University of Wisconsin
Chemistry 524
Spectroscopic Applications (GFAA, ICP, UV/Vis, Fluorescence)*

For this laboratory exercise, you will explore a variety of spectroscopic methods used in an analytical laboratory. Common to all of these methods is the use of a spectrometer to measure light at ultraviolet and visible wavelengths. Although the optical designs are unique for each instrument, all of the spectrometers used in this exercise include a monochromator as an integral component.

The methods that you will be using are: Fe determination using colorimetry measured with a UV/Vis spectrometer, As determination using a graphite furnace atomic absorption spectrometer, elemental analysis in water using an inductively coupled plasma atomic emission spectrometer, and quinine measurement in tonic water using a spectrofluorimeter.

Method 1: Fe Determination using a UV/Vis Spectrometer

Beer's law tells us that the amount of light absorbed by a sample is proportional to the concentration of the absorbing species in the sample. This provides the basis for many quantitative analytical methods, because if the proportionality constant is known, the measured absorbance can be used to calculate the concentration of the absorbing species.

Spectrometers can be made that measure absorbances in different regions of the electromagnetic spectrum. A visible spectrometer, wavelengths from 400 – 700 nm, is used to detect species that are colored. The energy transitions that occur in the visible region are due to electronic transitions in the atoms of or molecules of interest.

Only a small percentage of all species are colored, so visible spectroscopy is limited in the kinds of species that can be detected. However, some species that do not have color or are only weakly colored, can be complexed with another species, and the complex may be strongly colored allowing very sensitive detection at visible wavelengths. This technique is called colorimetry.

In this method, you will determine the amount of iron in either hard cider or fruit juice by complexing the iron with o-phenanthroline and measuring the absorbance of the complex with a visible spectrometer. The iron must be in the ferrous (Fe^{2+}) oxidation state to make the complex that absorbs at the appropriate wavelength so a small amount of ascorbic acid is added to reduce any ferric iron (Fe^{3+}) that may be present in the sample or standards. You will use the method of standard additions in this analysis. The method of standard additions is covered in your text in Chapter1.

The spectrometer that you will use for measurement is a Jasco 570 UV/Vis double beam spectrometer. A diagram of its optical layout is shown below. The TAs will help you with the operation of this spectrometer.

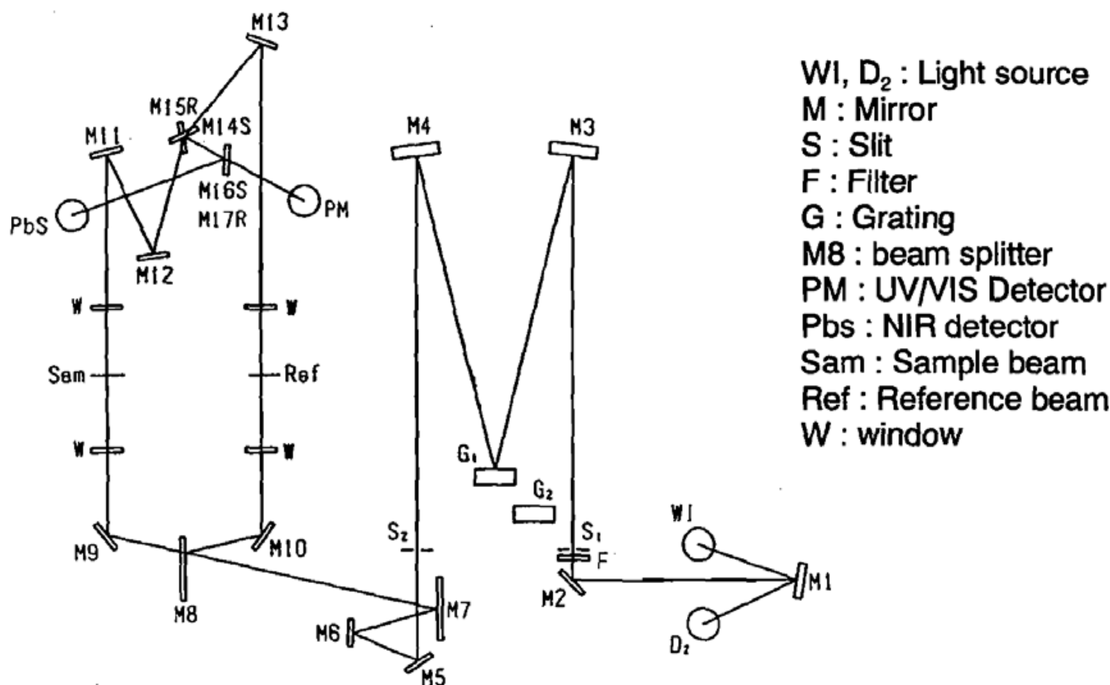

Fig 2.5 V-570 Optical system

Use the following procedure to prepare the samples.

Note: Add all components in the order specified.

-Pipet 10.00 mL of the sample into 6 separate 25.0 mL volumetric flasks.

-To flask #1, add 1.00 mL of the ascorbic acid solution, 1.00 mL of 0.3% o-phenanthroline solution, and dilute to the mark with water.

-To flask #2, add 1.00 mL of the ascorbic acid solution, 0.100 mL of 100 μg/mL Fe solution, 1.00 mL of 0.3% o-phenanthroline solution, and dilute to the mark with water.

-To flask #3, add 1.00 mL of the ascorbic acid solution, 0.200 mL of 100 μg/mL Fe solution, 1.00 mL of 0.3% o-phenanthroline solution, and dilute to the mark with water.

-To flask #4, add 1.00 mL of the ascorbic acid solution, 0.300 mL of 100 μg/mL Fe solution, 1.00 mL of 0.3% o-phenanthroline solution, and dilute to the mark with water.

-To flask #5, add 1.00 mL of the ascorbic acid solution, 0.400 mL of 100 μg/mL Fe solution, 1.00 mL of 0.3% o-phenanthroline solution, and dilute to the mark with water.

-To flask #6, add 1.00 mL of the ascorbic acid solution and dilute to the mark with water. This solution will be used in the reference cell.

If it is not on already, turn on the UV/Vis instrument and allow the instrument about 10 minutes to warm up. Use one of your samples to collect the absorbance spectrum of the colored complex. From the spectrum, choose a good wavelength for your quantitative analysis. Select the instrument to read absorbance, set the wavelength, and zero the instrument by placing the blank in two different cuvettes, and putting the cuvettes in the sample and reference beams of the instrument. Using the same sample cuvette, measure and record the absorbance value for each of the prepared solutions.

Analysis

Using Excel, plot absorbance vs. Volume of standard solution added. Use the slope and the intercept of the fitted line to **calculate the concentration in μg/mL of iron in the sample**. Refer to your text for help with the standard addition analysis.

Method 2: As Analysis Using Graphite Furnace Atomic Absorption You are going to use the GFAA to identify if the As present in treated lumber leaches out of the wood. You are going to test a sample soaking under slightly acidic conditions and compare to a sample soaking in tap water. The optical layout for the Perkin Elmer Model 3030 Atomic Absorption Spectrometer is shown below. The TAs will help you run the instrument but condensed instructions are included here for your information.

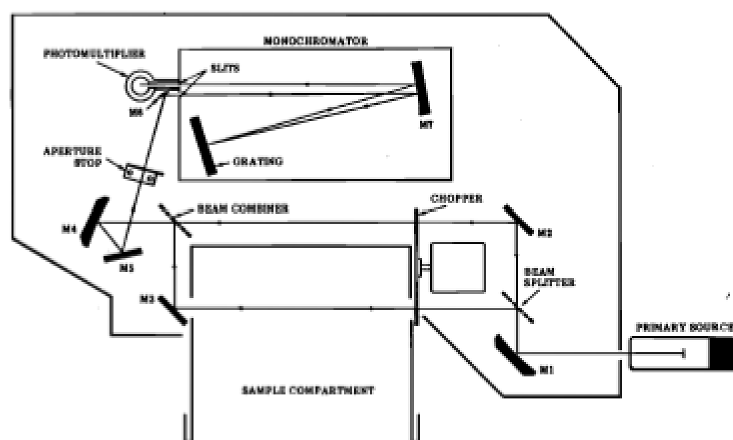

Figure 4-1 - Model 3030 Optical Schematic, AA Mode

Use the GFAA to run an H_2O blank, the two samples from the leaching solutions, and the two As standards. **In each case does the leaching solution contain more than 10 ppb of As? Does the acid affect the leaching?**

The following instructions are for the operation GFAA spectrometer.

Operation of the Perkin Elmer 3030 Graphite Furnace Spectrophotometer for As Determination

Setting up the spectrometer.

1. Turn on water and gas supplies. The Ar pressure should be 30 psi.

2. Turn on the spectrometer and allow it to warm up for at least 15 minutes.

3. Be sure the As lamp is in the turret. The As lamp uses the additional power supply located near the instrument.

4. Go to the program screen using [Prog] to input the parameters for your analysis. Toggle through the parameters using [backup] and [advance] buttons. Set up the parameters as follows:

1. Technique: AA	2. Lamp current: 25 mA
3. Signal Processing: Peak Height	4. Calibration: Auto Select
5. Nominal Weight: 1.0	6. Statistics: Single Reading
7. Time (seconds): 20	8. Read Delay (seconds): 0.0
9. Screen Format: Graphics	10. Printer: All Main Values
11. Recorder Signal: 0.2 Cont Abs	12. Recorder Exp. 1000

5. Run [Setup] to find maximum lamp intensity. Change the monochromator to about 193 nm and fine adjust the dial to maximize the energy. Align the lamp and optimize the booster current. (You usually get an energy reading near 30.) Use the [Gain] button to rescale.

6. Hit [Display graph] to see graphical output on screen.

Preparing the Heater Control Unit

1. Turn on the power to the unit.

2. Program the temperature run according to the following:

Step 1 involves setting up the solvent evaporation. .
Type 1 [step]

Type 120 then [temp]	Evaporation Temp.
Type 10 then [hold]	time to hold evaporation temp.
Type 5 then [ramp]	time to go from room temp to evaporation temp

Step 2 involves setting up the ashing temperature.
Type 2 [step]

Type 1500 then [temp]	Ashing temp
Type 10 then [hold]	time to hold the ashing temp
Type 10 then [ramp]	time to go from evaporation temp to ashing temp

Step 3 involves setting the atomization temp.
Type 3 [step]

Hit [stop flow]	Tells instrument to stop Ar flow
Hit [read]	Tells instrument to read absorbance
Type 2500 then [temp]	Atomization temperature
Type 5 then [hold]	time to hold atomization temp.
Type 0 then [ramp]	time to go from ashing temp to atomization temp.

Step 4 involves conditioning the furnace between runs
Type 4 [step]

Type 2700 [temp]	Carbon rod conditioning temp
Type 5 then [hold]	time to hold conditioning temp.
Type 0 then [ramp]	time to go from atomization temp to cond. Temp

3. Set the time constant to 46.

Running an As sample

Use the pipetter to add 40µL of the sample to the furnace.
Use the pipetter to add 10 µL of the matrix modifier to the furnace. (1 mg/mL $MgNO_3$ in 5 % HNO_3)
Start the data acquisition and press the Start/Stop on the heater unit to begin the run. .
When the run is complete stop the data acquisition and save the data.
Wait for the ready light to come on, this indicates that the furnace has cooled, before adding solutions for the next run.

Shutdown

1. Turn off the power for each unit.

2. Close the Ar tank and turn off the cooling water.

Method 3: Elemental Analysis in Water Using Inductively Coupled Plasma Optical Emission You will use the ICP to test for the common elements, Zn, Fe, Mn, Mg, Ca, Cu, Na, and K in the water from the second floor bubbler. You will need to run a blank, a series of standards, the water sample and the water sample diluted by a factor of 10. **Report the measured concentration of each of the elements and state your confidence in each result**. Dr. McClain will help you with this analysis.

Again, the TAs or Dr. McClain will help you run this instrument but condensed instructions are included for your information. The Perkin Elmer Optima 2000 DV uses an echelle monochromator for wavelength selection and a Charge Coupled Device (CCD) for its detector. More information about these components can be found in your text. The optical layout for the spectrometer is shown below.

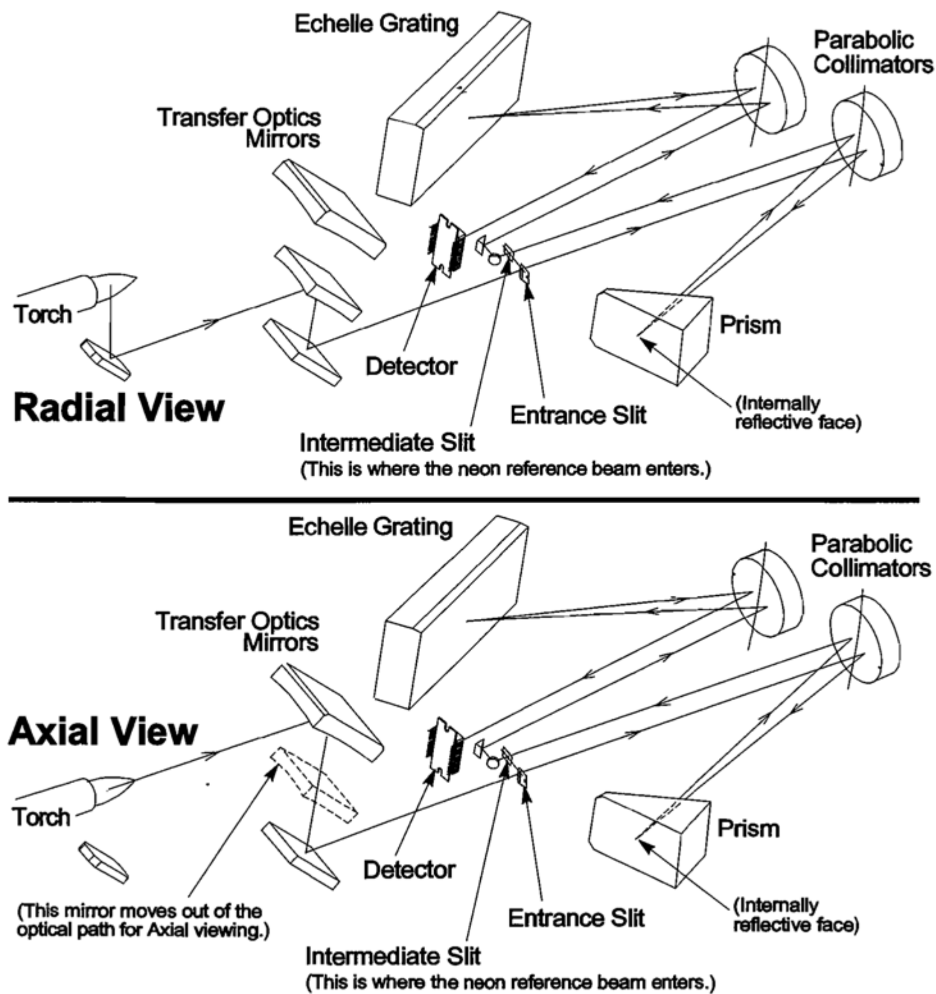

Figure 3-2 Schematic diagram of the optical system.

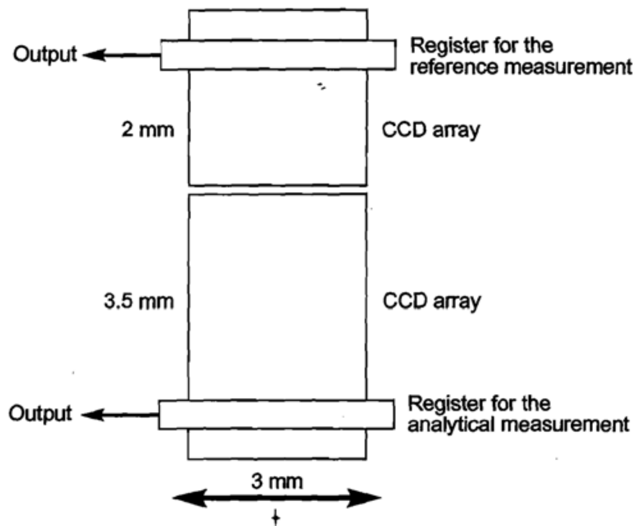

Figure 3-4 Detector

Startup

1. Turn on the instrument power, the chiller power, and plug in the autosampler if you are using it.
2. Open the air valve, and Ar valve, and the N_2 valve at the tanks.
3. Double click on the Winlab32 icon to start the ICP software. The instrument will go through a series of internal checks that takes a couple of minutes.
4. Attach the pump tubing, set the tension, and place the inlet into the wash solution.
5. Ignite the plasma by opening the plasma control window in the tools menu and clicking the ON switch.

Check the pump flow. The plasma needs about 30 minutes to stabilize before making measurements. The instrument will automatically optimize the optics after the plasma has been on for 20 minutes. When this optimization is complete, you may run your samples.

Running Samples

6. Load the appropriate method file, and sample file for your analysis. Run your samples either manually or with the autosampler. You can view the spectra while the acquisition is taking place by clicking on Spectra.
7. Print your results file.

Shutdown

8. Run a 5% nitric acid solution for a couple of minutes to clean out the system.
9. Run DI water for a couple of minutes to rinse the acid
10. Remove the sample introduction and run the plasma without sample introduction for a couple minutes to dry out the sample introduction system.
11. Turn off the plasma in the in the plasma control window.
12. Wait for the plasma to completely shut down then exit the software.
13. Close all tank valves, unplug the autosampler, disengage the pump tubing, turn off the chiller and the instrument.

Method 4 Measurement of Quinine in Tonic Water Using a Spectrofluorimeter
In this experiment[i], you are going to use molecular fluorescence to determine the quinine concentration in tonic water.

You will use the RF1501 Fluorimeter for your fluorescence measurements. This instrument uses 2 monochromators, one for the excitation wavelength selection and one for the emission wavelength selection. It also uses two PMT's so that fluctuations in light source can be corrected for in quantitative measurements. The optical design is shown below.

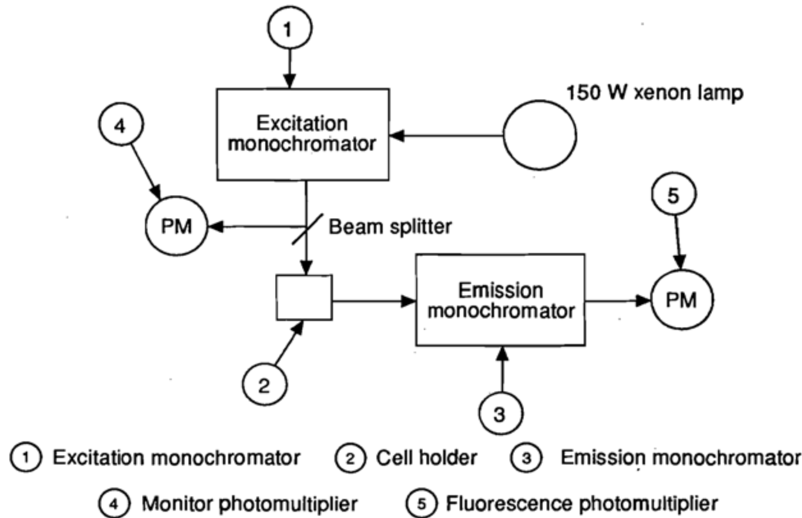

Figure 1 RF-150X arrangement

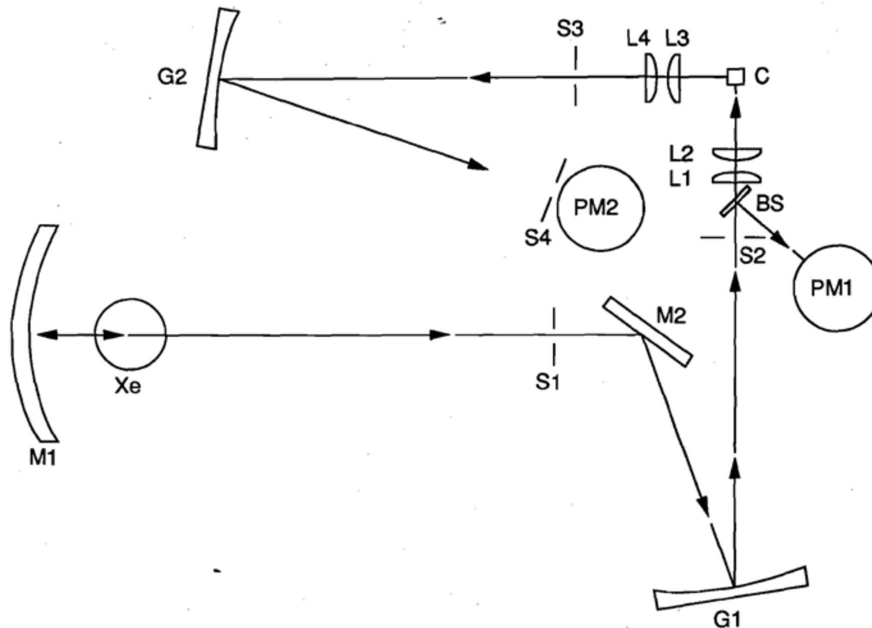

Figure 2 RF-150X Optical system

Use the following procedure for this analysis.

Power up the instrument If the instrument has not already been powered up, start the spectrometer by: a) set the Xe lamp switch to on; the lamp switch is a small black switch on the right side of the instrument b) turn the power switch on; wait about a minute for the instrument to go through its startup procedure c) double click on the Fluorimeter icon on the PC desktop to start the software.

Find a good wavelength for analysis Place the 0.05 µg/mL quinine sample in the cell holder. There are three different Acquire modes of operation; Spectrum, Quantitative, and Time Course. Start by collecting a spectrum for the quinine. Set up your collection parameters by choosing parameters in the configure menu. Quinine absorbs strongly at 350 nm so set up an emission scan from 350 nm to 700 nm. Other paramaters to set are: a) sensitivity: high b) scan speed: fast c) slits: 10 nm d) recording range: low 0; high 1000 e) response time: auto. Choose start on the toolbar to collect the spectrum. Your data will be saved in C:\PC150X\DATA. Look at your spectrum and select good wavelengths for your quantitative measurements.

Calibrate Switch to Quantitative mode of operation. To read the fluorescence value in this mode hit the "read" key. Measure the fluorescence of each of the five standards and a 0.05 M H_2SO_4 blank and make a calibration plot.

Analyze the sample Measure the fluorescence of the tonic water. Make suitable dilutions of the tonic water with 0.05 M H_2SO_4 to bring the fluorescence within the calibration range. Calculate the quinine concentration in the tonic water.

Power down the instrument If you are the last user of the day turn off the instrument by: a) turn off the lamp b) wait 5 minutes for the lamp to cool c) turn off the power switch d) log out of the computer.

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[*] R.L. McClain, February 2007

[i] Adapted from J.E. OReilly, J. Chem. Educ., 1975, 52(9) 610.