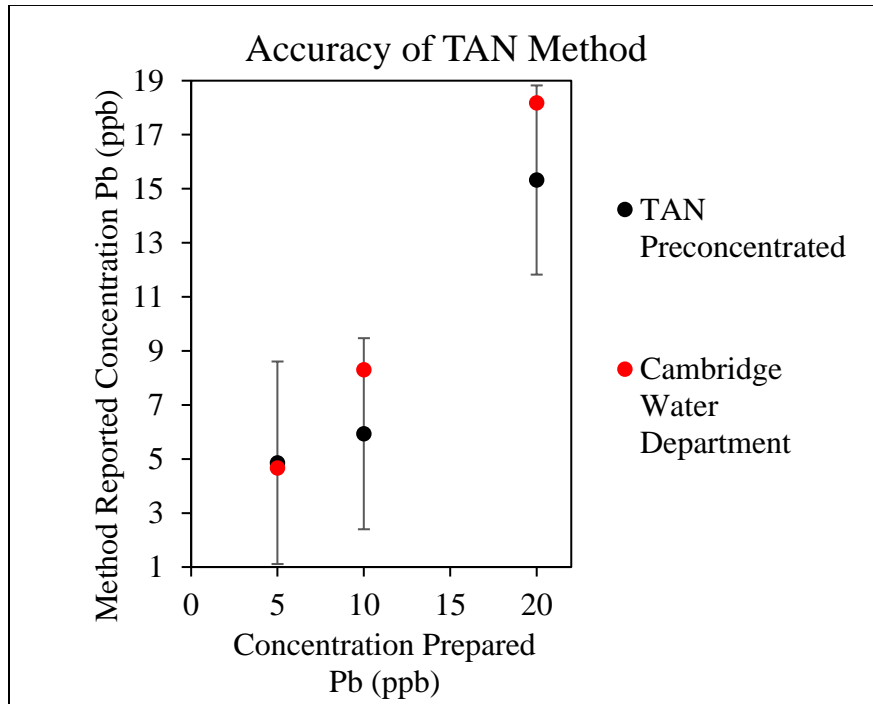
Figure 19. Graph of the lead concentration reported by TAN preconcentration and Cambridge Water Department versus the concentration that was prepared.

5.0 Discussion

5.1 Comparison of Chelating Agents

As shown below in Table 3, the TAN method resulted in the highest concentration factor along with being the easiest method to implement and the most reproducible. Based on these factors the TAN preconcentration method outlined in Appendix D is recommend for future drinking water analysis experiments. Additionally, because of the lack of signal increase, non-reproducibility, and difficulty of use future investigation of the PAN or Oxine methods described in Appendix B. Detailed PAN Procedure Appendix C. Detailed Oxine Procedure is strongly discouraged.

Chelating Agent	Concentration Factor (for a 50 ppb Pb sample, except ADPC which is 100 ppb)	Ease of Use	Estimated Sample Preparation Time	Reproducibility
ADPC	7.4	Time Consuming but Not Difficult	1 hour	Not Very Reproducible
PAN	1.3	Very Difficult	1 hour	Not Reproducible
Oxine	-0.1	Moderately Difficult	40 minutes	Unknown
TAN	30.7	Easy	50 minutes	Very Reproducible

Table 3. All four chelating agents evaluated based on the concentration factors they achieved along with method difficulty and reproducibility.

It is challenging to accurately compare the results of all these preconcentration experiments due to instrumentation complications along with the lack of a standard set of "unknown" lead samples across all experiments. When the experimental portion of this research began in October of 2019, it was not known that the lead hollow cathode lamp in the FAAS was in need of replacement, that the machine needed replacement O-rings, or that a high-solids capillary tubing was needed for this kind of work to prevent clogs. The hollow cathode lamp was

replaced, and the capillary tube was changed in early December 2019 for the final ADPC experiment. The FAAS was cleaned and the O-rings were replaced in late February 2020. All these instrumental issues would impact the signal intensity and relative standard deviation (RSD) of the data. The most accurate comparison of these methods would have required all of the maintenance to be completed before any samples were run and there would also need to be a consistent series of lead standards being preconcentrate with each method. However, even with these inconsistencies taken into consideration, the TAN method clearly resulted in the highest concentration factor, which in turn means that it lowered the FAAS limit of detection the most.

6.0 Future Work

6.1 TAN Sample Deterioration Study

While conducting the final TAN experiments the final concentrated TAN samples lightened in color over time as shown below in Figure 20. This could potentially be an indication of the solution changing in pH as it sits, and the concentrated surfactant phase is digested by the nitric acid. In the TAN method a similar color change was observed when the pH 8.6 buffer solution was added to the mixture of TAN, TX-114, and lead, which changed from a light orange to dark orange with the buffer. It is necessary to determine if this color change corresponds to a change in reported absorbance, if so, the samples could be time sensitive. In the event that this research is continued a timed sample deterioration study should be conducted. Ideally preconcentrated samples of 10 ppb lead would be allowed to sit for 6 hours, 12 hours, 24 hours, and 48 hours before all being analyzed at the same time to determine if there are any changes in the absorbance.
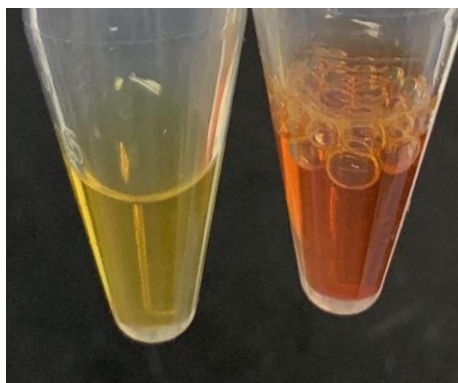
Figure 20. TAN color change over time. On the left a 10 ppb preconcentrated solution made the day before the photo was taken, on the right a 5 ppb preconcentrated solution made immediately before the photo was taken.

6.2 TAN Optimization

While the TAN method outlined in Appendix D was shown to have the highest concentration factor among all the chelating agents, the method could be optimized to concentrate the samples further. Similarly to what was done for ADPC in this paper, a set of experiments could be conducted to determine how the concentration factor varies with changes in the pH of the system, changes to the TAN concentration, and changes to the TX-114 concentration. A similar study was conducted by Chen and Teo in the original article however, the experimental setup at Simmons University could result in slightly different optimum conditions.[19]

6.3 Large Volume Study

As mentioned in section 3.3 many of the operational issues associated with micelle-mediated preconcentration were due to the resulting small volumes of concentrated phase, which were difficult to aspirate into the FAAS. One solution which is worth further investigation is starting with an increased volume of original aqueous lead sample, for example going from 50 mL to 100 mL or 1000 mL, which would then concentrate down into a larger final volume and aspirate easier into the FAAS. Unfortunately, it was not possible to conduct this experiment because the department's centrifuge capacity is limited to 6, 15 mL tubes. Access to a 50 mL centrifuge along with a larger water bath would allow a future student to investigate with larger volumes of drinking water samples. Larger sample volumes, and the resulting increased concentrated phase volume would allow for more FAAS sample replicates, and potentially improve the standard deviation, which in turn could further lower the limit of detection.

6.4 TAN LOD Study

A TAN preconcentration FAAS limit of detection study should be conducted in order to accurately compare this method against traditional water analysis techniques outlined in the introduction of this paper. Based on the DI water results from Cambridge Water Department it would be recommended to use purified water in all future experiments such as the ACS reagent water for ultratrace analysis from Sigma Aldrich. To determine the new FAAS LOD at least 10 samples of low lead concentration (5 ppb) would need to be concentrated with the TAN method and the LOD is defined as:[24]

$$LOD=\frac{3\times\text{average standard deviation of samples}}{\text{slope of calibration curve}}$$

6.5 DDTC Method

A final preconcentration method, which was unable to be explored due to time restrictions, is an adaptation of the diethyldithiocarbamate (DDTC) method proposed by Simmons thesis student Kavetha Ranjit.[25] The theoretical method would be conducted following these steps. A 500,000 ppb Pb solution would be prepared using lead nitrate and ultratrace water. 50 mL of this solution would be diluted to 500 mL with ultratrace water. 100 mL of that solution would then be transferred to a beaker along with 10 mL of 2% DDTC and the pH would be adjusted to 5 using HCl. The solution would be stirred for 1 hour. Theoretically 5 µg of Pb would precipitate out of solution in a complex with DDTC. This precipitate would then be filtered out using a Buchner funnel with glass filter paper and left on the filter paper to dry. The paper would then be transferred into a centrifuge tube and the precipitate would be digested with 2 mL of concentrated nitric acid before being aspirated into the FAAS.

6.6 Boston Public Drinking Water Analysis

The final phase of this research was intended to be a report of the lead levels in public drinking water around in greater Boston area. The preconcentration method that achieved the highest concentration factor (TAN) would have been used to analyze the drinking water samples. All water samples would have been collected from public drinking water fountains, with a focus on fountains which are frequently used by children such as those in schools, public playgrounds, and libraries. Samples would have been collected in plastic bottles which had been soaked for 24 hours in concentrated nitric acid to remove any potential contamination. Three replicates from each sample would have been analyzed and the average concentration would have been released in a report stating the locations of every sample. Unfortunately, this research was prematurely ended due to the COVID-19 pandemic but hopefully a future student will be able to see it through to completion.

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Nomenclature (in order of appearance)

FAAS: Flame Atomic Absorption Spectrometer

TX-114: Triton X-114

ADPC: Ammonium pyrrolidinedithiocarbamate

OXINE: 8-hydroxyquinoline

PAN: 1-(2-pyridylazo)-2-napthol

TAN: 1-(2-thiazolylazo)-2-napthol

Pb: Lead

CDC: Center for Disease Control

ppb: Parts per billion (µg/L)

EPA: Environmental Protection Agency

WHO: World Health Organization

LOD: Limit of detection

CMC: Critical micelle concentration

CPE: Cloud-point extraction

CF: Concentration Factor

NaOH: Sodium hydroxide

HCl: Hydrochloric acid

NaCl: Sodium chloride

HNO_3: Nitric acid

$Na_2B_4O_7 \cdot 7H_2O$: Sodium tetraborate heptahyrdrate

CWD: Cambridge Water Department

a.u.: Arbitrary units

abs: Absorbance

RSD: Relative standard deviation

ACS: American Chemical Society

DDTC: diethyldithiocarbamate

Appendices

Appendix A. Detailed ADPC Procedure

ADPC Procedure Materials:

- Methanolic Solution

    o 3.1 mL trace metal grade Nitric Acid and 50 mL Methanol.

- Dilutions of HCl

    o 0.1 M HCl

    o 1.0 M HCl

    o 4.0 M HCl

- Dilutions of NaOH

    o 0.1 M NaOH

    o 1.0 M NaOH

    o 4.0 M NaOH

- 10% (w/v) Pyrrolidinedithiocarbamic Acid Ammonium Salt (ADPC)

    o 10 g ADPC in 100 mL DI water.

- 100% Triton X-114

- Lead Standard Solutions

    o 10 ppb, 50 ppb, 100 ppb, 300 ppb, 1000 ppb

    o Prepared from 1000 ppm AA Lead Standard diluted to 100 mL.

- Digital pH Meter

- Hot Plate with Magnetic Stir Bar

- Disposable Plastic 15 mL Centrifuge Tubes

- Glassware soaked in 10% Nitric Acid for 24 hours

General ADPC Procedure:

1. Transfer 50 mL of water (containing lead) to a 125 mL glass bottle.

2. Add 1,500 µL 10% ADPC to the bottle.

    a. Sonicate for 1 minute.

3. Add 500 µL Triton X-114 to water solution.

    a. Sonicate for 1 minute.

4. Adjust the pH of the solution to 4.0 using NaOH or HCl.

    a. Sonicate for 30 seconds.

5. Place glass bottle into a 60 °C water bath for 10 minutes.

6. Divide water solution between 15 mL centrifuge tubes and centrifuge for 10 minutes at 3500 rpm.

7. Cool centrifuge tubes in ice for 5 minutes.

8. Invert all centrifuge tubes to decant the aqueous phases into one 50 mL centrifuge tube.

9. Transfer all organic phases from the 15 mL tubes into one 5 mL conical vial.

10. Place the conical vial into a 118 °C oven for 15 minutes to evaporate excess water.

11. Cool the conical vial for 15 minutes at room temperature.

12. Add 750 µL of methanolic solution to conical vial and vortex to combine.

13. Filter the final solution through a 0.22 micron aqueous filter before aspirating into FAAS.

Appendix B. Detailed PAN Procedure

PAN Procedure Materials:

- 0.01 M 1-(2-pyridylazo)-2-napthol (PAN)

    o 0.25 g PAN diluted in 100 mL ethanol.

- 0.3% (v/v) Triton X-114

    o First dilute to a 3.0% Triton X-114 by adding 3.174 g of Triton X-114 in 100 mL

      DI water.

    o Then dilute to a 0.3% Triton X-114 by adding 5 mL of the 3% Triton X-114 in 50

      mL DI water.

- 0.5 M HNO_3 in Ethanol

    o 1.6 mL of concentrated trace metal grade nitric acid diluted in 50 mL of ACS

      reagent grade ethanol.

- 3.5% (w/v) NaCl

    o 1.75g of NaCl diluted in 50 mL DI water.

- Acetic Acid/Sodium Acetate Buffer (pH 6)

    o Dilute 2.56 g sodium acetate and 0.11g acetic acid in 100 mL DI water and pH

      adjust to 5.78 with dilute NaOH and HCl.

- Digital pH Meter

- Hot Plate with Magnetic Stir Bar

- Styrofoam Support For 15 mL Centrifuge Tubes (diagram below)

- Disposable Plastic 50 mL and 15 mL Centrifuge Tubes

- Plastic Luer-Lock Syringes With 0.22 Micron Aqueous Filters.

General PAN Procedure:

1. Transfer 12 mL of water (containing lead) to a 50 mL plastic centrifuge tube.

2. Add 100μL of 0.01M PAN to centrifuge tube.

   a. Invert the tube twice and sonicate for 1 minute.

3. Add 1,000 μL of 0.3% Triton X-114 to centrifuge tube.

   a. Invert twice and sonicate for 1 minute.

4. Add 1,000 μL of 0.2M Acetic Acid/Sodium Acetate Buffer to centrifuge tube.

   a. Invert twice and sonicate for 30 seconds.

5. Add 1,000 μL of 3.5% NaCl solution to centrifuge tube.

   a. Invert tube twice and sonicate for 30 seconds.

6. Check the pH of the solution and adjust to 6.0 with dilute NaOH or HCl

7. Transfer the solution to a 15 mL centrifuge tube.

8. Heat the centrifuge tube in a 40°C water bath for 15 minutes.

9. Centrifuge the tube (with the cap on) for 5 minutes at 3,000 rpm.

10. Cool centrifuge tube in an ice bath for 5 minutes.

11. Pipette off the aqueous phase from the centrifuge tube into another 15 mL centrifuge tube.

12. Add 500μL of 0.5 M HNO_3 to the remaining organic phase.

    a. Vortex for 30 seconds to combine.

13. Aspirate directly into FAAS.

PAN Reaction Setup:

Styrofoam disk
with four holes
drilled through to
hold 15 mL
centrifuge tubes

15 mL Centrifuge Tube

1000 mL beaker
filled with DI water

Stir Bar
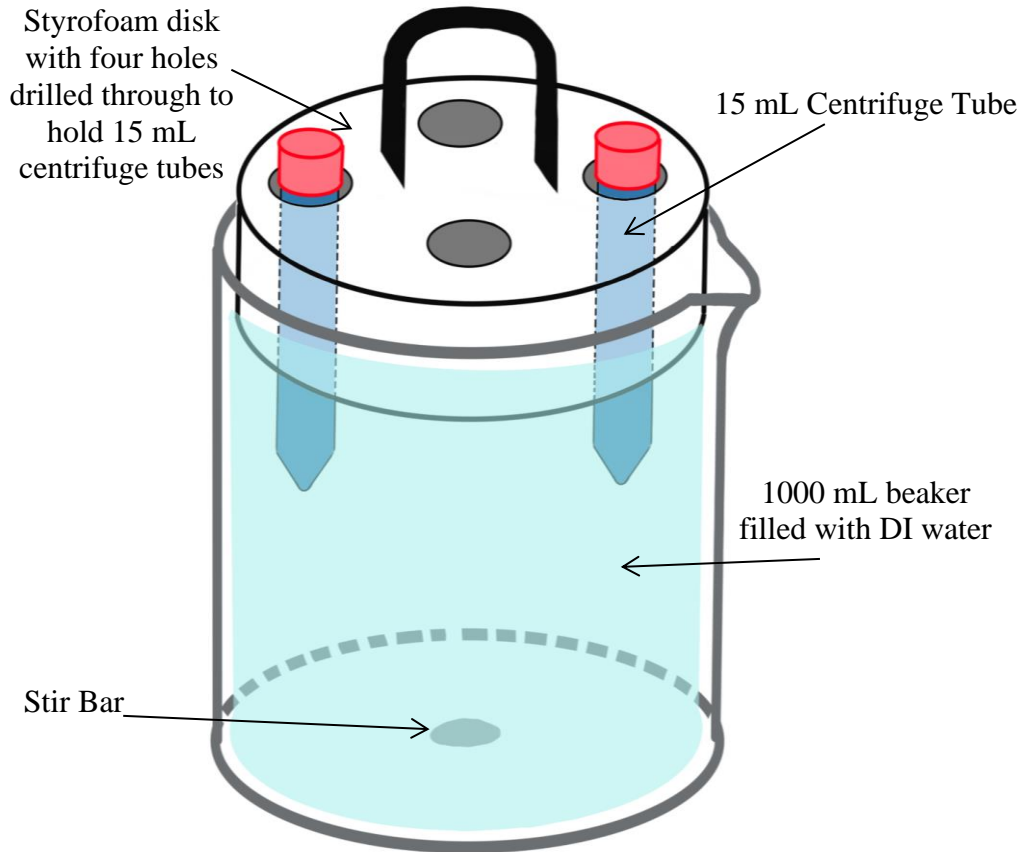
Appendix C. Detailed Oxine Procedure

Oxine Procedure Materials:

- Oxine Solution ($5x10^{-4}$ M)

  - 0.07250 g 8-Hydroxyquinoline dissolved in 10 mL 200 proof Ethanol and diluted to 100 mL with 0.01 M Acetic Acid.

- 0.1 M HNO_3 in Ethanol

  - 630 µL trace metal grade Nitric Acid diluted to 100 mL with 200 proof Ethanol.

- 10 % Triton X-114

  - 5 mL of 100 % Triton X-114 diluted to 50 mL with DI water.

- 50 ppb Lead Standard Solution

  - 1,250 µL of 10,000 ppb AA Lead Standard diluted to 250 mL with DI water.

- 0.1 M HCl

- 0.1 M NaOH

- Digital pH Meter

- Hot Plate with Magnetic Stir Bar

- Disposable Plastic 50 mL and 15 mL Centrifuge Tubes

- Styrofoam Support For 15 mL Centrifuge Tubes (same as PAN procedure)

- Glassware soaked in 10% Nitric Acid for 24 hours

Oxine Procedure:

1. Using a bubble pipette transfer 25 mL of water (containing lead) to a 50 mL centrifuge tube.

2. Add 20 µL Oxine Solution to the centrifuge tube.

   a. Sonicate for 1 minute.

3. Add 1.25 mL of 10% Triton X-114 to the centrifuge tube.

   a. Sonicate for 1 minute.

4. Adjust the pH of the solution to 7.0 with 0.1 M HCl or 0.1 M NaOH.

5. Divide the solution into two 15 mL centrifuge tubes.

6. Place the centrifuge tube in a 50 °C water bath (with stir bar) for 10 minutes.

7. Centrifuge for 10 minutes at 3,500 rpm.

8. Cool centrifuge tubes in an ice bath for 10 minutes.

9. Invert both centrifuge tubes to decant off the aqueous phases into one 50 mL centrifuge tube.

10. Add 100 µL of 0.1 M HNO_3 in Ethanol to the organic phase left in each 15 mL centrifuge tube.

    a. Vortex for 30 seconds to combine.

11. Combine the diluted organic phases into one 15 mL centrifuge tube.

12. Aspirate directly into FAAS.

Appendix D. Detailed TAN Procedure

TAN Procedure Materials:

- Solution of Triton X-114 and 1-(2-thiazolylazo)-2-napthol (TAN)

    - 100 mL of 2.6 % Triton X-114 (diluted in DI water)

    - 1×10^{-3} M TAN

        - 0.02553g TAN

- 0.1 M $Na_2B_4O_7 \cdot 10H_2O$ Buffer Solution

    - 3.8137 g $Na_2B_4O_7 \cdot 10H_2O$ in 100 mL DI water.

    - Adjust to a pH of 8.6 with 4 M HCl.

- 4 M HCl

- 0.1 M HNO_3 in Methanol

    - 320 µL of trace metal grade Nitric Acid diluted to 50 mL with Methanol.

- Lead Standard Solutions

    - 5 ppb, 10 ppb, 20ppb, 50 ppb, 100 ppb

    - Prepared from 1000 ppm AA Lead Standard diluted to 1 L.

- 1 M HCl

- 1 M NaOH

- Disposable Plastic 15 mL and 50 mL Centrifuge Tubes

- Glassware soaked in 10% Nitric Acid for 24 hours

General TAN Procedure:

1. Transfer 50 mL of water (containing lead) to a 125 mL glass bottle using a bubble pipette.

2. Add 1.0 mL of the TX-114 and TAN solution.

    a. Sonicate for 1 minute.

3. Add 1.0 mL of buffer solution.

    a. Adjust the pH to 8.6 using 1 M HCL and 1 M NaOH

    b. Sonicate for 1 minute.

4. Place the glass bottle into a 40 °C water bath for 15 minutes with a stir bar in the water at 350 rpm.

5. Divide the solution into 4, 15 mL centrifuge tubes and centrifuge at 5,000 rpm for 15 minutes.

6. Cool centrifuge tubes in ice for 10 minutes.

7. Invert all centrifuge tubes to decant the aqueous phases into one 50 mL centrifuge tube.

    a. Place the 15 mL tubes back into the ice and allow any remaining aqueous phase to settle to the bottom before inverting again into the 50 mL tube. Repeat this a second time.

8. Add 50 µL of the HNO_3 solution to each 15 mL centrifuge tube.

    a. Vortex the tubes until the organic layer and HNO_3 are homogeneous.

9. Combine all the organic layers from the 15 mL tubes into one tube.

10. Aspirate directly into FAAS.

Appendix E. ADPC Results

Results from the FAAS run on 10-30-19. Samples were analyzed at 217 nm. Only 1 replicate was taken for each sample, so no RSD data was collected.

Calibration Curve (not preconcentrated)

$y = 4E\text{-}05x + 0.0063$
$R^2 = 0.9985$

ADPC Preconcentration Study
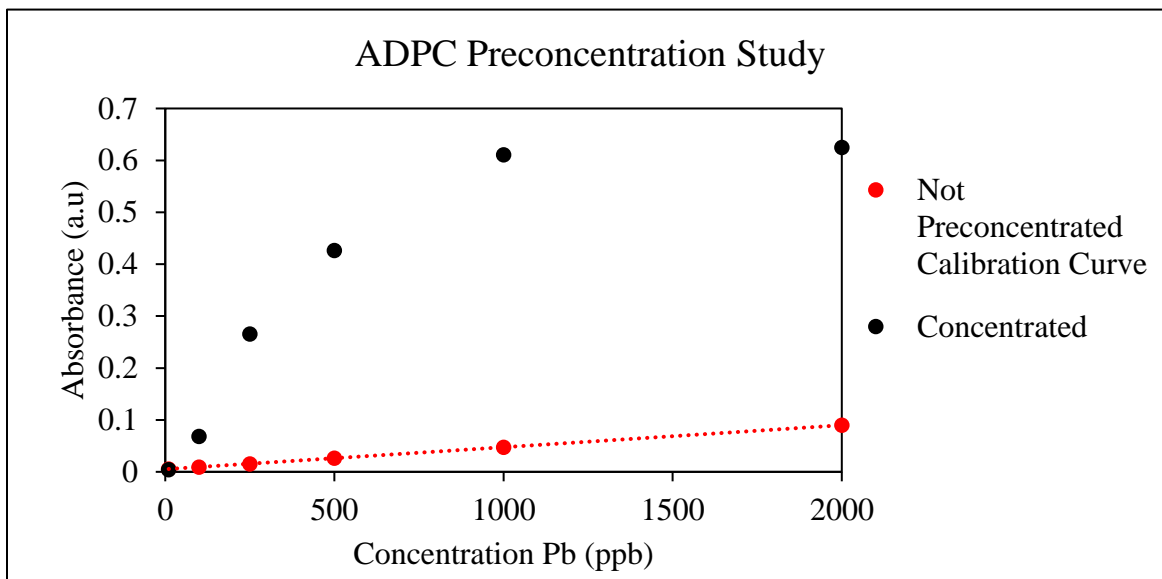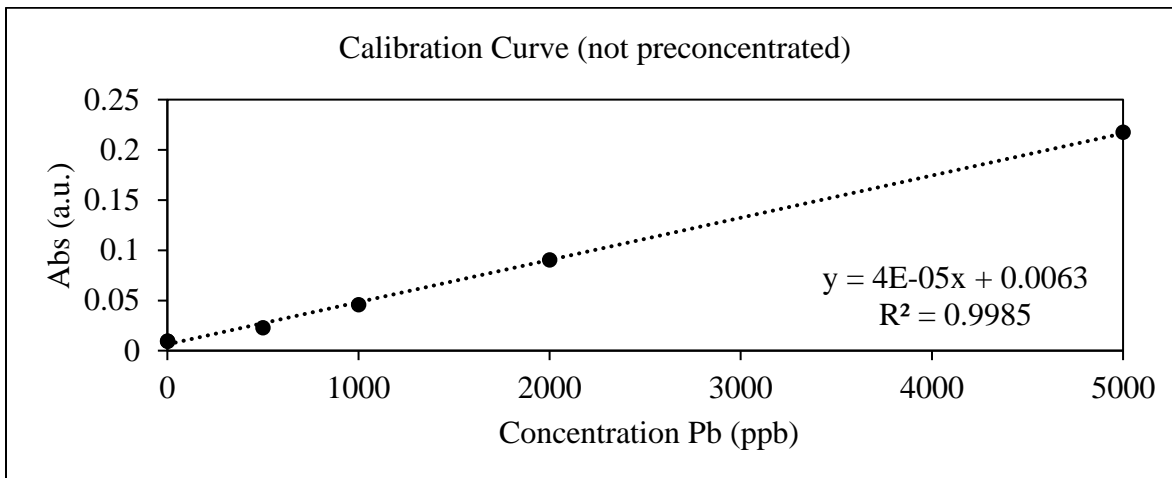
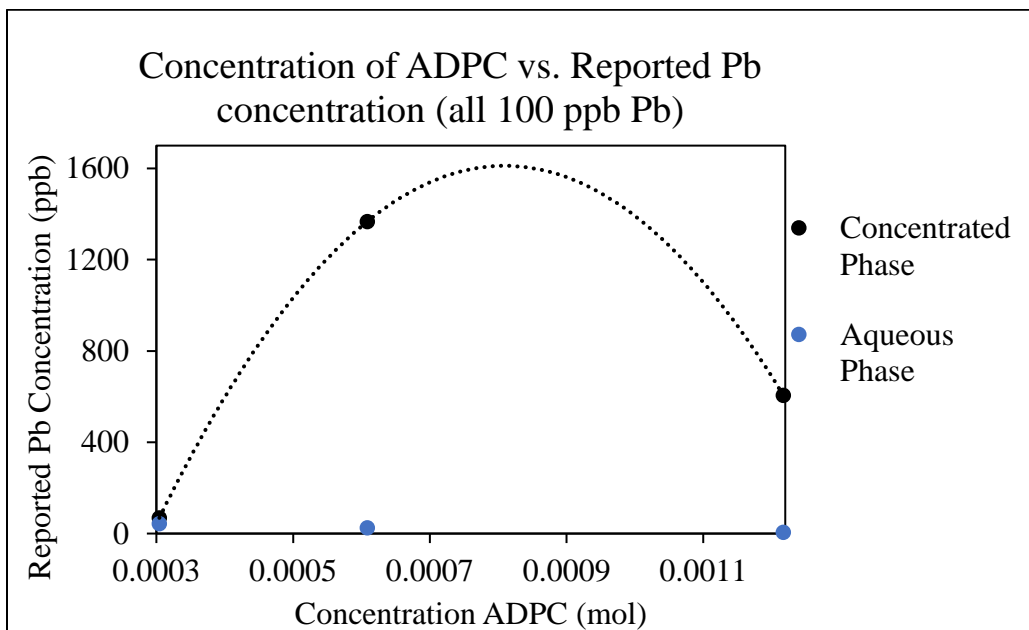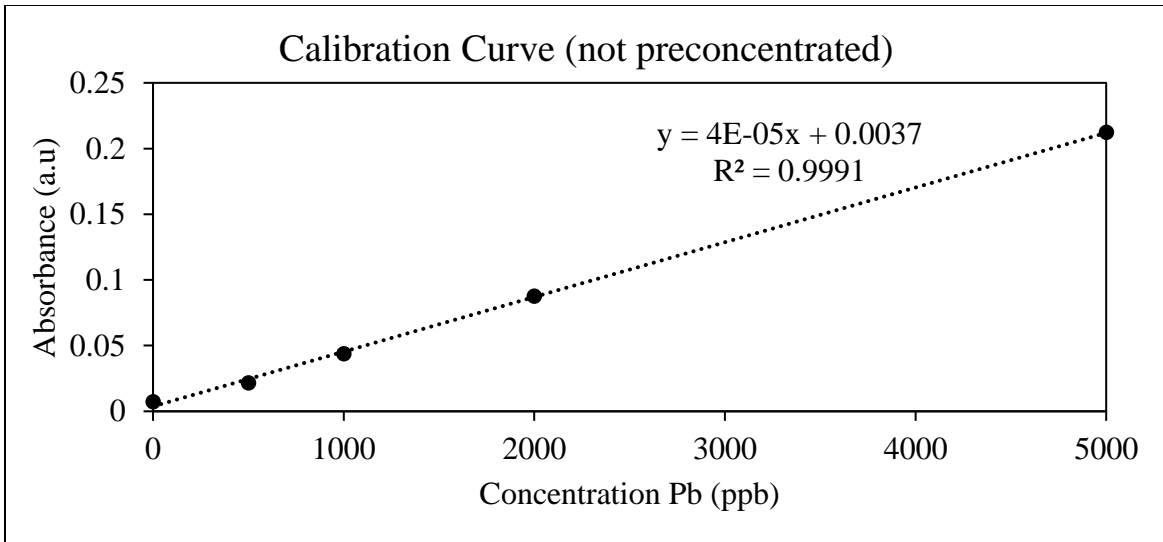| Sample ppb	Expected Abs based on Calibration Curve	Actual Abs	Concentration Factor
10	0.0004348	0.0045	10.35
100	0.004348	0.0685	15.75
250	0.01087	0.2654	24.42
500	0.02174	0.4268	19.63
1000	0.04348	0.6109	14.05
2000	0.08696	0.6253	7.19

Results from FAAS run on 11-12-19. Samples were analyzed at 217 nm. Only 1 replicate was taken for each sample, so no RSD data was reported.

Calibration Curve (not preconcentrated)

$y = 4E\text{-}05x + 0.0037$

$R^2 = 0.9991$

Absorbance (a.u) vs. Concentration Pb (ppb)

Concentration of ADPC vs. Reported Pb concentration (all 100 ppb Pb)

Reported Pb Concentration (ppb) vs. Concentration ADPC (mol)

● Concentrated Phase

● Aqueous Phase

Concentration of ADPC vs. Absorbance (in 100 ppb Pb)
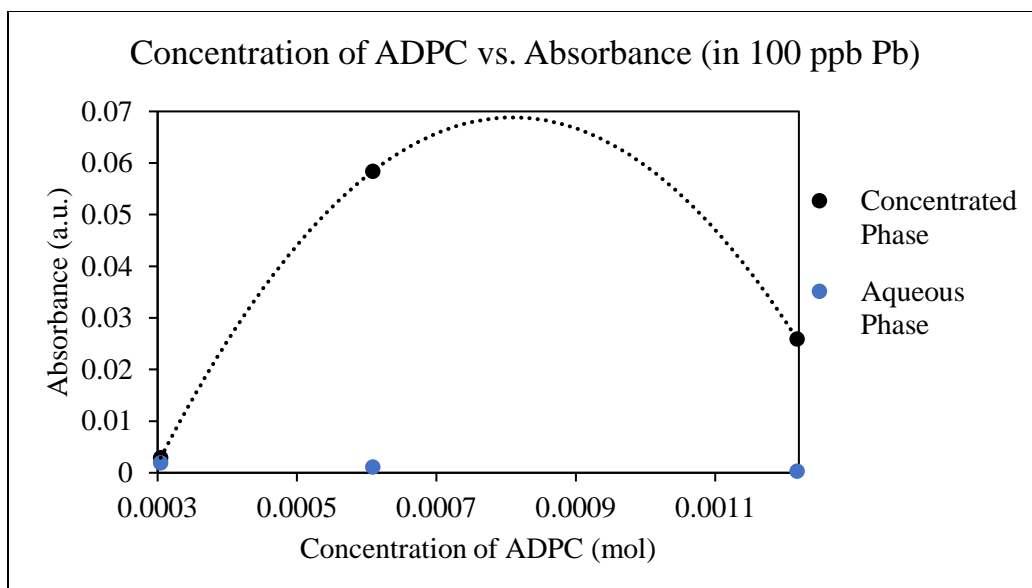
Sample	mL ADPC	Mol ADPC	Mean Abs
Concentrated 1	2	0.00121	0.0259
Concentrated 2	1	0.00061	0.0584
Concentrated 3	0.5	0.00030	0.0029
Aqueous 1	2	0.00121	0.0003
Aqueous 2	1	0.00061	0.0011
Aqueous 3	0.5	0.00030	0.0019

Appendix F. PAN Results

Results from FAAS run on 1-27-20. Samples were analyzed at 283.3 nm.
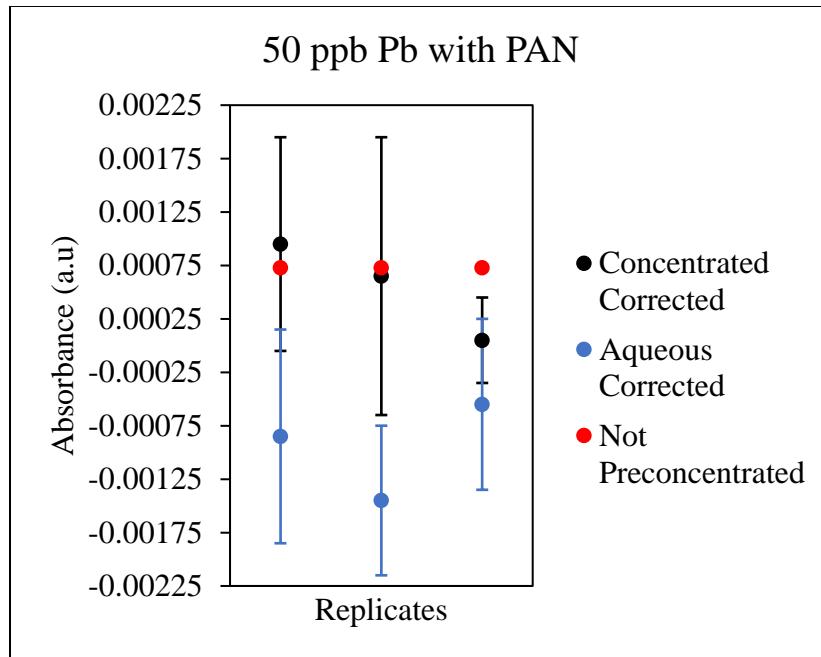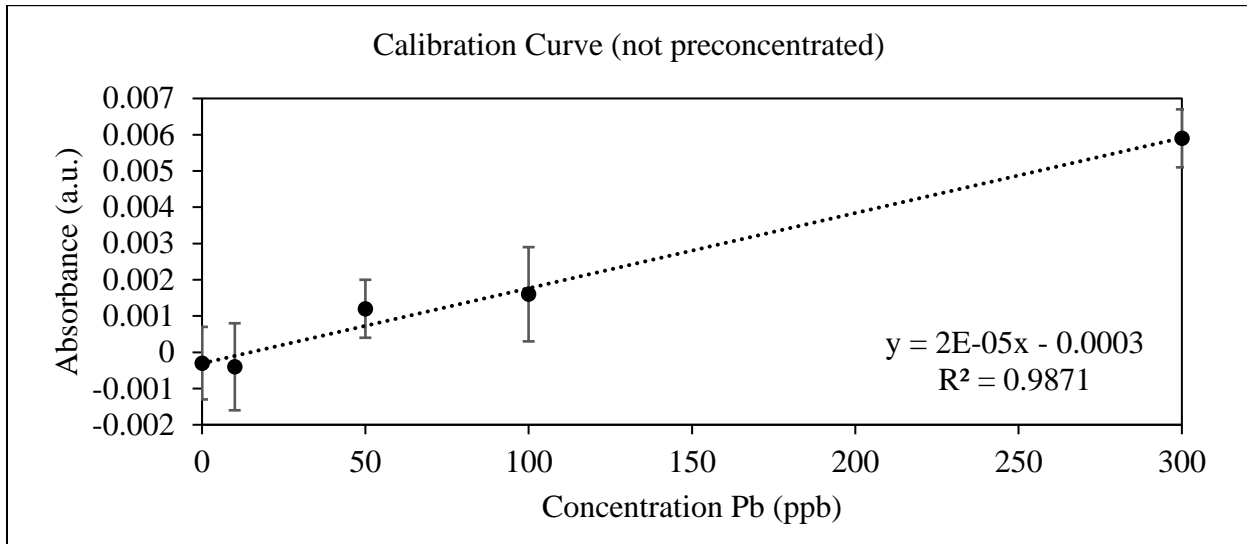
Sample	% RSD	SD	Mean Absorbance
50 ppb concentrated 1	14.5	0.001	0.0067
50 ppb concentrated 2	19.7	0.0013	0.0064
50 ppb concentrated 3	7.4	0.0004	0.0058
50 ppb aqueous 1	75.6	0.001	0.0013
50 ppb aqueous 2	96.3	0.0007	0.0007
50 ppb aqueous 3	50	0.0008	0.0016

Results from FAAS run on 2-7-20. Samples were analyzed at 217 nm.

Calibration Curve (not preconcentrated)

$y = 4E\text{-}05x + 0.0004$
$R^2 = 0.9937$

50 ppb Pb with PAN
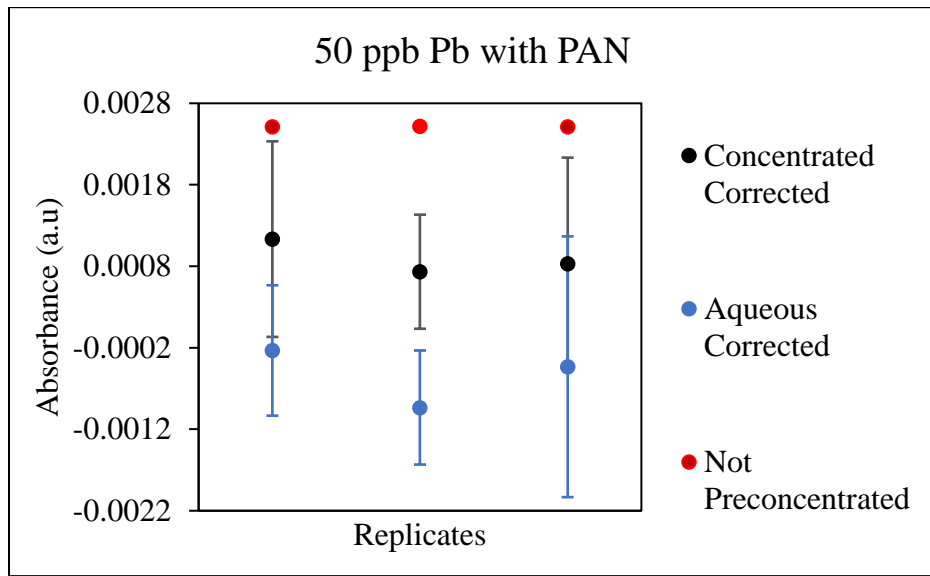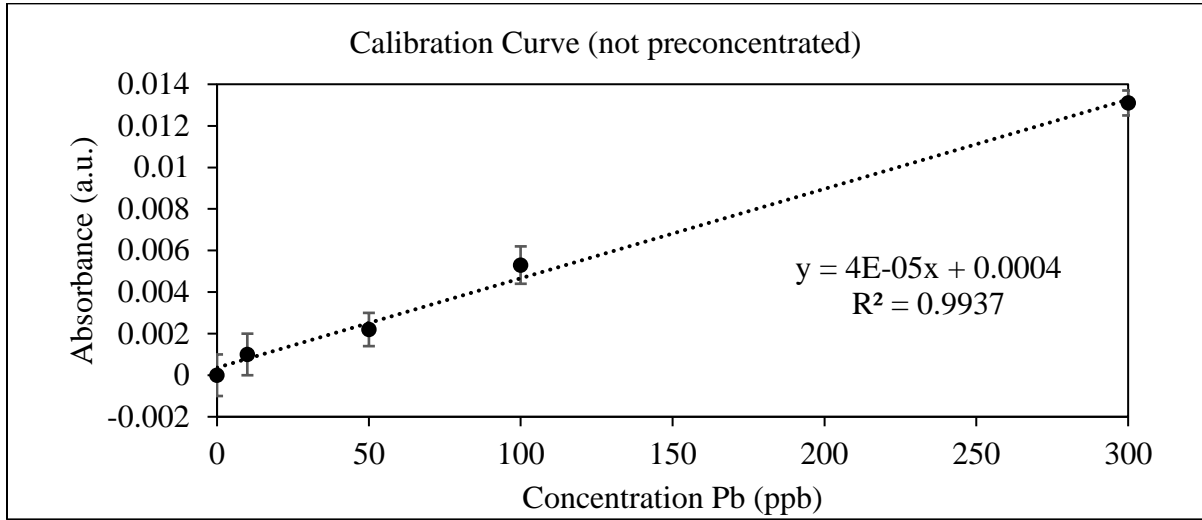
Sample	%RSD	SD	Mean Abs	Abs-Method Blank Abs	Expected Abs from Calibration	Concentration Factor
50 ppb Concentrated 1	23.5	0.0012	0.0051	0.0011	0.0025	0.4509
50 ppb Concentrated 2	15.4	0.0007	0.0047	0.0007	0.0025	0.2917
50 ppb Concentrated 1	26.6	0.0013	0.0048	0.0008	0.0025	0.3315
50 ppb Aqueous 1	30.3	0.0007	0.0022	-0.0016		
50 ppb Aqueous 2	43.5	0.0009	0.0022	-0.0016		
50 ppb Aqueous 3	41.8	0.0009	0.0021	-0.0017		

Appendix G. TAN Results 1

Results from FAAS run on 2-28-20. Samples were analyzed at 217 nm.

Calibration Curve (not preconcentrated)

$y = -6E-07x^2 + 0.0001x - 0.0004$

$R^2 = 0.9813$

Absorbance (a.u.) vs. Concentration Pb (ppb)

50 ppb Pb with TAN

Corrected Absorabance (a.u.) vs. Replicates

● Concentrated Corrected

● Aqueous Corrected
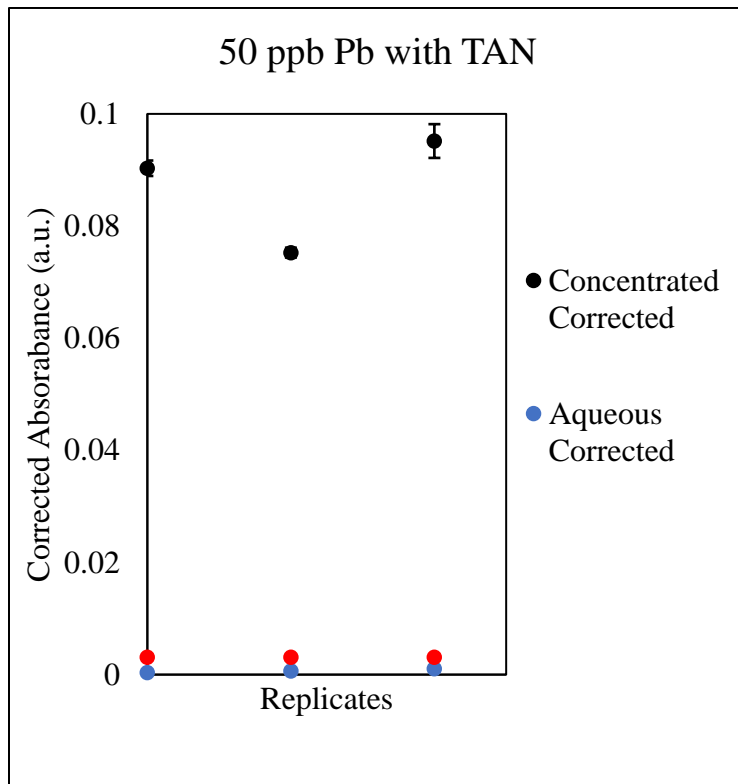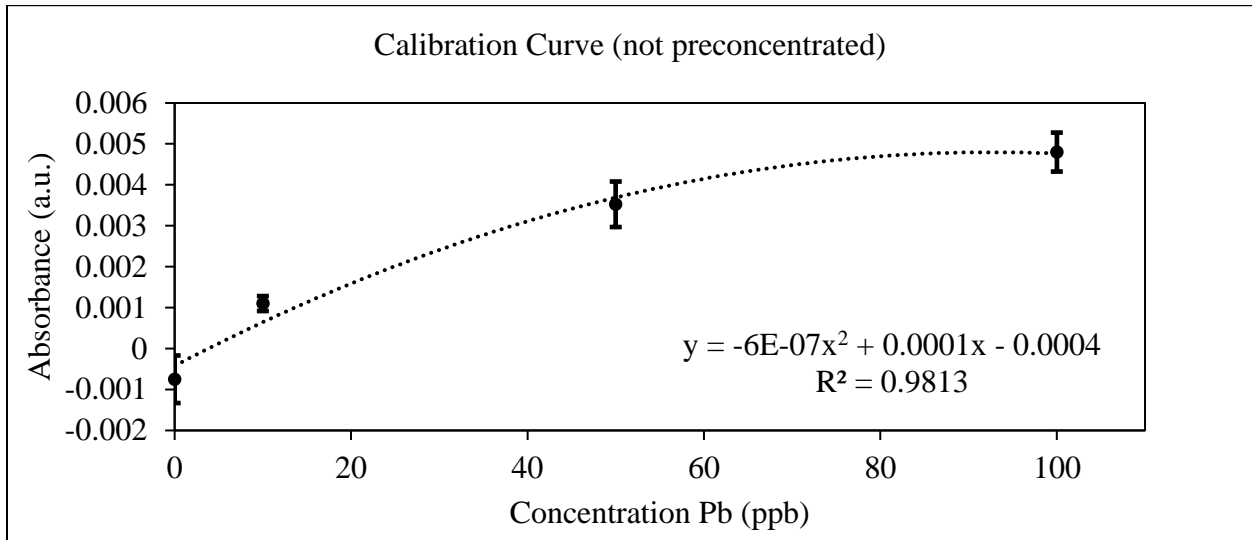
Sample	Mean Absorbance	SD	%RSD	Adjusted Abs	Expected Abs	Concentration Factor
50 ppb Concentrated 1	0.09265	0.00138	1.49	0.09031	0.0031	29.13
50 ppb Concentrated 2	0.07760	0.00088	1.13	0.07526	0.0031	24.28
50 ppb Concentrated 3	0.09750	0.00301	3.09	0.09516	0.0031	30.70
50 ppb Aqueous 1	0.00118	0.00033	28.12	0.00041		
50 ppb Aqueous 2	0.00145	0.00037	25.50	0.00068		
50 ppb Aqueous 3	0.00185	0.00039	20.94	0.00108		

Appendix H. TAN Results 2

Results from FAAS run on 3-6-20 and Cambridge Water Department. Samples were

analyzed at 217 nm.
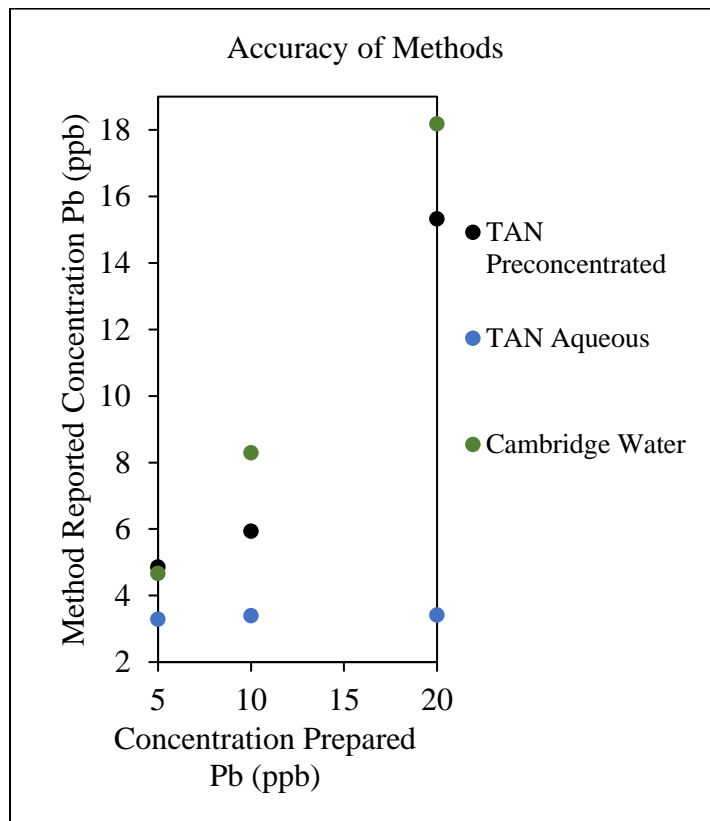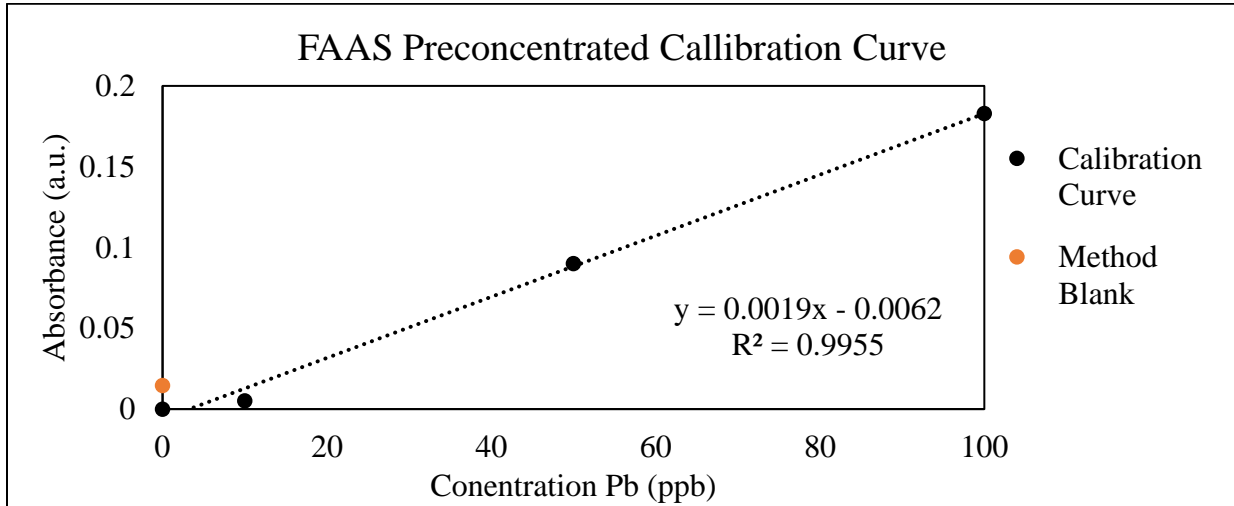
FAAS Data

Sample	%RSD	SD	Mean Abs
5 ppb Concentrated 1	59	0.0018	0.0030
5 ppb Concentrated 2	11.2	0.0005	0.0040
5 ppb Concentrated 3	23.8	0.0005	0.0021
10 ppb Concentrated 1	5.9	0.0003	0.0052
10 ppb Concentrated 2	19.4	0.001	0.0050
10 ppb Concentrated 3	6.8	0.0003	0.0050
20 ppb Concentrated 1	3.6	0.0008	0.0214
20 ppb Concentrated 2	1	0.0003	0.0288
20 ppb Concentrated 3	3.4	0.0006	0.0179
5 ppb Aqueous 1	>100	0.0002	-0.0001
5 ppb Aqueous 2	>100	0.0003	0.0000
5 ppb Aqueous 3	66.9	0.0002	0.0003
10 ppb Aqueous 1	>100	0.0003	0.0001
10 ppb Aqueous 2	52.9	0.0001	0.0002
10 ppb Aqueous 3	84.6	0.0004	0.0005
20 ppb Aqueous 1	>100	0.0007	0.0001
20 ppb Aqueous 2	88.3	0.0003	0.0004
20 ppb Aqueous 3	84.4	0.0003	0.0004

Appendix I. Exporting Data from Simmons' Agilent 240 FAAS

In the Spectra AA Software:

1. Save the FAAS worksheet after finishing the run.

2. Close out of the worksheet.

3. Open the "Reports" section from the Spectra AA startup page.

4. In the "worksheets" tab select the file you wish to export.

5. In the "settings" tab select the specific data you wish to be exported (RSD, error messages etc.)

   a. Unless you need them, it is best to NOT select error messages, it makes formatting easier later.

6. In the "report" tab first choose "print" which should open a pdf report, an example is shown below. To save this go to "File", "Print", and "save as pdf" instead of selecting a printer.

SpectrAA Report.   3:29 PM 1/17/2020   Page 2 of 10

| Sample ID	Conc ug/L		%RSD	SD	Mean Abs	BG Abs	
CAL ZERO	0.00		>100	0.0010	-0.0005	0.0004	
	Readings						
	-0.0008	0.0000		-0.0002		0.0007	-0.0020
	1/17/2020	1:48:38 PM					
STANDARD 1	10.00		42.9	0.0016	0.0036	-0.0016	
	Readings						
	0.0037	0.0057		0.0045		0.0021	0.0021
	1/17/2020	1:49:00 PM					
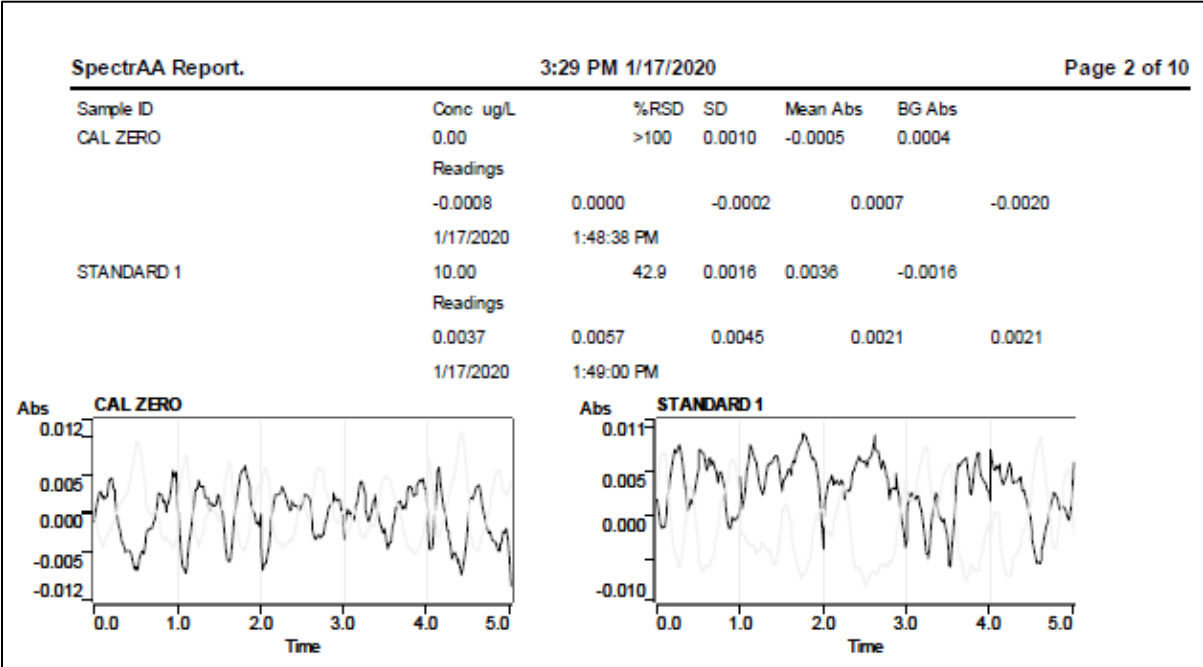
7. Then return to the Spectra AA software and under "reports" select "write to a text file" and save the file to the computer. It should save as a .txt file.

8. These two files from steps 6 and 7 can then be transferred onto a flash drive and uploaded onto a computer with Microsoft Excel.

Below is an example of a pdf report and how to read the information it contains. Headings are only placed at the beginging of each page but the same pattern of information is follwed througout the page. First is the reported concentration, shown here in red. Next is the units of concentration, shown in blue. Then the %RSD, shown in green. Followed by the SD, shown in yellow. Then the mean absorbance, boxed in pruple. And the background absorbance shown in dark orange. Finally the readings are presented along the bottom, boxed in black. A dashed line has been placed across this page to demonstrate the seporation between the samples. All of the data above this line belong to the sample "CAL ZERO". The amount and type of data presented in the report may be differnet depending on what was selected under "settings".
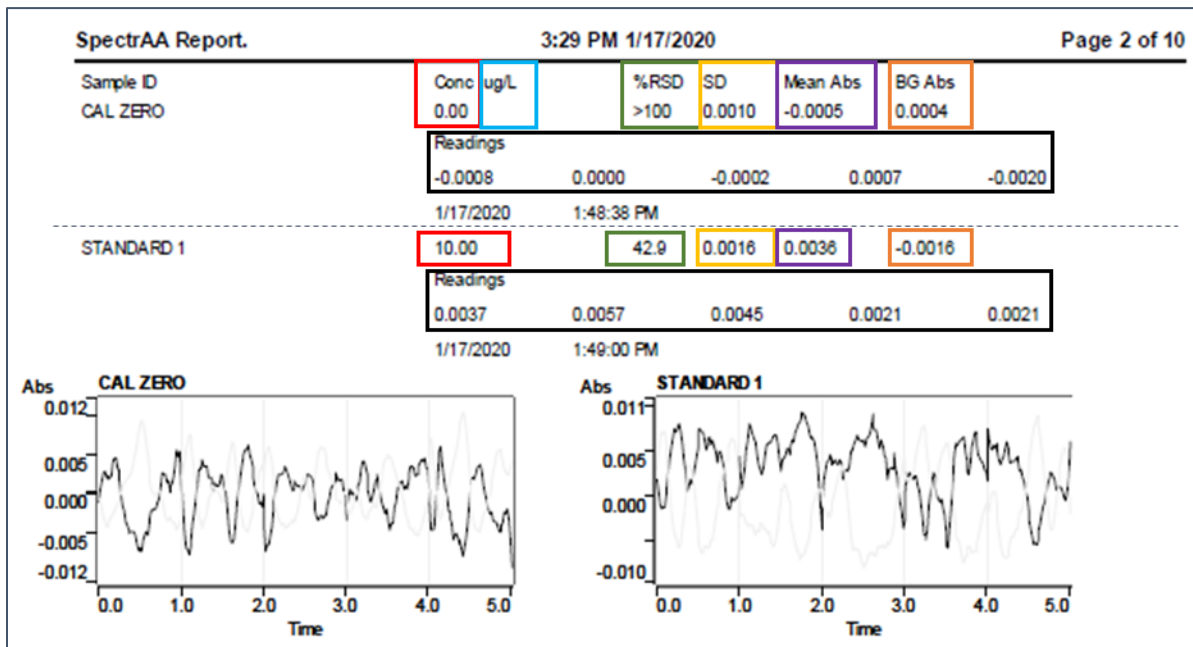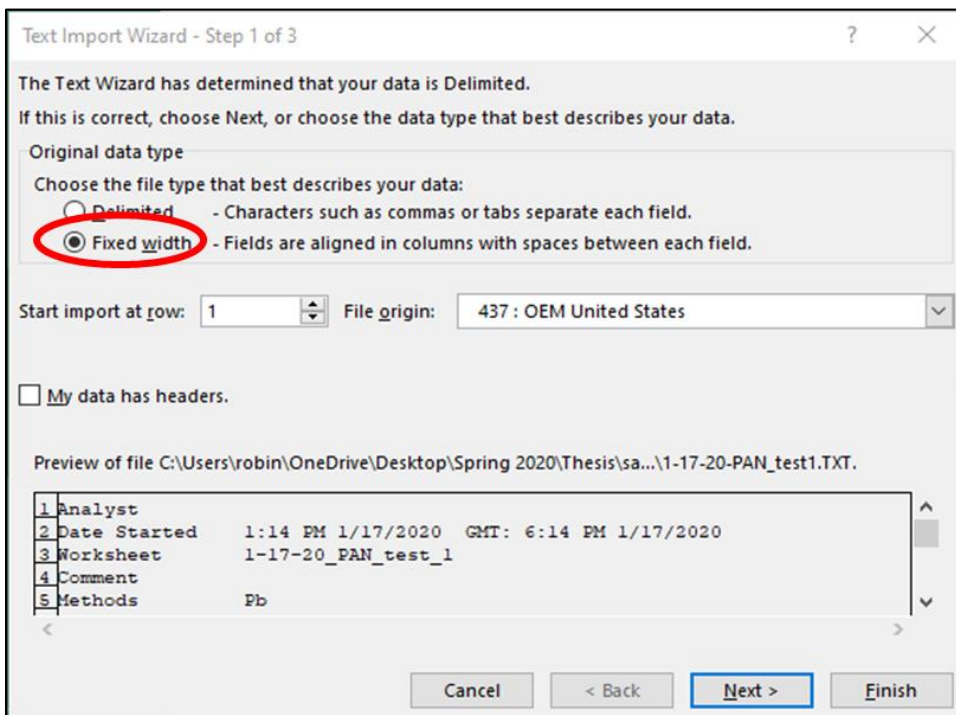
In Excel:

1. Open excel and go to "Open", then "Browse" and make sure "All Files" is selected in the

    bottom righthand corner in order to see your .txt files.
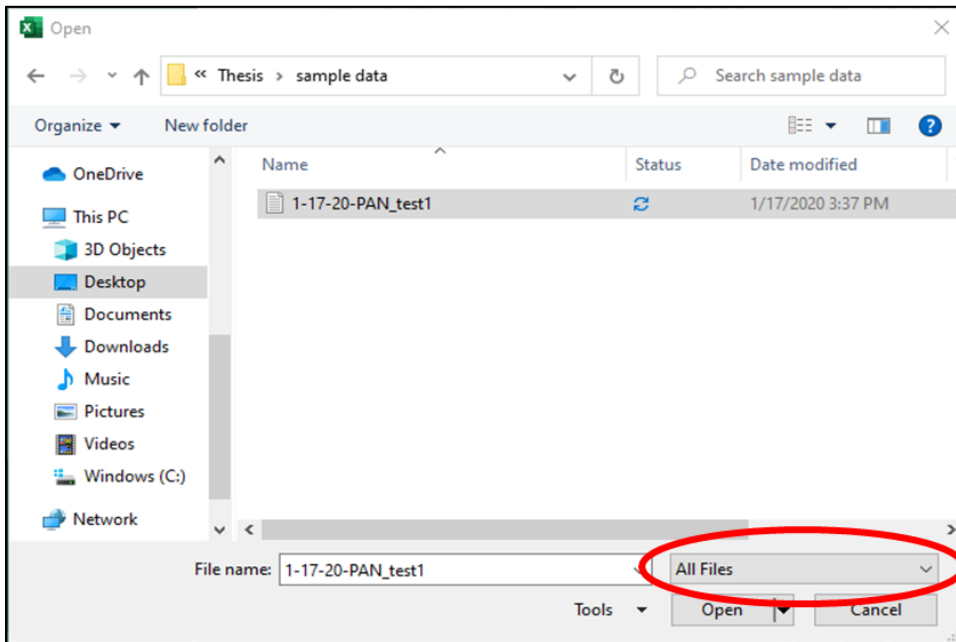
2. In the "Text Import Wizard" popup window select "fixed width", then click next. Also

    open the pdf version of the data as in a second window for reference.

3. Using the pdf report as a guide, place a line break between each column of data by clicking once. Pay close attention to the SD and RSD column, the .txt file does not split them into two obvious columns.

    a. On my files I found that breaks needed to be added at 27, 45, 56, 66, 77, 82, 90, 100, 111, 124, 135, and 144. An example of breaks 56-124 are shown below and labeled with the corresponding pdf label.
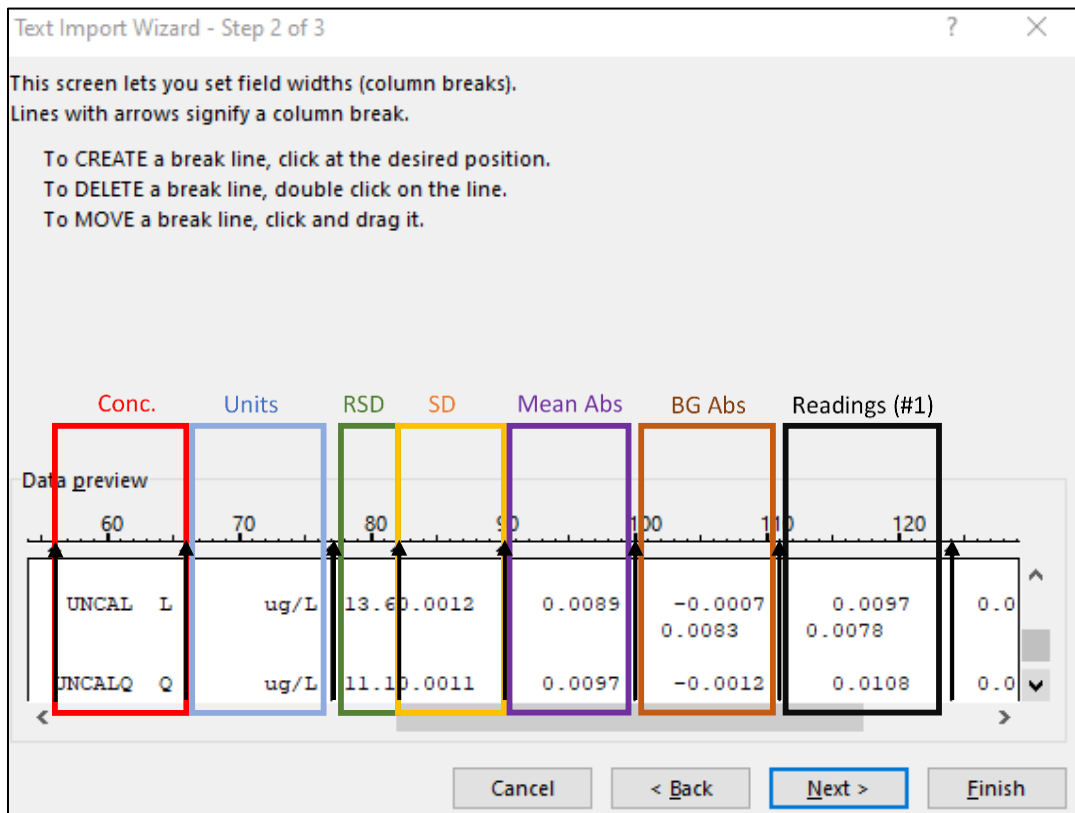
4. Then click "Next" and "Finish".

5. The excel file should then open, some of the text (especially the file name) may be broken up between multiple column. They can be re-written if you are unable to read them. What is important is that the data has all been separated properly.

6. Label the top of the data columns, follow along with the pdf report if you are unsure of what the numbers represent.

	C	D	E	F	G	H	I	J	K	L	M	N
158												
159		Conc	Units	RSD	SD	Mean Abs	BG Abs	Readings (#1)	Readings (#2)	Readings (#3)	Readings (#4)	Readings (#5)
160		UNCAL L	ug/L	13.6	0.0012	0.0089	-0.0007	0.0097	0.0106	0.0081		
161								0.0083	0.0078			
162												
163		UNCALQ (	ug/L	11.1	0.0011	0.0097	-0.0012	0.0108	0.0094	0.0105		
164								0.0099	0.0081			
165												

7. If there are more than three readings (replicates) they will have been placed into a new row under the rest of the data. It is recommended to select that data and move it up next to the other replicates so all the data for one sample is in one row.

	C	D	E	F	G	H	I	J	K	L	M	N
158												
159		Conc	Units	RSD	SD	Mean Abs	BG Abs	Readings (#1)	Readings (#2)	Reading (#3)	Readings (#4)	Readings (#5)
160		UNCAL L	ug/L	13.6	0.0012	0.0089	-0.0007	0.0097	0.0106	0.008	0.0083	0.0078
161												
162												
163		UNCALQ (	ug/L	11.1	0.0011	0.0097	-0.0012	0.0108	0.0094	0.0105		
164								0.0099	0.0081			

8. SAVE THE FINAL FILE AS AN EXCEL WORKBOOK! The default setting for these imported data files is to save as a "Text (tab delimited) (.txt)" file which will NOT save any of the formatting work you have done or any graphs that are made. Before closing out of the window it MUST be saved as an excel workbook file. Go to "File", "Save As", and instead of "Text (tab delimited) (.txt)" Select "Excel Workbook (.xlsx)".