

OREGON STATE UNIVERSITY

DEPARTMENT OF CHEMISTRY

## Experiment 4

Integrated Laboratory Experiment - CH 461 &amp; CH 461H

**ICP EMISSION AND FLAME ABSORPTION ATOMIC SPECTROMETRY**

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## EXPERIMENT 4

**ICP EMISSION AND FLAME & FURNACE ABSORPTION SPECTROMETRY**

## I. INTRODUCTION

A. *Atomic Spectrometry*

The development of analytical **atomic spectrometry** began in the 18th century with the observation of colored radiation from flames containing alkali salts. Initially all atomic spectrometers were atomic emission spectrometers because they were based on thermal excitation of atoms to their excited states and monitoring of their characteristic emission lines. Due to the thermal nature of excitation, the magnitude of the emission signal is extremely dependent on the temperature of excitation source (flame, plasma, etc.). The first **flame atomic emission (AE) spectrometers** for quantitative analysis appeared before 1950 and improvements were made in instrumentation including higher temperature and more stable flames and improved monochromators. Emission spectrometry based on arc and spark instrumentation also flourished for analysis of metals in alloys and steel. Although flame atomic emission can be used to determine most metals at concentrations of  $\mu\text{g/mL}$  (and below in many cases), it did not provide adequate detection limits for many important metals because of the limited excitation energy of flames. By the 1970's, flame emission spectrometry (AES) was largely replaced by **flame atomic absorption (AA) spectroscopy** (today AES is sometimes used for determination of alkali metals in clinical samples).

The phenomenon responsible for atomic absorption was first observed in 1802 with the discovery of the Fraunhofer lines in the sun's spectrum. It was recognized that the phenomenon of atomic absorption is responsible for non-linearity in emission methods of analysis (i.e., self-absorption and self reversal). In 1955, Walsh proposed that atomic absorption could advantageously be used for quantitative chemical analysis and the initial growth of analytical **atomic absorption spectroscopy (AAS)** was rapid as it replaced flame AES spectrometry.

It provides better detection limits than flame emission spectrometry for many elements (e.g. Zn, Be, Cd). Flame atomic absorption spectrometry is subject to the same chemical and physical interferences as flame atomic emission spectrometry; however, it is less affected by spectral overlap interferences. **Electro-thermal atomizers** are also widely used as atomization

sources for AAS and provide superior detection limits relative to flames but yield lower sample throughput. This technique is referred to as Graphite Furnace AAS (or GFAA).

Atomic absorption spectrometry is based on the absorption of radiation from an external excitation source by ground state atomic vapor. Under proper conditions, Beer's law is followed and the absorbance of the atomic vapor is directly proportional to the solution concentration of the element determined. Usually line sources such as **hollow cathode lamps** (HCL) are used for atomic absorption spectrometry. Normally a different HCL is required for each element, although some multi-element lamps are available.

In the 1980's, plasma sources for atomic emission spectrometry (AES) were refined and combined with high resolution monochromators. These plasma atomic emission spectrometers have now replaced flame atomic absorption spectrophotometers in many cases, although flame AAS is still probably the most popular single-element technique for analysis of trace amounts of metals.

The most common plasma source is an **inductively coupled plasma (ICP)** which is an argon plasma that receives its energy from a radio frequency (RF) coil. The high excitation temperature (about 6000 K) provides ng/mL detection limits for many elements and calibration linearity extends over 4 or 5 decades with a PMT detector. ICP atomic (optical) emission spectrometers (ICP-AES or OES) are considered multi- element instruments. With high-resolution, computer-controlled monochromators, instruments can perform rapid sequential determinations of many elements in a relatively short time with little operator attention. Instruments that perform simultaneous analysis instead of sequential analysis use CCD detectors.

## B. *Scope of Experiment*

In this experiment, you will study:

- the emission of several elements in a plasma, including Ca, Cu, Fe, Mg, Pb, and Zn
- element of your choice from the commercial standard by ICPAES
- the absorption of Cu, Fe, Pb will be measured by flame AA, and Pb with and without a modifier by graphite furnace GFAA .

For all elements, data are obtained to construct calibration curves, to estimate the detection limit, and to determine the analyte concentration in real samples including: tap water, a vitamin/mineral tablet, a dairy product (e.g., milk, yogurt), and a biological sample (e.g., liver, clams, or oysters), and a Standard Reference Material from NIST for oysters.

## II. Solution Preparation

### A. Stock solutions and Standards.

**Stocks.** The following stock solutions are available in the laboratory:

1. a commercial multi-element standard containing 100  $\mu\text{g/mL}$  of Ca, Cr, Cu, Fe, Mg, Pb, Zn, and other elements (As, Be, Cd, Co, Li, Mn, Mo, Ni, Sb, Se, Sr, Ti, Tl, and V) for ICP work provided for you.
2. various multi-element standards that are dilutions of stock solution 1 above including 5000, 500, 100, and a 50.0  $\text{ng/mL}$  levels in 2%(v/v) nitric acid for ICP work provided for you.
3. a 100  $\mu\text{g/mL}$  multi-element standard for AAS flame work containing equal concentrations of Cu, Fe, and Pb in 2% nitric acid.
4. a 50  $\text{ng/mL}$  standard for GFAA containing Pb is provided for you. Also a solution of  $\text{PdCl}_2$  used as the modifier in GFAA is provided.

**Standards.** Each team makes 4 standards for the AAS flame work and the remaining standards are provided for you. Make your standards from the 100  $\mu\text{g/mL}$  stock #3 above and quantitatively prepare the following test solutions in 100-mL volumetric flasks for the Flame AAS measurements. Make a 10.0, 5.00, 3.00, and a 1.00  $\mu\text{g/mL}$  standard solution using volumetric pipets and flasks. Dilute to volume with 2% (v/v) nitric acid which was made in DI water and available in large labeled carboys on the side reagent shelf.

### B. Synthetic unknown, Sample Reference Material, and Real Samples.

**Synthetic multi-element unknown.** Quantitatively transfer the contents of the vial of unknown to a 50-mL volumetric flask and dilute to volume with 2%(v/v) nitric acid. Record the number of your team's unknown in your notebook.

**Standard Reference Material (SRM)** from National Institute of Standards and Testing (NIST). A 2.xxx g sample diluted to 250 mL with 2%  $\text{HNO}_3$  of 1566b Oyster will be provided for you. Record the actual mass used and later calculate the expected elemental composition using the information on the Certificate of Analysis posted on the web site.

**Real samples.** There are four types of real samples analyzed in this experiment. Be sure to record all relevant product information that is listed on the containers: serving size, mass / serving, and % DV (daily value) of all the metals listed on the product containers.

- (1) **oysters or clams, (note if you used the water or the oil packed oysters)**
- (2) **Centrum multivitamin/multimineral tablet,**
- (3) **tap water** and
- (4) **yogurt.**

### **Sample Preparation Protocols:**

#### **Oysters and Clams (each group selects one of these)**

Oysters and clams are a challenging samples for analysis. They have high mineral content and are considered a good source of Cu, as is liver and crab. The typical Cu content of oysters is 1 to 3 mg per oz (1 oz = 28.35 g) and in clams is 0.1 - 0.2 mg per 3 oz. In this experiment, the oysters and clams will be dry ashed in a muffle furnace. The first day you will weigh them into a pyrex beaker and put the beaker in a designated spot in the muffle furnace in the main lab. The next lab period you will weigh the beaker and ash then dissolve the resulting ash after heating it in acid. You will prepare two solutions for chemical analysis: one concentrated solution for the AAS analysis, and one diluted solution for the ICPAES and GFAA analyses.

#### **Oysters:**

Take a couple of oysters out of the can and pat the oil/and liquid off with some Kimwipes. Weigh the oysters to 0.1 mg and transfer to a small beaker. The total mass should be between 3 and 6 g. If the mass is not in this range, add or remove an oyster. Somewhat chop up the oysters in the beaker (don't lose any mass). The instructor will help you put your team's beaker in a designated spot in the muffle furnace. Over the weekend the muffle furnace will be ramped up to 500 °C and the oysters roasted for at least 24 hr at to produce a white ash. At first the temperature is set to 100 °C and then increased to 500 °C so avoid excessive organic smoke at the start that could cause losses.

**Clams:**

Take several clams out of the can and drain and pat the oil/and liquid off with some Kimwipes. The total mass should be between 30 and 40 g. If the mass is not in this range, add or remove a clam. Weigh the clams to 0.1 mg and transfer to a medium beaker. Somewhat chop up the clams in the beaker (don't lose any mass). The instructor will help you put your team's beaker in a designated spot in the muffle furnace. Over the weekend the muffle furnace will be ramped up to 500 °C and the clams roasted for at least 24 hr at to produce a white ash. At first the temperature is set to 100 °C and then increased to 500 °C so avoid excessive organic smoke at the start that could cause losses.

**For both the oysters and the clams:**

The next lab period, add a few milliliters to the beaker of 50% (v/v) nitric acid (i.e., 50% concentrated  $\text{HNO}_3$ , 50% MP water that you make yourself). Gently heat and swirl for 5 min- don't let the solution and material boil out of the flask. Do not use boiling chips. Neutralize up to between pH 2 and 7 by adding a drop of con  $\text{NH}_3$  (aqueous) and swirl carefully to mix. Check the pH with indicator paper using a drop of solution on the paper, and add more base if needed. Keep the volume to a minimum and keep a record of how much base is added. Gravity filter the solution directly into a 50-mL volumetric flask using Whatman Qualitative "1" filter paper, 5.5 cm. Rinse the beaker with a small amount of 2% (v/v) nitric acid and quantitatively transfer to the flask through the filter paper, CAUTION-DON'T exceed 50 mL of washings. Bring the flask up to the 50 mL volume with 2% (v/v) nitric acid. This is the S1 concentrated seafood solution (label it Seafood 1) and it will be analyzed by flame AAS. Next, quantitatively prepare a 1 to 10 dilution of S1 concentrated solution using 2% (v/v) nitric acid. This is S2 and label it Seafood 2. S2 solution will be used for the ICP, and also for the GFAA experiments to analyze for Pb with and without a matrix modifier (e.g.  $\text{Pd}(\text{NO}_3)_2$ ).

### **Multi-vitamin/Multi-mineral Tablet**

Prepare two Centrum Multivitamin & Multi-mineral tablets as follows. You will have four solutions at the end of this procedure. Weigh each whole Centrum tablet to 0.1 mg and then crush each one in a separate mortar and pestle. Transfer about 0.15 g of each powdered tablet to weighing paper. Weigh and quantitatively transfer all the powder into a medium test tube, then re-weigh the paper to determine the mass transferred to the test tube. CAREFULLY add 5 mL of concentrated nitric acid to both tubes and heat them in the dry block heater at 60 °C for 1 hr under a hood. Quantitatively transfer the contents of each test tube into a separate 100-mL volumetric flask and bring up to volume with MP water (the solution will now be 5% (v/v) HNO<sub>3</sub>). These solutions are denoted as vitamin sample concentrated solutions: Multi Vit C1 and Multi Vit C2.

Next, make a 1 to 100 dilution of each Multi Vit C solution in the 2 % (v/v) HNO<sub>3</sub> stock solution and denote these two diluted solutions as Multi Vit D1 and Multi Vit D2.

### **Tap Water (not DI or MP water)**

Clean two 100-mL bottles. You can collect these from anywhere you want. Note the location of the tap or water fountain, the time and date of collection, and the sampling conditions that you choose to use (e.g., first sample of the day, sample after extensive flushing, etc.). Collect two samples of the same tap water by filling two bottles and stopper these (use one for AAS and one for ICPAES & GFAA analysis). **Do not use DI or MP water for this part.**

### **Yogurt**

Yogurt, a fermented, semifluid milk product, is used extensively as a health food. It is prepared from fresh whole or skim milk, boiled and concentrated by evaporation. Fermentation is caused by the addition of cultures of bacteria, usually *Lactobacillus bulgaricus* and *Streptococcus thermophilus*, and sometimes *Lactobacillus acidophilus*. This type of fermented milk has long been an important constituent of the diet in southeastern Europe and in Asia Minor and is popular in the U.S.

*Procedure for using the CEM Microwave Digestion System*

You will use the MDS 2000 microwave digestion system in GBAD 309 to digest a yogurt sample for elemental analysis with ICPAES. The total digestion time will take about 50 min so that you should set this part up to run while you work on making the other solutions for Exp. 4.

**Wear eye protection and latex gloves.** Be prepared to clean up any spills immediately with sodium bicarbonate and wash with water. If you get nitric acid on your skin, flush with copious amounts of water and don't be surprised if you see yellow stains from the reaction of the acid with certain amino acids in the skin.

The dissolution process is carried out as follows.

- 1) Record serving size and %DV of Ca. Task is to quantitatively mass a viscous sample. One method is as follows: draw up sample in plastic pipet, weigh whole thing on analytical balance, transfer 0.15 to 0.25 g of yogurt by dropping (about 0.25 mL to 0.50 mL) directly into the bottom of the Teflon vessel (avoid splashing sample on the inside walls). Re-weigh the plastic pipet with left over sample, and determine by difference the amount of yogurt added to the Teflon vessel. If you added too much or it spilled during the transfer, wash out the bomb vessel, rinse with DI water, and start over. If you have a better idea, try it! You just need to make an analytical transfer for the sample.
- 2) Working under the hood, carefully add 10 mL of concentrated  $\text{HNO}_3$  (70%) so that it covers the sample. Let the vessel liner stand open in the hood for 15 min before sealing. This pre-digestion step allows the sample digestion process to begin before sealing the vessel and helps to avoid pressure overruns during the closed vessel step.
- 3) Put the Teflon cover on the liner and seal the cap on the threaded ring.
- 4) Carefully insert and seat one new rupture membrane inside the grey vent fitting. Tighten the vent fitting over the knurled outlet on the cover. The membrane is designed to blow if the pressure builds up too high in the sample vessel.
- 5) Place the liner in the sleeve and put the entire bomb in a marked position of the turntable. Record the position that you are using in your notebook. After all the sample vessels are assembled and in the turntable, the turntable will be loaded in the microwave oven (instructor). At this time each vessel is fitted with a vent tube through the ferrule nut. The vent tubes go into a

collection vessel in case the reaction is so vigorous that the excess pressure breaks the membrane and hot acid solution spews out.

6) The instructor will load the temperature probe into the sensor vessel and fit it with the special microwave valve. The pre-programmed pressure method called "Yogurt" below has been loaded in memory and will be used. The digestion program is given below. Press the Start button on the front panel of the oven when ready to start. This procedure takes about 1 hour..

**MicroWave Digestor File Name: Yogurt**

Standard Digestion

stage (1) (2) (3) (4) (5)

POWER (% 630 W) 60% 60% 60% 60% 60%

PRESSURE (psi) 20 40 85 130 175

RUN TIME (min) 8:00 8:00 10:00 10:00 10:00

TIME @ P (min) 3:00 3:00 5:00 5:00 5:00

TEMPERATURE °C 120 140 160 180 200

FAN SPEED 100% 100% 100% 100% 100%

NUMBER OF VESSELS: 6

VOLUME PER VESSEL: 11 mL

SAMPLE WEIGHT: 0.2 TO 0.3 g

ACID: HNO<sub>3</sub>

After the digestion is finished (next lab period):

7) The instructor will remove the turntable with vessels to a position under the hood. The vessels must cool down to room temperature before they are vented.

**8) Safety Note: After the vessels have cooled to room temperature, work under the hood and wear eye protection and gloves. Carefully vent by slowly opening the vent fitting under the hood while pointing the vent away from you or others. Expect a small amount of NO<sub>2</sub> gas to be emitted.**

9) Quantitatively transfer your sample solution into a clean 50-mL beaker. Rinse out the Teflon vessel with a minimum of Millipore water (not more than about 20 mL). Do this under the hood, wear eye protection and gloves and use caution.

10) Quantitatively transfer the sample solution in the beaker to a 200-mL volumetric flask and carefully dilute to volume with Millipore water.

11) Rinse all Teflon parts immediately with hot water. The imide cap and threaded ring should be rinsed and dried. Return all parts to the instructor immediately.

**Waste Disposal.** Excess solutions containing 2%(v/v) nitric acid should be neutralized with  $\text{NaHCO}_3$  (baking soda) before disposal down the drain. To neutralize, transfer acidic solutions into a large beaker on ice, place in hood, and slowly add  $\text{NaHCO}_3$  until evolution of  $\text{CO}_2$  ceases.

### III. Instrumentation

This experiment will be conducted with three commercial spectrometers. The flame absorption measurements for three elements will be done on the Agilent 240 Fast Sequential flame absorption spectrometer. The GFAA measurements for three elements will be done on the Agilent 240Z electrothermal absorption spectrometer. Both of these are in GBAD 314. For multi-element emission measurements, the JY 2000 ICP emission spectrometer in GBAD 313 will be used. One of the instructors will demonstrate the use of these spectrometers when you are ready to use them. An additional document with more detailed instructions about the operation of each instrument will be provided to you. The instrument manuals are usually found next to each instrument and are also available for reference. Review the pictures of the ICP torch on the course supplementals page.

### IV. Experimental

#### A. *Inductively Coupled Plasma Emission-Optical Emission (ICP-OES)*

Use the following initial instrumental settings on the JY 2000 in room GBAD 313:

plasma power	1050 W
sample flow rate (pump speed)	20 (1 mL/min)
plasma gas (Ar) flow rate	12 L/min
auxiliary gas flow rate	0
sheath gas flow rate (gainage)	0.2 L/min
nebulizer pressure	3.2 bar
nebulizer gas flow rate	~0.7 to ~1 L/min (regulated by neb)
entrance slit width	20 $\mu\text{m}$ (10 $\mu\text{m}$ is also available)
exit slit width	15 $\mu\text{m}$ (80 $\mu\text{m}$ is also available)
integration time	3 s except 1 s for Mg
measurement mode	Max (1/1)
no. of measurements for calibration	3
no. of measurements for recalibration	3
flush time	30 s at fast pump speed

An instructor will take you through some of the primary operations of the instrument which is computer controlled and will provide details about the software, ICP-JY v . 5.4. Use the 2% HNO<sub>3</sub> blank solution as the Global Rinse solution when prompted for this. You should perform the following steps:

1. **Initial Plasma Setup and Zero Order Search** - Learn about how to start the plasma and to adjust plasma conditions such as RF power, plasma gas flow rate, and sheath gas flow rate. Once the plasma has started and stabilizes, minimize the Automatism window (don't close it or the plasma will go off in 7 min!). Next run the zero order search under the Instrument-Configuration-Run- from the top menu bar to find the search zero order position for the stepper motor. Open the Communication task window on the task bar so you can watch the progress. The software automatically optimizes the search for zero order. Record the zero order position in the ICP log book and in your notebook. Select Stop when the search is done and close the Communication window.

2. **Select a Method** - You will choose a method file which has been configured for this experiment ("CH\_461\_2017"). Load the Analytical Method from the top menu bar.

**Immediately save this general course method with a new name that identifies your team.**

When a "method" is developed, the user specifies which elements are to be determined and the measurement conditions for each element selected (e.g., analytical wavelength, entrance and exit slit widths, PMT and gain parameters, integration time, number of repetitive measurements, measurement mode). The background-correction wavelength is set after the slow scan of the peak region or profile task is done.

3. **New Method Development and Setup**-Before a method can be applied, several steps or tasks called **peak search**, **autoattenuate**, and **profile** must be carried out for each element in the method. Peak Search counts out the steps from zero order to the element wavelength and stores it; Auto-Attenuate optimizes the HV and Gain at the peak wavelength for the PMT detector for each element and stores it. Profile uses the peak search and autoattenuate results and scans through each element line and store this scan of the peak. You will use the profile scan to identify a background point as reference and store this. We have already run these tasks to

prepare this method for your use for the 5 main elements, however, to illustrate what steps are involved with setting up a method, you will edit your team method and re-run these three tasks for one element, so use the additional element you chose to add to your method from the commercial 21 element standard for quality control (QC).

***Edit Method to Add New Element:***

- i. Add your additional element of choice to the established method. To do this open your Method if not already open. (See step 2 above). Select Edit from right tool bar, then Add/Delete button at the bottom. A periodic table should open. Click on the element that you are adding to the method. Set “concentration high” to 5000 ng/mL for this element.
  
- ii. Select the analytical line for the new element by clicking on the Lines tab. There is a database in the software that will bring up the suggested lines for analysis of this new element. It is usually better to pick a line that is more toward the visible region if there is a choice.
  
- iii. Next you will need to set the Acquisition mode. Select the new element from the Lines list and select the Modify button at the bottom of the screen. Go to Acquisition tab. Select Customized (big button); Analysis mode: Max; measure points 1, calculate points 1; Integration time, 3 s; entrance slit 20 and exit slit 15; increment 0.002 nm. Then OK.
  
- iv. Add the set of calibration standards already setup for the other elements to your new element. Click on Standards tab, select the element symbol, check all 4 standard boxes. Select the Standards button lower left, and enter the values for the standards (5000, 500, 50, 0). Select Save and Close method.

#### 4. Running the Instrument:

Click on the Sequences & Tasks icon from the Top Menu. You need to associate your method with a Sequence. To do this, look near top of the form on the left side and select your method from the drop down list. Next, select New from the right side menu and build a sequence of the three setup tasks (or steps): **peak search, autoattenuate, and profile**. Your method name will also be listed in the middle column in the list of tasks (check this before you go on). Click on the task ICON at the start of a row and **select only the new element you added to the class method.** The other elements have already been run this year.

**Read the overview below of what the three setup Tasks will do before you actually start the run. Note that two different standard test solutions are used in the steps below :**

- i.. **Peak search** - This task is carried out to check that the monochromator is properly calibrated to find the wavelengths for all elements selected (finds and stores the correct number of stepper motor steps). For your new element, use the **5000 ng/mL** multi-element standard for peak search.
- ii. **Autoattenuate** - This task automatically adjusts the PMT bias voltage (HV) for an optimal gain and adjusts the electronic gain (1, 10, or 100) to optimize the absolute magnitudes for the peak signals so they will be on scale. For your new element, use the multi-element standard containing **5000 ng/mL**
- iii. **Profile** - This task provides a scan of each element line spectrum over a very narrow wavelength range around the peak for the element. Later you will use the profile spectrum to select a background correction wavelength point for each element. For your new element, use the multi-element standard containing **500 ng/mL**. The profile is setup to run with a 0.3-s default integration time per point (don't change this). After the profile is done running, get a print out by selecting Print near the bottom of the open sequence window.

**Now you are ready to run the Sequence of Tasks you just built.** Select the Red arrow (top left side of menu) and when prompted give your Sequence a unique file name that includes your initials and group number. Once the sequence is started you will be prompted to use a

Global Rinse (use the 2% HNO<sub>3</sub>) between samples and standards. You should always wipe the sipper tube with a kimwipe when switching test solutions to avoid carry over. To monitor the progress of the run, maximize the ICP Analyst window and click on the Instrument Status window on the task bar. To check the intensity of the signal for each element as the run proceeds, click on the lightning-bolt icon next to the element symbol.

iv **Set Background point:** After running the setup Sequence, close Sequences and go back to the Methods screen. Open the Lines tab, then select Edit (right side), then select Modify (bottom) and then Background tab. Select Profiles and pick the profile filename that you just collected for your new element. Find a point on the baseline, either to the left or to the right of the main peak where the baseline intensity is low and constant. Set this as the background point by double clicking (select only one background point). If the background point has already been assigned, you can use the pre-assigned value or set a new value yourself (you have to delete the old one). Now for all analysis runs, the software will automatically calculate the net peak intensity based on the value at the peak maximum and the value at this background point. Later you will re-run a profile scan on the same (new) element using the multivitamin sample solution to check for possible interferences from other elements in the real sample in the region of this element peak.

5. **Print a copy of the Analysis Parameters** - Print the important method parameters for all elements. **To do this, go to Method and choose Print Method BUT check only the following: User Acquisition Parameters, Background, and Plasma Parameters.**

6. **Calibration** - Return to the Sequences screen and select the Calibration task. **Run this task for all the elements in the method.** Run the 2% HNO<sub>3</sub> blank solution as the lowest standard and the three multi-element standards as follows in this order:

STDLOW	blank (2% HNO <sub>3</sub> ) - be sure it is freshly taken from the carboy
STDA	50 ng/mL multi-element
STDB	500 ng/mL multi-element
STDHIGH	5000 ng/mL multi-element

Check List for getting a good calibration curve:

- The standards must be run in the order listed above
- Do not forget to give your Calibration task a unique group file name when you are prompted to do so.
- Look at the average intensity of the signal for each element line immediately as it is generated to confirm that the repetitive measurements are consistent within each standard and that the intensity increases with concentration. For example, the signal for 5000 ng/mL is 10 times the signal for 500 ng/mL, and the 500 ng/mL is 10 times the signal for 50 ng/mL. If not consult with the instructor before continuing. **Sometimes Cu doesn't work well on the blank and the 50 ppb standard. If these values are nonsense, rerun just Cu (ask if you can't figure out how to do this).**

After all the standards are run, the program will autoprint a calibration summary which includes the average net corrected signal and the RSD for each element in each standard. Check that the RSD values are reasonable. If these are too high, investigate the causes. The summary also includes the equation for the calibration curve based on a linear regression fit for each element in the form (the software automatically determines if more than a first order fit is required):

$$\text{conc} = (\text{intercept}) + (\text{slope}) \text{Intensity} + (\text{coefficient}) (\text{Intensity})^2 + \text{higher orders}$$

The calculated concentrations should be near the expected values for STD A, B, and HIGH and the blank (STDLOW) should be within 10 ng/mL of zero for most elements. You may be prompted to accept the calibration. If the calibration is not good, rerun the elements and/or concentrations that don't match (you don't have to run all elements and all concentrations).

Now return to the Methods screen and select the Calibration tab to view a plot of the calibration curve for each element. If one of the standards is not reasonable it can be eliminated from the calibration, or you may choose to rerun that element calibration if it is really bad. The general course method is setup to use a weighting factor for each standard (1/square root of standard deviation) in the regression; the weighting factor helps to ensure that the low-concentration samples aren't overwhelmed by the high-concentration samples in the regression

over the wide range of concentration data. The program also decides whether a polynomial fit is better than a simple linear fit. You can change this if you don't like it.

**7. Analysis and Detection Limit** - Return to the Sequence window, select New, and add one Analysis Task for each sample listed below (bulleted items) and save the Sequence. Edit the name for each task to indicate the sample name and put your team number in the name. Determine all the elements in the method except where indicated below. The method you are using is setup to run each element for each sample in triplicate. You should change the number of replicates for the blank measurement to 15 to get better statistics for the DL.

The analysis samples are:

- 2 % HNO<sub>3</sub> blank (STDLOW) - run this first and use this data to find the detection limit for each element. You will need to change the number of measurements in the this task to 15 replicates for each of the elements: select Edit from the menu bar on the right, then left double click on the task icon and change the number of Replicates from 3 (the default) to 15.

**Change the number of Replicates back to 3 (triplicate - not 15) for the following samples:.**

- tap water (all elements in your method)
- synthetic unknown (all elements in your method)
- 1 µg/mL mixed standard that you made - to test your solution preparation skills. To select only the elements in this standard, select Edit from the menu bar on the right, then left double click on the task icon, go to Elements (a periodic table should appear on the screen), **deselect elements that you do not want to run this time for this sample.**
- yogurt for all elements in your method
- vitamin tablet solution **Multi VitD1** filtered with 0.22 or 0.45 µm nylon disk filter -run all elements in your method
- vitamin tablet solution **Multi VitD2** filtered with 0.22 or 0.45 µm nylon disk filter -run all elements in your method
- oyster or clam - **only the diluted 1 to 10 (labelled S2)** solution and filtered with 0.22 or 0.45 µm nylon disk filter - run all elements in your method

- SRM solution and filtered with 0.22 or 0.45  $\mu\text{m}$  nylon disk filter for all elements in your method
- add one more Task and make it a Profile run of the **Multi VitD1** solution and set the software just to run the new element that you ran a profile on earlier using a standard. Get a hard copy of this using Print before leaving the Sequence window.

Before you start the Sequence, save it with a unique name using your initials and group number. After each sample is run, the program will export the data to an Excel file: the mean net or baseline corrected signal, the estimated concentration in the sample based on your calibration data, the calibration fit equation for each element, the SD, and the RSD.

**8. Check Data before leaving. Check your data against the criteria below to make sure it is consistent and if not, re-run the solution that doesn't fit:**

- a. For your analysis samples, the element concentrations should be within the standard range and the RSD should be reasonable (better than 5%) in most cases.
- b. For your blank measurement, note that this signal for the blank and its SD ( i.e., the blank noise ( $s_{\text{bk}}$ ) ) is **reported directly in concentration units** and not in signal units. This means that the SD reported by the program is the standard deviation of the signal automatically divided by the calibration curve slope. Later you will use the SD in concentration units to calculate the detection limit for that element. **Check that the individual peak intensities taken for the DL measurement are consistent** with each other. For example, if the first point is high and distorts the standard deviation, recalculate the blank SD yourself using the remaining points. Also check that the readout resolution is adequate. **If the standard deviation shows as zero**, you should adjust for more resolution (decimals for readout) or later calculate the standard deviation from individual results given in the spreadsheet of the exported data. Note: use the by element listing in the excel output.
- c. For the sample Multi Vit D1 profile, go back to the Method screen and look at the Profile of the Multi Vit D1 solution you just ran and compare with the original profile run on the standard solution. Note any differences. Get a hard copy of both profiles (the standard and the Multi Vit D1 before you leave).

### ***B. Flame Atomic Absorption Spectrophotometry***

Before you start, check your samples. If the sample solutions are turbid, you will need to filter first with filter paper and then with the 0.22 or 0.45  $\mu\text{m}$  nylon syringe filter or centrifuge about 10 mL of each of solution before analysis. Complete this step before going to the instrument. **This is important as small particles can clog the aspirator. If this happens you will need to work with the instructor to disassemble the aspirator and clean it out.**

The standards and samples that you will run are:

- 1, 3, 5, and 10  $\mu\text{g/mL}$  mixed standard containing Cu, Fe, Pb;
- the synthetic unknown solution;
- tap water;
- the filtered concentrated oyster or clam extract solution, S1 (not the 1/10 dilution);
- yogurt;
- concentrated vitamin solutions (Multi Vit C1 and Multi Vit C2);
- SRM solution provided for you
- 2%  $\text{HNO}_3$  blank for detection limit.

Take all your standards and test solutions to the Agilent 240 FS AA in room 314. If available, you may use the autosampler for this experiment since you have several test samples and several standards and run the instrument in the fast sequential mode (all elements in the method will be measured on one solution before going on to the next solution). The instructor will help you with the setup. However before you use the autosampler, you will do some manual runs on Cu using one of your prepared standards to get the idea of how the instrument works. Use the following analytical wavelengths for each element:

Cu	324.8 nm
Fe	248.3 nm
Pb	217.0 nm

For Cu make sure the instrument is set to use 324.8 nm (not 327.4 nm, a different Cu line that is nearby). Other important instrument parameters are automatically set for each element and will be recorded in the method. Before you leave, obtain a hardcopy of the method that gives these main experimental parameters, e.g., HCL lamp current, measurement time (integration time), air flow rate, acetylene flow rate, number of replicates. Turn on the autosampler (do this before loading Spectra AA) and check the DI water supply and the tubing is engaged on the pump. The Instructor will demonstrate how to load Spectra AA and open the class method for Cu, Fe and Pb. Check the alignment for each lamp and make sure that the light beam is focused over the burner head. Light the burner by pressing the auto striker button by the on/off button on the front of the instrument. . **Reminder: record all file names and other parameters and data in your laboratory notebook.**

To start with, aspirate some millipore water into the flame by placing the free end of the small white plastic sipper tube leading to the burner into a small plastic sample cup containing water. Notice the change in sound as the water reaches the burner. Briefly dip the end of one of your fingers in the water. The small amount of sodium from the salt on your finger will cause the flame to become yellow. What does this suggest concerning the way in which you prepare your samples? Next, fill a 10-mL graduated cylinder with millipore and measure the time for aspiration of a known volume (about 5 mL) and record this in your notebook. A typical rate should be 5-6 mL/min. Adjust the knurreled collar around the nebulizer to adjust flow rate.

Next you will run your 5  $\mu\text{g}/\text{mL}$  standard. First aspirate the 2% nitric acid blank solution and press **the Zero** button to zero the absorbance reading. Do not re-zero the instrument as you continue; a significant drift in the zero indicates a problem such as a clogged burner head. While the instrument is still aspirating blank solution, press the **Read** button and record three replicate readings for the blank. Next aspirate the 5  $\mu\text{g}/\text{mL}$  standard and press the **Read** button and record three sequential readings in your notebook. The net absorbance for this solution for Cu should be about 0.4-0.6. If it is not, work with the instructor to check the optical alignment of the burner head and the aspiration rate. Turn the flame off first!

Once the instrument is tuned up, the instructor will help you load the multi-element method called **CH461\_Mutielement**. Save a copy of this "with new data sheet", otherwise it will have the last user's data recorded. If the autosampler is available use it, if not do this manually. Make sure that you have added three sample labels to the list of samples: DL1, DL2, DL3. These will be used to determine the detection limit for AAS. This will give you a total of three runs on the blank for a total of nine measurements to use to determine the DL for each element.

Autosampler:

Load the four standards you made and the test samples in the autosampler (standards go in the big tubes at the back of the autosampler with the blank first, then increasing concentrations to the right). The test sample solutions go in the small plastic tubes from right to left (the spaces are labeled in numerical order). Turn the autosampler on and connect the sipper tube on the aspirator to the tubing on the autosampler. In the method, enter labels for your samples in the order they will be run. When the run is done, print a copy of the results and save these to a txt file so you can open in Excel later. If you are the last group for the day, turn off the flame.

**C. Graphite Furnace AA (revised 2018).** The standards are between 0-50 ppb Pb are made on the fly by the autosampler. The instructor will guide you through the GFAA experimental technique after you finish with the flame AA experiment. Observe the operation of the Agilent 240Z GFAA in room 314 (details on running instrument are given below). We will run the class SRM solution as one sample. Record the number in the rack for your group's samples. This analysis is only operated with an autosampler and auto-diluter and takes time to run. The SRM sample will be loaded for you to observe. You will get the output for this analysis later.

**GFA Start-Up Procedure:**

1. Check the water level of the Automatic Liquid Sampler (ALS). Refill with Millipore water if necessary.
2. Turn on Lytron chiller.
3. Turn on GTA120.
4. Turn on 2407 AA.
5. Turn on Ar gas and check cylinder pressure.
6. On the PC, start the SpectrAA program.

**Tray Loading Procedure:**

Solutions should be loaded into the ALS tray in the following configuration:

<b>Solution</b>	<b>Position</b>
50 ppb Bulk Standard	49
Millipore water (make-up solution)	48
Pd modifier (for Pb analysis only)	47
20 ppb check standard	1
Tap water	2
Samples	3 +

**ALS Alignment and Pre-cleaning Procedure:**

The alignment of the ALS sipper arm should be checked whenever the ALS is removed or re-hung on the front of the instrument, or if the ALS is bumped or jarred.

1. If not already running, start SpectraAA on the PC.
2. Select <New from>
3. Select template CH461 - Pb and give your worksheet a new unique name by adding the date to the suggested name. (e.g., CH461 - Pb - 2015-11-14)
4. Click on the Analysis tab.
5. Click on the Instrument menu and select Furnace Facilities.
6. Click on rinse to rinse the sipper tube. Repeat 2x.
7. Raise the mirror shield and check that the graphite tube is properly aligned. The hole should be centered and facing directly up.
8. Click on Align.
9. The sipper will first dip into a sample container. Check that the tube is centered in the sample well and that the tube goes to the bottom of the sample container. If the tube is not centered, gently push on the sipper arm to center it.
10. Click OK. The sipper arm will move to the graphite tube. Watch to ensure that the sipper tube enters the hole in the graphite tube cleanly.
11. If adjustment is necessary, first loosen the lock knob on the bottom of the ALS. There are two adjustment knobs on the side of the ALS; one for the x-direction and one for the y-direction. Adjust slightly and check new alignment by manually lifting and lowering the sipper arm. Adjust until the sipper tube enters cleanly.
12. Close mirror cover.
13. Click OK.

14. Rinse sipper tube twice. Be sure you allow the rinse cycle to complete before clicking on the Rinse button a second time.
15. Click on tube clean to run the tube clean furnace profile. Repeat.
16. Close the Furnace Facilities window.

### **SpectrAA Calibration Procedure:**

Calibration must be performed anytime the instrument is to be used for a block of samples. The best working procedure is to: start the instrument, run calibration methods until a suitable calibration curve is obtained, add sample metadata to the sample log table and then run all samples in one block. **The cal curve generated in a worksheet can only be used as long as the instrument is kept continuously running, with the SpectrAA worksheet open.**

### **Procedure for Pb and Pb plus PdCl<sub>2</sub> (modifier):**

1. Select template CH461 - Pb and give your worksheet a new unique name by adding the date to the suggested name. (ex. CH461 - Pb - 2015-09-14) This worksheet will be used for analysis of lead.
2. Select the Analysis tab.
3. Select the Pb column of the sample table.
4. Select CAL ZERO row of the standards table (lower right of the screen).
5. Click the Start GTA button to run a blank.
6. Ensure that the mirror cover is closed and click OK.
7. The instrument will perform two blank runs, which will take ~ 4 minutes.
8. Pb blanks should have an absorbance of < 0.05 with an RSD of < 10%. If these benchmarks are not met, return to the Furnace Facilities window and repeat sipper tube rinse and graphite tube clean. If blanks still do not meet benchmarks, replace make-up solution and

container with fresh Millipore water and a new vial. If benchmarks are still not met, trim ~ 1 cm off the end of the sipper tube and repeat ALS alignment procedure.

9. Once suitable blank values have been obtained, click the Start button and the instrument will generate calibration curves and run the 20 ppb check standard. This will take about an hour.
10. When the calibration sequence has finished, select the Labels tab.
11. Click on the Total Rows button.
12. Set the total number of samples to load. Select a row to change the sample name to identify the sample to be placed in the corresponding numbered well on the ALS. Include a 20 ppb check standard after every five samples.
13. Click the Edit Sequence button.
14. Change the "Starts With" column from "calibration" to "solution" for Pb and Pb plus modifier.
15. Select the Analysis tab.
16. Click on Start. Analysis will take ~ 5 min per sample.

## **Reports**

Save reports as .prn files. If you change the extension of a .prn file to .csv (for example, mydata.prn becomes mydata.csv), you get a file that can be opened and edited in Excel.

**GFA Shut-Down Procedure**

A calibration curve is only useable as long as a worksheet is kept open, but is useable for around 50 sample runs if the ALS tray is not removed. The instrument should be left switched on until all samples to be run on a given cal curve are run. If you wish to save a cal curve for a while, but not run samples, the Ar gas can be turned off at the cylinder. This will cause a pop-up error in SpectraAA. Restart the gas and click OK when ready to continue.

To fully shut down the instrument:

1. Save worksheet and close SpectrAA.
2. Turn off 2407 AA.
3. Turn off GTA120.
4. Turn off Ar gas.
5. Turn off Lytron chiller.

**Misc notes:**

1. Calibration stock solution on the ALS tray should be changed every day to avoid any possible evaporative loss. Samples should be run within a few hours of being placed on the tray.
2. Reusing the plastic sample containers is not recommended, as they seem very prone to contamination!
3. Whenever results seem odd, go to the Furnace Facilities window, clean the graphite tube and rinse the sipper tube twice. This will usually remove any contamination.
4. If contamination persists, The Tube Condition profile will clean the graphite tube more aggressively than the Tube Clean profile. Trimming the sipper tube may also be necessary.

## V. LAB REPORT

The goal for this part of the project is to take the large amount of raw data generated by the commercial instruments, sort through it, and organize it into a tabulated form that is easy to reference and use. This is exactly what you do when using a modern instrument on an research project. This report is a team effort because of the large amount of data that needs to be organized with results into 15 Tables; **however, each team member is expected to prepare and submit their own copies of tables 5 and 6 including requested sample calculations.** The report should include an abstract and responses to questions posed in the lab manual. Use the table numbers and column letters requested below to identify the information and give each table a descriptive title. Include a table of contents as a first page when you have completed the tables for your report, and please number all pages of your report at the right bottom position of each page (page numbers can be written). Present a labeled sample calculation for one element in each case where called for (insert your values in the equation and show how you did this calculation). Always include units and proper significant figures in reporting values (e.g, 2 to 4 significant figures & scientific notation as needed). You should be able to copy and paste much of the information called for in the tables using the data reports from the instruments, either as ASCII format (text), or Excel. If Text format you can open the file in Excel and select “data-text to columns” and Excel helps interpret which data goes in which column but sometimes you still have to edit this to make it useful. **You should not be typing values into tables “by hand” except occasionally.**

**PART I. ICP-AES (section IVA)**

1. **Raw data generated by ICP-AES.** Each group member will have a copy of the Excel files generated during the run. Cut and paste as required from these spreadsheets to create the requested tables below.

**Table 1. Instrumental Method Parameters.** Include the following parameters which are found in the spreadsheets, except where noted, for each element in your method. Use the following **six column** designators in Table 1:

- A. wavelength for the analytical line
- B. wavelength for the background correction
- C. peak integration time (from method setup-Acquisition)
- D. slit widths (from method setup-Acquisition)
- E. PMT bias voltage
- F. PMT electronic gain

**Table 2. Calibration Method Information.** Note that the program automatically determines the best calibration curve equation and that it may be a higher order polynomial fit. Include the following parameters which are found in the spreadsheets, except where noted, for each element in your method. Use the following **five column** headers in Table 2:

- A. element
- B. nominal concentration of standard
- C. net intensity for standard
- D. calculated concentration for standard (obtained from printed output for calibration)
- E. calibration equation (e.g., slope, coefficients, intercept values) for each element (in both printed output from calibration run, and in each spreadsheet for each sample analysis)

**Table 3. Analysis Results** for each real sample and for each element in your method that you analyzed for. Use the following six **column** headers in Table 3:

- A. sample name
- B. element name
- C. average net intensity for element (NetInt.)
- D. average calculated concentration for element in ng/mL(Conc)
- E. standard deviation for conc for element (SD)
- F. relative standard deviation for conc for element (RSD)

**Table 4. Detection Limits.** This is based on the standard deviation for each element as determined using the 2% HNO<sub>3</sub> blank run with 15 replicates for each element in your method. For the JY ICP, the software reports the blank standard deviation **in concentration units** (the  $\text{stdev}_{\text{blank}}$  is already divided by the slope). Hence,  $\text{DL} = 3 \times \text{stdev}_{\text{bk}}$  in concentration units. Include a sample calculation as a footnote to the table for one element. Use the following three column headers for Table 4:

- A. element name
- B. SD in ng/mL
- C. DL in ng/mL

## 2. Presentation of Analysis Calculations.

**Table 5 .** See format next page. For each type of sample, report the information specified in the Table 5 template below from the ICP-AES analysis. If the measured value for the concentration of a particular element in a test solution is below the calculated detection limit, report the concentration as 'not detected' by giving **ND** for that element in the table. This includes negative numbers - they are negative due to deviations around zero - so any reported concentration that is negative is listed as "0" and obviously below the DL. Use the proper number of significant figures. For example, the number of significant figures is rarely ever greater than 3 for concentration data, and only 2 significant figures should be reported for SD, RSD, and the DL.

**Table 5. Detection Limits and Elemental Concentrations in ng/mL for Test Solutions including % RSD from ICP-AES.**

Elements Tested	DL (ng/mL)		Syn Unk	Mixed Std- 1 µg/mL you made	Tap water	Multimineral tablet 1	Multimineral tablet 2	Oyster	Yogurt	SRM
Ca		Conc								
		STD								
		RSD								
Cu		Conc								
		STD								
		RSD								
continue table with Fe, Mg, Pb, Zn and your group's element of choice		Conc								
		STD								
		RSD								

3. **Final Sample Results.** Prepare Tables 6- 8 as described below. Back-calculate to relate your results to the values listed on the original containers or given in a published reference, e.g. USDA web page. For the oyster, back calculate to the wet weight and to the ash weight. As a footnote to Table 6, give one complete sample calculation for one element for each type of sample in Table 6. Use proper units and significant figures. See format for Table 6 on next page.

Table 6. Mass in a “Typical Serving” or Tablet found from your data. Include at least the following five columns for each sample and list for each element studied for that sample:

<b>Sample Name:</b> <b><u>Yogurt</u></b>	<b>Original Sample Mass.</b> <b>wet weight (g):</b>	<b>Test Solution Total.</b> <b>Volume (mL):</b>		
<b>Element Name :</b> (make separate row for each element)	<b>Test Solution Conc., ng/mL:</b>	<b>mg Element / g Sample:</b>	<b>Typical Sample Serving Size, g :</b>	<b>mg Element /Serving:</b>
Cu				
Ca				
Zn				
etc,,,,,,,,,				
<b><u>Multi</u></b> <b><u>Vitamin 1</u></b>	<b>sub-sample (g):</b>  <b>whole tablet (g):</b>	<b>Test Solution Total.</b> <b>Volume (mL):</b>		
<b>Element Name :</b> (make separate row for each element)	<b>Test Solution Conc., ng/mL:</b>	<b>mg Element/ g Sample:</b>	<b>Typical Sample Serving Size, g :</b>	<b>mg Element /Serving:</b>
Cu				
Ca				
Zn				
etc,,,,,,,,,				

<p align="center"><b><u>Multi Vitamin 2</u></b></p>	<p align="center">sub-sample (g): _____</p> <p align="center">whole tablet (g): _____</p>	<p align="center"><b>Test Solution Total. Volume (mL):</b></p>		
<p><b>Element Name :</b> (make separate row for each element)</p>	<p align="center"><b>Test Solution Conc., ng/mL:</b></p>	<p align="center"><b>mg Element/ g Sample:</b></p>	<p align="center"><b>Typical Sample Serving Size, g :</b></p>	<p align="center"><b>mg Element /Serving:</b></p>
<p align="center"><b><u>Oyster</u></b></p>	<p align="center">wet weight: _____</p>			
<p><b>Element Name :</b> (make separate row for each element)</p>	<p align="center"><b>Test Solution Conc., ng/mL:</b></p>	<p align="center"><b>mg Element/ g Sample:</b></p>	<p align="center"><b>Typical Sample Serving Size, g :</b></p>	<p align="center"><b>mg Element /Serving:</b></p>
<p align="center"><b><u>Oyster</u></b></p>	<p align="center">ash weight: _____</p>			
<p><b>Element Name :</b> <b>(make separate row for each element)</b></p>	<p align="center"><b>Test Solution Conc., ng/mL:</b></p>	<p align="center"><b>mg Element/ g Sample:</b></p>	<p align="center"><b>Typical Sample Serving Size, g :</b></p>	<p align="center"><b>mg Element /Serving:</b></p>

**Table 7.** Comparison to expected mass values for Vitamin Tablet. Report in Table 7 for each of the eight elements (Ca, Cu, Fe, Zn, Pb, + element of your choice), the mass (mg/tablet) found for multivitamin 1 and multivitamin 2 (from Table 6), the average of these two values, the absolute value of the range between these two values, the percent deviation from the mean for duplicate measurements ( $\% \text{ deviation} = (\text{range} / 2) / \text{average} \times 100$ ), the expected value for mg element/tablet listed on the container, and the percent difference between the average value you determined and the expected value for mg element/tablet on the container.

- Show a complete sample calculation with units and proper significant figures for one element listed in Table 7.
- Offer some explanation for any elements that do not agree very well for the observed mass for each element you found with the expected mass for each element in the Vitamin Tablet. Consider the differences between observed and expected mg/tablet to the % deviation in replicate measurements, as appropriate.

**Table 8.** Comparison to “%Daily Value” or “%DV”. Report in Table 8 the sample name, name of element measured, the value for %DV for that element based on your results, %DV for that element listed on the sample container, and % difference between your value and the container value. Use the same samples as those in Table 6. If the element is not listed on the container show NA (not available) in the table entry. If there is no Food Labeling Guide Reference Value (FLGRV) for the element, show NA in the table entry. The FLGRV values are “daily values” recommended by the FDA. Turn to Appendix F. at:

<http://www.fda.gov/food/guidanceregulation/guidancedocumentsregulatoryinformation/labelingnutrition/ucm064928.htm>

Calculate the Percent Daily Value (DV) for the Appropriate Nutrients in your study.

Based on your results for mass (in mg) per serving listed in Table 6, calculate your experimental values for the %DV for each element as follows:

$$\%DV = \text{mass per serving (mg)} / \text{FLGRV daily value (mg)} \times 100$$

- Show a complete sample calculation with units and proper significant figures for one element listed in Table 8.
  - Briefly discuss how well the results for each of the real samples compares to what is expected for each element and offer some explanation for any elements that do not agree between measured %DV and expected %DV.
4. **Spectral interferences on ICPAES.** Compare the spectral profile only for the additional element that your group chose to analyze from the standard solution and the profile run on your vitamin tablet solution. Is there evidence **from the vitamin tablet profile** that suggests that there might be other elemental spectral interferences in this same region of the spectrum? If yes, how might this affect the final reported concentration for the additional element? (Hint: what is the wavelength for the background correction point you used to determine the net peak intensities for this added element and does it fall in the profile wavelength range?)

**PART II. AAS (section IVB)** Work up the data according to the following instructions and turn in the results. Label each item with the numbers and titles given below. **Show a sample calculation with actual values and proper units and significant figures.**

2. **Table 9. Linear Calibration in Excel versus SpectrAA software.** First, make a calibration graph of AU vs concentration in  $\mu\text{g/mL}$  in Excel and determine the coefficients in the linear calibration equation for Cu and Pb only. Report the calibration equation and give the slope in AU per  $\mu\text{g/mL}$  and report the intercept. Calculate a predicted absorbance value for each standard solution based on the linear calibration equation you found in Excel. Make a **Table 9** and give the % difference for the elemental concentrations for the Cu and the Pb standards calculated using the linear model above compared to the values reported for these two elements directly by the SpectrAA software. **Note:** SpectrAA is set to use the “New Rational” algorithm to find a calibration equation. This method is not the direct linear method we use in CH 461 but is accepted as industry standard for AAS and better represents the dynamic range of calibration for flame AAS. In Excel, does the data deviate from linearity at higher concentrations? What are two possible causes that can cause non-linearity in AAS?

3. **Table 10. Concentrations of test solutions from AAS.** Report the concentrations in ( $\mu\text{g/mL}$ ) based on the values as reported by the SpectrAA software for the test samples: (a) synthetic unknown, (b) tap water, (c) oyster or clam, (d) Multi VitC1, (e) Multi VitC2, and (f) SRM.
  
4. **Characteristic Concentration.** The **characteristic concentration** ( $m_A$ ) for atomic absorption is defined as the concentration of the element that results in a transmittance of 99% T or absorbance of 0.004365. If the intercept from the least squares linear fit is non-zero, the characteristic concentration is the concentration which yields an absorbance 0.004365 **above the absorbance at the intercept**. Hence,  $m_A = 0.004365 / (\text{calibration curve slope in AU per concentration unit})$ . Calculate and report the characteristic concentration for each element in  $\text{ng/mL}$ . The characteristic concentration for Cu should be less than 100  $\text{ng/mL}$ . If the characteristic concentration value is reported by the SpectraAA software, also report it here for comparison.
  
5. **Detection Limit.** Report the repetitive measurements of absorbance from the blank (reference) solution, plus the mean and the standard deviation (use at least 4 place precision). Calculate and report the AAS detection limits in concentration units ( $\text{ng/mL}$ ) for each element measured. For Cu this should be above zero but well below 100  $\text{ng/mL}$  and it is normally higher for Pb.

### Part III. Graphite Furnace Atomic Absorption Spectrometry

1. Prepare **Table 11. Concentrations for Pb and Pb with modifier using GFAA**. Report the concentration of the test solutions in columns given by the SpectraAA software, and include the reported SD and RSD for the SRM, and the DL with and without the modifier. Note concentrations are given in ng/mL (i.e., ppb). Give the results for the back calculation of the test solutions to mg Pb/g for 1 g of the SRM oyster. Make a statement to compare and contrast the precision and the accuracy of results for Pb without the modifier and Pb with the modifier.

### Part IV. Overall Comparison for Three Techniques.

1. **Table 12.** List results for all three techniques (flame AAS, ICPAES, and GFAA) **for Pb only** (you already have these values in other tables) in the SRM. Give the concentration of the test solution in ng/mL and include DL, SD, and RSD for each. Include in Table 12, the expected values for Pb in the SRM with given SD, and RSD according to the SRM certificate posted on the course web page for comparison. Give the calibration equation for Pb for each method in a footnote to Table 12. If you needed to determine trace levels of Pb accurately and precisely - based on your results presented in Table 12- which technique would you use and why?
2. **Table 13.** Present the expected and measured values **for only Cu and Fe** from ICPAES and flame AAS for the **SRM oyster sample** so a comparison can be made for the quality of the precision and accuracy from these two techniques for Cu and Fe. You will want to include the SD and RSD and DL's. Based on your results presented in Table 13- if you need to determine trace levels of Cu and Fe accurately and precisely which of these techniques would you use for each of these elements, and why?

3. **Table 14:** Rank flame AAS, ICPAES, GFAA techniques with a 1, 2, or 3 (where 1 is the best) for the following reasons for **Pb only**:
  - a. DL's to determine sensitivity of technique
  - b. Results for SRM to determine accuracy of technique
  - c. RSD to determine precision of technique.
  
4. **Table 15.** Report the concentration range over which the calibration is linear for Pb using each of the three techniques (flame AAS, ICPAES, GFAA). That is, what is the highest concentration and what is the lowest concentration for the standards used in this study that produce a linear response for Pb for each technique?
  
5. **Lab Notebook pages.** Turn in your duplicate pages from your lab notebook with all sample preparation information, file names, method names, and other information. These pages should be initialed by an instructor each day.