

# ATOMIC ABSORPTION SPECTROSCOPY

**The object of this experiment is to gain an understanding of the use of atomic absorption spectroscopy for the analysis of low concentrations of metallic elements.**

## REFERENCES

Gary D. Christian and James E. O'Reilly, Editors, "Instrumental Analysis", 2nd ed., Allyn and Bacon, Boston, 1986, Chapter 10. (available from the instructor)

R. B. Beaty, "Concepts, Instrumentation, and Techniques in Atomic Absorption Spectrophotometry", Perkin-Elmer, 1988. (to be used in the laboratory only)

Perkin-Elmer, "Running the 3100: Operating Instructions", Jan., 1990. (to be used in the laboratory only)

Basically, this experiment consists of preparing a series of standard solutions containing the element to be determined (iron in this case) and of preparing solutions of metallic alloy samples and then measuring the absorption of light by the atoms of the element when the solutions are aspirated into a flame. You will be using a Perkin-Elmer Model 3100 Atomic Absorption Spectrophotometer. Normally the alloy samples are dissolved directly in acid. The concentrations of the element in the sample solutions are determined from curves of absorbance versus concentration determined from measurements on standard solutions or directly by the spectrometer.

If you not familiar with atomic absorption spectroscopy, read the appropriate material in the first two references. Read all of the instructions thoroughly before beginning any experimental work. **Do not attempt to operate the spectrometer or to light the burner until you have been briefed by the instructor.**

## EXPERIMENTAL

**A) Preparation of the Standard Iron Solutions.** Prepare five standard solutions covering the range 2-16  $\mu\text{g}/\text{ml}$  in 100 ml volumetric flasks. One of the solutions should contain 5.0  $\mu\text{g}/\text{ml}$  of iron. This solution is used to check the performance of the instrument. To make up the five standard solutions, make one stock solution (0.1000g/1000 ml) and dilute aliquots of it. Clean

the iron wire with emery cloth and dissolve the wire in a minimum amount of a 1:1 v/v mixture of concentrated nitric acid and water. Heating will be necessary but avoid boiling during the dissolving process. When the iron wire is dissolved boil the solution briefly to remove oxides of nitrogen. Transfer the solution quantitatively to a 1000-ml volumetric flask and dilute to volume with distilled water. Use the volumetric pipettes provided to take aliquots of the stock solution. Deliver the aliquots to 100-ml volumetric flasks and dilute to volume with distilled water.

**B) Preparation of the Sample Solutions.** You will receive four copper-based alloy samples to analyze for the iron content. The first sample will be a standard sample of known iron content. Using the known amount of iron calculate the amount of sample that must be taken to prepare a solution in a 100-ml volumetric flask whose concentration lies in the middle of the standard solution concentrations. The second sample will be a prepared unknown sample. You will be told the amount to weigh-out. The third and fourth samples will be "archaeological" objects from which you will take samples of approximately 0.2 g from object #1 and 0.1 g from object #2 to prepare solutions in 100-ml volumetric flasks. Dave in the shop will help you to prepare the samples from the objects. All four samples should be weighed to 0.1mg. With gentle heating, dissolve each weighed sample in a 250-ml beaker with a 1:1 v/v mixture of concentrated hydrochloric acid and water. If necessary, add a minimum amount of a 1:1 v/v mixture of concentrated nitric acid and water to aid in the dissolving process. Avoid boiling during the dissolving process; however, when the sample is dissolved, boil briefly to remove oxides of nitrogen. Transfer the sample solutions to the appropriate volumetric flasks and dilute to volume with distilled water. **Wear your safety goggles while you are dissolving your samples.**

**C) Preparation of the Instrument.** Verify that an iron lamp has been installed in the instrument and that water is present in the drain system float assembly container. Open the vent for the exhaust above the instrument and close the hood door. The acetylene tank pressure should be 75 psig or greater. We use welding grade acetylene in which the acetylene is dissolved in acetone. When the total tank pressure drops below 75 psig, the partial pressure of acetone in the tank becomes unacceptably high and the tank must be replaced. Set the monochromator to 248.3 nm, the slit width to 0.2, and the slit height to HIGH.

**D) Turning on the Instrument and Preparation for Measurements.**

- 1) Turn on the instrument. The power switch is located on the right-hand side of the instrument. The message SELF TESTING MODE will appear on the display. When this message disappears, the instrument has passed a series of diagnostic tests and you may proceed.
- 2) Define the operating parameters by pressing the *Param Entry* function key. You will be prompted to define a series of parameters in the given order. After each entry of a parameter

you will need to press the *Entry* function key to proceed to the next parameter.

- a) **Lamp Current.** Enter 30, the operating current in mA for the iron lamp. It is important that you have verified that an iron lamp is present before completing this step. A 30 mA current might damage other types of lamps. When the lamp current has been entered, the amplifier gain will be automatically set. The message AGC APPLIED will appear on the screen.
- b) **Integration Time.** Enter 1. Each measurement will be made for 1 s. You may wish to change this number later. The allowed range for the integration time is 0.1-60 s.
- c) **Replicates.** Enter 5. Each measurement will be repeated 5 times. You may wish to change this number later. The allowed range for the number of replicates is 1-99.
- d) **Calibration Type.** Enter 1 for non-linear. The 3100 allows three types of calibration: non-linear (1), linear (2), and the method of additions (3).
- d) **AA technique.** Enter 1 for flame. The other options apply to accessories which we do not have.
- e) **Standard 1.** Enter the concentration of the first (most dilute) standard in  $\mu\text{g/ml}$ . Use the format nn.mm. For example, if the concentration is  $2.12 \mu\text{g/ml}$ , enter 02.12 .
- f) **Standard 2.** Enter the concentration of the next most concentrated standard in  $\mu\text{g/ml}$ .
- g) **Standard 3-n.** Continue the process for your n standard solutions.
- h) **Standard n+1.** Do not enter a number in this case. Simply press the *Enter* key.
- i) **Reslope Standard.** If you wish to use the reslope feature, enter the concentration of the reslope standard. Otherwise simply press Enter. You are now back at the beginning of the parameter menu. You may verify if you wish the values of the parameters by going through the menu. If you do not wish to change a value, simply press the *Enter* key. You can change the parameters at any time by pressing the *Param Entry* function key.
- 3) Optimize the power reaching the detector. Press the *Energy* function key. The display will show the energy screen. The Counts (CTS) and Energy (EN) parameters provide a measure of the amount of radiation reaching the detector. Maximize Counts by making small adjustments in the wavelength setting. The adjustment of the wavelength should be small. The final wavelength should not be 247 nm. There is an iron line at 247 nm but it has lower intensity than the 248 nm line. If the bar graph which gives a visual measure of Counts goes off scale, the gain will be automatically adjusted. If the gain is not automatically adjusted, press the Gain function key to bring the bar graph back on scale. Once the wavelength has been adjusted, adjust the two black alignment screws on top of the lamp for maximum Counts. When the optimization procedure has been completed, record the values of Counts and Energy. Then convert the instrument to Automatic Baseline Correction mode by pressing the *AA-BG* function key. You will hear the movement of a mirror in the optics and the AGC routine will be applied.

4) Ignite the flame. Use common sense and pay attention to the details outlined below. Acetylene is a hazardous combustible gas which can be used safely if one follows the lab protocol. **Before you ignite the flame, put on your safety goggles and continue to wear them until you turn off the flame.**

a) Open the valves of the compressed air and acetylene cylinders. Adjust the air (oxidant) outlet pressure to 60 psig and the acetylene (fuel) outlet pressure to 14 psig.

b) Turn the Oxidant Switch on the pneumatics control panel to Air. Adjust the air flow to 4 units on the right (Oxidant) flowmeter using the Oxidant Control Knob. Adjust the acetylene flow to 2 units on the left (Fuel) flowmeter using the Fuel Control Knob. Since there is a strong coupling between acetylene flow and acetylene pressure, also adjust the acetylene pressure with the acetylene regulator to 13 psig. The acetylene pressure should not exceed 15 psig.

**NOTE:** If the gases are left on for 15 sec without ignition or if the acetylene pressure drops below 12 psig, the red interlock light comes on and the system turns off the gas flows. If this happens, turn the gas control switch to Off and then back to Air.

c) When the gas flows and pressures have been adjusted, press the Ignite button until the flame appears. When gases are first turned on, the flame may not light the first time due to air in the supply lines. If the flame does not ignite, turn the gas control switch to Off, then back to Air, and press the Ignite button.

**E) Measurement of Absorbance.** Fill the 5" test tubes provided with blank solution (distilled water), wash solution (distilled water), the standards in order of increasing concentration, and the samples. This should be done prior to adjusting the gas flows and igniting the flame so that you can go directly from ignition to measurement. For a given run attempt to place the capillary aspiration tubing in a reproducible manner in each test tube and try to have the height of the solution the same in each test tube. Check the acetylene pressure and maintain it close to 13 psig.

**I) Manual Calibration.** In this series of measurements, you will measure the absorbances of the standard solutions and construct a standard curve using the data from the standards. You will then measure the absorbances of the sample solutions and determine the concentrations of the sample solutions from the standard curve. You should make a plot of the standard curve on mm graph paper as you are taking the data in order to see if there are any gross errors in your standard solutions. If there are, remake the ones that are in error. Remember that 0,0 is a defined point. The curve will probably be nonlinear. Hence, when you prepare a computer plot of your data use an appropriate nonlinear curve fitting program.

a) Enter the data collection mode by pressing the *Data* function key. The message on the screen should read **ABS (MEAN):-----** (mean absorbance), **SD:-----** (standard deviation), **RSD:-----** (relative standard deviation).

b) Aspirate the blank and let the flame stabilize for 30-60 seconds. Then press the *A/Z* (absorbance zero) function key. Five measurements of the absorbance of the blank solution will be made. Each measurement will require 1 s. The 3100 microprocessor will use the average of the 5 measurements to adjust the absorbances of the other solutions.

c) Read the absorbances of the standard and unknown solutions by dipping the aspiration tubing in the solution, waiting a few seconds, and pressing the blue *Read* function key. Five measurements of the absorbance will be made. Each measurement will require 1 s. The final display will give the average absorbance and the standard deviation of the measurement. After aspirating a solution, aspirate some of the wash solution for a few seconds before moving on to the next solution. Collect the data in a random order. The absorbance of the 5  $\mu\text{g/ml}$  solution should be between 0.1 and 0.2. ; if it isn't, see the instructor before continuing the experiment.

d) If either of the "archaeological" samples gives an absorbance value that is well beyond the absorbance value for the most concentrated standard solution, make an appropriate dilution of the solution and measure the absorbance for the diluted solution.

**2) Automatic Calibration.** The measurements will be repeated and the automatic calibration routine of the instrument will be used.

a) Enter the data collection mode by pressing the *Data* function key.

b) Aspirate the blank solution for a few seconds and then press the *A/Z* function key.

c) Aspirate the most dilute standard and press the *Calib* function key. The absorbance of the this solution will be measured and will be assigned to the first concentration that you entered when the parameters were defined. When this step is completed, aspirate some of the wash solution for a few seconds.

d) Aspirate the second most dilute standard, press the *Calib* function key a second time, and follow by aspirating some of the wash solution. Continue in this way with all of your standards. It is important that you measure the standards in order of increasing concentration.

e) Aspirate each of the unknowns. This time press the *Read* key to initiate measurement of the absorbance. The concentration of the solution and its standard deviation will be calculated automatically.

f) The 3100 software is unforgiving if you make the measurements out of order. Consult Chapter 6 of the 3100 manual for remedies if you make a mistake and have to repeat the measurements.

**3) Determination of Number of Standards.** In sections 1 and 2 you used five standard solutions to establish your calibration curves. Since the "Running the 3100" operating instructions manual states that usually only two or three standards are necessary. Repeat section 2 using three standards and then two standards to determine the concentrations of your unknowns. Use your manual calibration data to decide which standards to use. Obviously you will have to change the standard value parameters for each determination. For a long report discuss the significance of these results.

**4) Integration Times and Number of Replicates.** In order to determine whether or not the given integration time of 1 sec. and the number of replicates of 5 are satisfactory, try several shorter and longer integration times and different numbers of replicates. Use the 5 $\mu$ g/ml standard solution and measure absorbance through the manual mode. Be sure to include the given integration time of 1 sec. and the number of replicates of 5 in these measurements.

**5) Flow Rate of Gases.** In order to see the importance of stable flow rates, put the instrument in the continuous mode and observe the changes in absorbance of the 5 $\mu$ g/ml standard solution as you vary the flow rates of the gases. Do not make large changes in flow rates, just large enough to observe changes in absorbance.

**F) Shut-Down.** When you have completed all your measurements, aspirate wash solution for several minutes. This will purge the burner of acid solution. Then turn the gas control switch on the Pneumatics Control Panel to Off. Close the valve on the acetylene cylinder and give the acetylene regulator several clockwise turns so that the pressure exceeds 15 psig. Note the flame is out at this point. Turn the gas control switch to Air to allow the acetylene to bleed. The red interlock light on the Ignite button should turn on. When it does, turn the gas control switch to Off and then to Air. Repeat this procedure until the acetylene pressure has been minimized. The high pressure gauge should read close to zero and the low pressure gauge should read ca. 10 psig. Now give the acetylene regulator several turns in a counter-clockwise direction to release pressure on the diaphragm. Close the valve on the air cylinder. **Note that the air valve is the last one closed and that the fuel line pressure is reduced as much as possible.** This is always the procedure in working with combustible gases. Finally, turn off the power switch on the instrument.

## REPORT

**A) Short Report.** For a short report, turn in your duplicate notebook pages, printout of any calculations, calibration graphs, and the report sheets in duplicate. (Make a Xerox copy of your report sheet after you have entered your results.) Be sure to comment briefly about the agreement among the values for the Wgt.%Fe determined using the different calibration procedures and about the agreement of the known Wgt.%Fe for the standard sample with the value determined experimentally. A simple statistical check is to average the four Wgt.% Fe for each sample and see whether any sample value lies more than one standard deviation from the average. Also comment about your results for the different integration times and number of replicates.

**B) Long Report.** The style for this long report is the style of an article for *Analytical Chemistry*. The theory of atomic absorption spectroscopy, a description of how the instrument enables the measurement of absorbance to be made in the flame, and the possible sources of error should be discussed in the introduction. Since theoretically atomic absorption spectroscopy is based upon Beer's Law, a linear relationship, include in your discussion why we use the non-linear parameter. In the Experimental Section reference can be made to the laboratory manual for detailed experimental instructions. In *Analytical Chemistry*, Results and Discussion are combined as one section. In this section, give all of your results in tabular form in the proper location and include a copy of your manual standard curve. Be sure to discuss the significance of all your data. Compare your computer generated equation for your standard curve with the equation used by the instrument as given in the Perkin-Elmer "Running the 3100 Operating Instructions". In the Conclusions section, compare the applicability of atomic absorption spectroscopy for elemental metal analysis to other common methods of analysis along with any conclusions you can draw from your experimental results. Remember to begin your long report with an Abstract that summarizes in a concise manner the report. For ease of preparation the references can be listed as a separate section at the end of the report. The report should stand alone, i.e., the Short Report form is not to be included as part of the long report.

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NAME \_\_\_\_\_ Date Experiment \_\_\_\_\_  
 Completed \_\_\_\_\_  
 Element \_\_\_\_\_ Date Report Submitted \_\_\_\_\_  
 \_\_\_\_\_  
 Wavelength \_\_\_\_\_ Weight of Standard Sample \_\_\_\_\_  
 \_\_\_\_\_  
 Unknown Sample Number \_\_\_\_\_ Weight of Unknown Sample \_\_\_\_\_  
 Wgt.% Fe Standard Sample \_\_\_\_\_ Weight of Archaeological Sample 1 \_\_\_\_\_  
 \_\_\_\_\_  
 Weight of Archaeological Sample 2 \_\_\_\_\_

### MANUAL CALIBRATION

| Standard Soln<br>Conc. ( $\mu\text{g/ml}$ ) | Absorbance | Rel Std Dev, % | Statistical Data |  |  |
|---|------------|----------------|------------------|--|--|
| _____                                       | _____      | _____          |                  |  |  |
| _____                                       | _____      | _____          |                  |  |  |
| _____                                       | _____      | _____          |                  |  |  |
| _____                                       | _____      | _____          |                  |  |  |
| _____                                       | _____      | _____          |                  |  |  |

  

| Sample %             | Absorbance | Rel Std Dev, % | Conc ( $\mu\text{g/ml}$ ) | Wgt.% Fe | Rel 95% C.I. of the Mean, |
|----------------------|------------|----------------|---------------------------|----------|---------------------------|
| <i>Standard</i>      | _____      | _____          | _____                     | _____    | _____                     |
| <i>Unknown</i>       | _____      | _____          | _____                     | _____    | _____                     |
| <i>Archaeolog. 1</i> | _____      | _____          | _____                     | _____    | _____                     |
| <i>Archaeolog. 2</i> | _____      | _____          | _____                     | _____    | _____                     |

Relative Error of Standard Wgt.% Fe Compared to Known Value, % \_\_\_\_\_

### AUTOMATIC CALIBRATION

| Sample   | Conc ( $\mu\text{g/ml}$ ) | Rel Std Dev, % | Wgt.% Fe | Rel 95% C.I. of the Mean, % |
|--|---------------------------|----------------|----------|-----------------------------|
| <b>Automatic Calibration with Five Standards</b> |                           |                |          |                             |
| <i>Standard</i>                                  | _____                     | _____          | _____    | _____                       |

*Unknown* \_\_\_\_\_  
*Archaeological 1* \_\_\_\_\_  
*Archaeological 2* \_\_\_\_\_

Relative Error of Standard Wgt.% Fe Compared to Known Value, % \_\_\_\_\_



### Automatic Calibration with Three Standards

|   |       |       |       |
|---|-------|-------|-------|
| Conc. of Standards ( $\mu\text{g/ml}$ ) | _____ | _____ | _____ |
| <i>Standard</i>                         | _____ | _____ | _____ |
| <i>Unknown</i>                          | _____ | _____ | _____ |
| <i>Archaeological 1</i>                 | _____ | _____ | _____ |
| <i>Archaeological 2</i>                 | _____ | _____ | _____ |

Relative Error of Standard Wgt.% Fe Compared to Known Value, % \_\_\_\_\_

### Automatic Calibration with Two Standards

|   |       |       |
|---|-------|-------|
| Conc. of Standards ( $\mu\text{g/ml}$ ) | _____ | _____ |
| <i>Standard</i>                         | _____ | _____ |
| <i>Unknown</i>                          | _____ | _____ |
| <i>Archaeological 1</i>                 | _____ | _____ |
| <i>Archaeological 2</i>                 | _____ | _____ |

Relative Error of Standard Wgt. % Fe Compared to Known Value, % \_\_\_\_\_

### INTEGRATION TIME AND NUMBER OF REPLICATES

| Time, sec. | Absorbance | Rel Std Dev, % | Number | Absorbance | Rel Std Dev, % |
|------------|------------|----------------|--------|------------|----------------|
| _____      | _____      | _____          | _____  | _____      | _____          |
| _____      | _____      | _____          | _____  | _____      | _____          |
| _____      | _____      | _____          | _____  | _____      | _____          |
| _____      | _____      | _____          | _____  | _____      | _____          |
| _____      | _____      | _____          | _____  | _____      | _____          |

### FLOW RATE

| Air Flow Rate | Acetylene Flow Rate | Absorbance |
|---------------|---------------------|------------|
| _____         | _____               | _____      |
| _____         | _____               | _____      |
| _____         | _____               | _____      |
| _____         | _____               | _____      |
| _____         | _____               | _____      |

**On the back of this sheet, comment briefly about the agreement among the values for Wgt.% Fe and about the agreement of the known Wgt.% Fe for the standard sample with the value determined experimentally and about your results for the different integration**

**times and number of replicates.**