

Atomic Absorption Spectroscopy

Introduction

This experiment is in three parts. In the first, the emission spectra of Cu and Ca hollow cathode lamps are collected and the emission peaks are identified. Next, the experiment focuses on the use of AA for the determination of metal concentrations. Two potential sources of interference: organic solvents and strong ligands are examined in parts 2 and 3, respectively. The principle concepts and instrumentation used for atomic absorption spectroscopy are presented in Chapter 9 of Skoog, Holler, and Neiman (5th edition).

The Varian SpectrAA-200 Spectrometer in Room 35 will be used for this experiment. Instructions for its use are on the last page of this handout.

Part 1. Emission Source Characteristics

The ideal source for atomic absorption (AA) would be an intense, stable, monochromatic source. In many respects the hollow cathode discharge tube fulfills these requirements. For atomic absorption, the line width of the source is narrower than the absorption line width and is therefore completely available for absorption. In this experiment, the spectrum of a typical hollow cathode lamp will be taken in order to identify the predominant lines and to illustrate the monochromator requirements in AA spectrometry.

Emission Spectra of Ca and Cu Lamps:

Follow steps 1-3 of the operating instructions. The worksheet is entitled "Calcium and Copper."

Make sure that the Ca lamp is in position 1 and the Cu lamp is in position 2.

Optimize the Ca lamp (see #3 in the instructions at end of this handout). You do not have to adjust the burner or light it for this part of the experiment.

Highlight the box next to Sample 001 under "Ca."

Click on "Instrument" on the menu bar (far-left option) and choose "Wavelength Scan," then "Lamp Scan."

Set the scan range to 185 – 900 nm and hit "OK" to collect the spectrum.

When the spectrum is complete, print it by clicking the right mouse button and selecting print.

Rotate the Cu lamp into the analysis position and optimize it.

Highlight the box next to Sample 001 under "Cu" and collect its spectrum over the same wavelength range and print it.

The spectra can be displayed by clicking on the appropriate box next to Sample 001. Holding the left mouse button down while drawing a box will expand the desired region. The wavelength of an emission peak can be determined by placing the cursor on it.

In lab:

1. Zoom in on the region of each spectrum around the most intense peaks below 500 nm. Print and determine the wavelengths and FWHM's (full width at half the maximum height) of the two most intense peaks.
2. Blowup the region between 600 and 750 nm in each spectrum and print. Determine the wavelength of several of the most intense peaks in this region of both spectra. Are the peaks the same in both spectra? What are they due to?

Part 2. Effect of Organic Solvents on Copper Absorbance

The physical properties of a solution can have a strong influence on the intensity of the absorbance measured with AAS. Properties such as density, viscosity, and surface tension are altered by the presence of dissolved solutes, surfactants, and miscible organic solvents. For this reason, it is inappropriate to use a calibration curve determined from pure aqueous standards for samples containing significant concentrations of these substances. In this part of the experiment the effect of a miscible organic solvent (ethanol) on the intensity of the Cu absorbance will be examined. In addition the physical properties of the solution which have the strongest influence on the signal intensity will be identified.

Use the Cu hollow cathode lamp for this analysis. Prepare the following solutions in 100-mL volumetric flasks from a stock solution of Cu 1000 ppm and ethanol (EtOH).

| | Cu (ppm) | EtOH (% Volume) |
|---|----------|--------------------|
| 1 | 10 | 0 |
| 2 | 10 | 10 |
| 3 | 10 | 35 |
| 4 | 10 | 60 |
| 5 | 10 | 80 |
| 6 | 10 | 99 |

Adjust and light the burner as describe in the instructions. Then, using distilled water, make sure that the aspiration rate is about 4 – 5 mL/min. To do this, put water in a 10-mL graduated cylinder and measure the time needed to aspirate a given volume of the solution. The TA will show you how to adjust the uptake valve, if necessary. Once the desired aspiration rate is obtained, lock the uptake valve in place with the locking ring. Do NOT make any further adjustments.

Measure the Cu absorbance of all six solutions prepared and determine each solution's aspiration rate. Which line in the copper emission spectrum is used for this analysis?

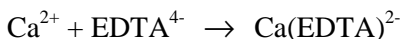
Part 3. Phosphate Interference and Releasing Agents in Ca Determination

In this experiment you will examine the effects of phosphate ions (PO_4^{3-}) on Ca absorption in atomic absorption spectroscopy. In addition, two methods of counteracting the phosphate interference will be examined. Phosphate ions, which are present in blood serum, interfere with the determination of Ca by AAS. At elevated temperatures, such as those that occur in the air-acetylene flame, Ca and other alkaline earth elements react to form pyrophosphate:



Since calcium pyrophosphate ($\text{Ca}_2\text{P}_2\text{O}_7$) does not fully decompose in the flame, the absorbance of free Ca atoms will be reduced. Therefore, an error is introduced which results in a systematically low Ca concentration measurement. The magnitude of this error is dependent on the PO_4^{3-} concentration in the sample.

The phosphate interference can be minimized with the use of a releasing agent. For example, several lanthanide elements form stronger complexes with phosphate than do the alkaline earth elements. If an excess of La or Sr is added to the sample it will preferentially react with the phosphate and act as a releasing agent for calcium. Another releasing agent is ethylenediaminetetraacetic acid (EDTA), which forms a more stable complex with calcium than phosphate does.



Because the $\text{Ca}(\text{EDTA})^{2-}$ is readily decomposed at high temperatures, the calcium absorption is not suppressed.

Procedure

Obtain 13 100-mL volumetric flasks and the following stock solutions from the TA:

- 400 ppm (0.01 M) Ca^{2+} solution
- 10^{-2} M $\text{NaH}_2\text{PO}_4 \cdot \text{H}_2\text{O}$ (phosphate solution)
- 0.04 M EDTA
- 0.04 M Sr^{2+}
- 0.4 M EDTA
- 0.8 M NaCl
- pH 10 ammonium chloride buffer solution

Put 10 mL of Ca^{2+} solution and 10 mL of the pH 10 buffer solution into each of the flasks. Add the following to each numbered flask and then dilute to volume with distilled water and mix thoroughly.

- 1) Add distilled water only.
- 2) 2 mL phosphate solution
- 3) 4 mL phosphate solution
- 4) 6 mL phosphate solution
- 5) 8 mL phosphate solution
- 6) 10 mL phosphate solution
- 7) 15 mL phosphate solution
- 8) 20 mL phosphate solution
- 9) 10 mL phosphate solution and 2.5 mL 0.04 M EDTA solution
- 10) 10 mL phosphate solution and 10 mL 0.04 M EDTA solution
- 11) 10 mL phosphate solution and 40 mL 0.40 M EDTA solution
- 12) 10 mL phosphate solution and 10 mL 0.04 M Sr^{2+} solution
- 13) 10 mL phosphate solution and 40 mL 0.80 M NaCl solution

Move the Ca lamp into the analysis position and determine the Ca absorbance of all solutions. Which line is used for the analysis? Is it a neutral atom line or an ion line?

Data Analysis

Identify the most intense lines in the Cu and Ca emission spectra. Note whether they are due to transitions in neutral atoms or in ions.

Make a double-y plot of Cu absorbance and aspiration rate vs. % EtOH. From the literature (identify your source), obtain the surface tension and viscosity of ethanol-water mixtures. Make a double-y plot of surface tension and viscosity vs. % EtOH.

Based upon the concentration of the stock solutions, make a table showing the molar concentrations of: Ca^{2+} and phosphate in all solutions, EDTA in solutions 9 – 11, Sr^{2+} in solution 12, and Na^+ in solutions 11 and 13.

Make a plot of Ca absorbance vs. phosphate concentration using the data collected for solutions 1 – 8 in Part 3 of the experiment.

Questions

Why are narrow line sources advantageous in AA?

Why might there be intense ion lines in the spectrum of one lamp and not in another?

Discuss what happens to the aspiration rate as the % EtOH increases. Explain any trends in terms of the physical properties of the solutions (i.e. surface tension, density, and viscosity). Based on the graphs made, give plausible scientific reasons why ethanol should influence the Cu absorption in the manner observed.

What is happening chemically to account for the observed trends in the Ca absorbance vs. PO_4^{3-} concentration curve?

Discuss the effect of EDTA on the Ca absorbance. Comment on the concentration of EDTA necessary to significantly counteract the phosphate interference.

Discuss the influence of sodium on the Ca absorbance and suggest a possible explanation for the effect that you observe.

The EDTA solution was prepared from the disodium salt. Based upon the results of the analysis of your samples, do you think that the presence of the high concentration of sodium had a significant effect on the Ca absorbance for sample 11?

Discuss the effect of Sr upon the Ca absorbance intensity.

References:

- Ingle, J.D. and Crouch, S.R. *Spectrochemical Analysis*; Prentice Hall: Upper Saddle River, NJ, 1988.
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Rocha, F.R.P. and Nobrega, J.A. *J. Chem. Ed.* **1996**, 73(10), 982.
West, A.C. and Cooke, W.D. *Anal. Chem.* **1960**, 32(11), 1471.
Dinnin, J.I. *Anal. Chem.* **1960**, 32(11), 1475.

Varian SpectraAA-200 Spectrometer Operating Instructions

The following are general operating instructions for the Varian SpectraAA spectrometer used in this experiment. Please consult with your assistant for specific details.

1. Power up the spectrometer and then boot the SpectraAA software.
2. Select “Worksheet,” then “open” and choose the appropriate worksheet for the experiment (your TA will provide the name). The experimental parameter can be examined by editing the method (click on the tab marked “2. Develop”).
3. **Lamp optimization procedure:** Click on the tab marked: “4. Instrument” and on the “Optimize” button. Select the lamp to optimize – this will bring up an “Analysis Checklist.” Open the instrument cover and check to see that the lamp is in the proper slot, that it is rotated to the analysis position, and that the slit is set to the indicated width. The lamp will light and after about 10 sec a green bar will appear in the slot on the left indicating the signal intensity. Adjust the setscrews at the base of the lamp to maximize the signal. Pressing the “Rescale” button resets the maximum intensity to 100. Note that as the position of the lamp is optimized, the PMT voltage decreases. When the lamp is aligned, press “Rescale,” close the lamp compartment, and exit the “Optimization” window.
4. Adjust the burner so that the light from the lamp falls within the center of the target on the card provided. There are three different adjustments possible: burner height, front to back position, and orientation angle.
5. Open the air and acetylene (fuel) tanks.
6. Check to see that the tanks still contain gas and that the line pressures are correct (~11 psi for acetylene and ~50 psi for air).
7. To light the burner, the burner door must be down. Close the ventilation snout. **MAKE SURE THAT YOU HAVE YOUR SAFETY GLASSES ON.** Open the air valve on the front of the instrument (note: you have no control over the flow rate). When the igniter button is pressed a tongue of flame will shoot out above the burner. Hold down this button and simultaneously open the acetylene valve. Increase the flow of acetylene until the burner lights and then back it off to a flow rate of about 2 mL/min. **OPEN THE VENTILATION SNOUT FULLY.**
8. Aspirate deionized water whenever the flame is on. Avoid sucking air through the aspirator, when possible.
9. Measure the absorbance of a blank (deionized water or other appropriate solution as instructed) by pressing the “READ” button. (Never press “START” – this is for analysis with an autosampler only and the computer will crash if there is no autosampler). Follow the instructions in given in the analysis window.
10. Measure the samples you prepared making sure to rinse with deionized water between samples. Pressing “READ” will start each analysis. The absorbance is shown in the upper right hand corner of the screen.
11. Press “STOP” when the analysis is done. If “READ” is then pressed after “STOP”, you will be prompted to re-measure a blank solution before measuring the absorbance of the next sample.
12. To turn off the AA, first close the fuel valve. Let the air flow through the burner for a few minutes, then turn off the air flow.
13. Close the air and acetylene tanks.
14. Turn off the instrument and close the software.